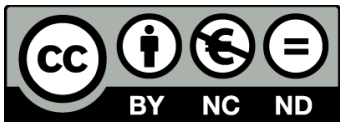
International University of Catalonia
School of Architecture

Doctoral Thesis

Embedding Microorganisms in Interior Design elements to achieve Design Ecology (Empirical study on achieving energy self-producing systems)

Yomna Mohammed Abdallah



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Embedding Microorganisms in Interior Design Elements to Achieve Design Ecology
(Empirical study on achieving energy self-producing systems)

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Preface

In The Name of God the Most Gracious, the Most Merciful

O people! Here is a parable, so listen to it. Those whom you call upon apart from Allah cannot create even a fly, though they may all join hands for it. And if the fly should snatch away something from them, they cannot recover it from it. Feeble indeed is the seeker and (feeble) the sought after.

Al Hajj, chapter 22, verse 73, Holly Quran.

Herby the author witnesses the ultimate capacity and power of creation is only owned to God the All mighty, and that all efforts that have been done and will be done by humankind will never rise to the pure definition of creation, even for the tiniest creature that is in reality sophisticated and exquisite.

In this context, all efforts done in this study or referred to in literature review is directed to exploit Gods' creatures for humankind prosperity, moreover this highlight the fact that all earths' resources are not owned by mankind, thus imposing responsibility to maintain these resources as augmented as possible.

In the way trying to survive against harsh natural forces, adjusting and exploiting it. We confused our purpose to enhance our lives with obsession and control over all our mother earth. We get lost in the purpose of proving our ego; through control, proclaiming that we seek humankind prosperity. Shortly we ruined the noble purpose by greed and arrogance. Now, when we thought we have reached the highest point of advancement and control over nature, nature fires back and our planet is collapsing.

However, nature always finds a way to repair and resist. Emerging from its creation secret, that God the All-Mighty is the only source of it, we just have to conscious our limits, accept it, and try to listen to nature, learn from it, and integrate our striving efforts towards what we really need, to live "In" nature, not "by" nature.

This thesis is hopefully a step on the right track of focusing the research efforts towards harmonizing and sharing control with nature, trying to restore its soft and powerful qualities of regeneration and survival; and to make a reasonable use of its resources to reinforce the eco system.

I dedicate this work to…
The reason, the aim, my role model, My Mother & Father.
To my shining star, my tree, my Love

Acknowledgment

Foremost, I am debit to God the all-mighty with all what was achieved through this research, for his miraculous grace and blessing making it possible to complete this work successfully. Putting me in the right path and in good hands as well as blessing me with patience, consistency, and strength to achieve this work.

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me with an extensive training in biochemical experimentation that optimized the empirical study of self-sufficient bioelectricity producing systems in this work. It would not be possible without your help and support.

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Methodology

Introduction

R. Buckminster Fuller, 1960s, demonstrated how design could play a central role in identifying major world problems. That included following contents*:* ***Review and analysis of world energy resources, defining more efficient uses of natural resources,*** and integrating machine tools into efficient systems of industrial production.

In the 1992 conference, "The Earth Summit Strategy to Save Our Planet "emphasized that designers should challenge for facing human problems. ***These problems included; the efficient use of natural resources, protecting the global commons, managing human settlements, the use of chemicals and the management of human industrial waste***. [a]•

Thus, developing eco-friendly technologies have been and remains of great industrial value and world's short listed priority issues, due to the world's current energy crisis and the threatening effects of the use of fossil fuels. The most alarming effects are: the global warming, non-renewable energy resources depletion and green cover depletion. As exhibited in its Fifth Assessment Report 2014, the Intergovernmental Panel on Climate Change concluded that more than 95 % of human activity is responsible for the escalation of global warming crisis over the last century; the burning of fossil fuels has increased the concentration of atmospheric carbon dioxide (CO_2), along with the biodiversity depletion. [b]•

Despite the growing number of mitigation polices addressing climate change, annual emissions grew on average by 2.2% per year from 2000 to 2010 and this was reported the highest in human history.***CO_2 emissions from fossil fuel combustion contributed about 78% of the total emission increase from 1970 to 2010***, with a similar percentage contribution for the period 2000–2010. Fossil fuel-related CO_2 emissions grew further by about 3% between 2010 and 2011 and by about 2% between 2011 and 2012. ***With this increase in total emissions directly coming from energy supply (47%), and buildings (3%) sectors***. Direct CO_2 emissions from the energy supply sector are projected to almost triple by 2050 compared to the level of CO_2/year in 2010, unless energy intensity improvements can be significantly accelerated.

Increased use of other energy sources has reversed the long-standing trend of gradual de-carbonization of the world's energy supply. However, without additional efforts to reduce emissions beyond those in place today, emissions growth is expected to persist. Baseline scenarios result in global mean surface temperature increases in 2100 from 3.7°C to 4.8°C. In addition, an increase in emissions that exceed 450 parts /10^6 of CO_2 by 2030 and reach CO_2 concentration levels between 750 and more than 1300 ppm CO2 by 2100. [c]•

In the majority of low-stabilization scenarios, the share of low-carbon electricity supply (comprising renewable energy (RE)) increases from the current share of approximately 30% to more than 80% by 2050, and fossil fuel power generation is phased out almost entirely by 2100. Thus decarbonizing electricity generation is a key component of cost-effective mitigation strategies in achieving low-stabilization levels of CO_2eq; as de-carbonization is more rapidly achievable in electricity generation than in the industry, and transport sectors. [c]•

• **[a] https://en.wikipedia.org/wiki/Ecological_design.**
• **[b] https://climate.nasa.gov/causes/.**
• **[c] IPCC Fifth Assessment Report/ 2014.**
• **Ibid.**

Recently, many RE technologies have demonstrated a potential candidate for low-carbon electricity generation, with substantial performance improvements and cost reductions, and a level of maturity to enable deployment at significant scale. Regarding electricity generation alone, RE accounted for just over half of the new electricity-generating capacity added globally in 2012, led by growth in wind, hydro and solar power. ***However, many RE technologies still need direct and/or indirect support. Challenges for integrating RE into energy systems and the associated costs vary by RE technology, regional circumstances, and the characteristics of the existing background energy system.*** [c]•

For instance, in Egypt, although the majority of Egypt's electricity supply is generated from thermal and hydropower stations, electricity production rates from non-renewable sources, especially fossil fuels have recorded very high rates as in 2013, 91% of power generation relied on oil & gas , 7.5% from hydro power and 1.5 % from wind and other resources. ***The current Hydro installed capacity represents 12% will become less than 8% by the year 2020.*** [d]•. ***Moreover, 12% contribution from renewable sources other than hydro need to be added by 2020. Thus, the current energy strategy in Egypt (adopted by the Supreme Council of Energy in February 2008) is to increase renewable energy generation up to 20% of the total mix by 2020.*** [e]•

Employing bioenergy offers energy supply with large-scale net negative emissions, which plays an important role in many low-stabilization scenarios, especially options with low lifecycle emissions (e.g., sustainable use of biomass residues) and outcomes that are site-specific and rely on efficient integrated biomass-to-bioenergy systems. However, to employ bioenergy options, ***there are issues to consider, such as the sustainability of practices and the efficiency of bioenergy systems. Bioenergy entails challenges and risks; these include the upstream large-scale development of bioenergy system including concerns about provision of the biomass that is used in the system, installation and handling of the bioenergy systems lifespans and related infrastructure.*** [c]•

Thus, adopting bioenergy scenarios demands extensive study of methodology and practices of designing and implementation of bioenergy systems in built environment to achieve both transition to efficiency in renewable bioenergy-dependent sustainable living environments and formal design and manipulation of these systems as an integrated organ of these spaces.

Corresponding to this issue it is necessary to move to energy self -production clusters that are focused on achieving self-sufficiency of its estimated required power rates (electricity consumption). One of the most rising eco-friendly technologies in this aspect is the use of microorganisms as biocatalyst in microbial fuel cells to achieve clean electricity production systems. Microbial fuel cells (MFCs) that have emerged in recent years as promising contributors to the transition to a low-carbon society can produce chemical energy from several classes of wastes, with the potential to effectively and directly convert into electrical energy several non-purified organic substrates. [f]•. This bioenergy production and embedding approach aims to localize and clustarize renewable bioelectricity production, by direct

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• Ibid

• [d] Mediterranean Energy Regulations -reporting methodologies -how to collect data and monitor regulated entities -Egypt ERA-www.egyptra.org-milan/10/10/2017.

• [e] https://tradingeconomics.com/egypt/electricity-production-kwh-wb-data.html/1/9/2017

• [c] IPCC Fifth Assessment Report/ 2014.

• [f] E. M. Milner, et al., Microbial fuel cells with highly active aerobic biocathodes, Journal of Power Sources, Pages 8-16,2016

embedding of bioreactors as a part of the built environment to achieve coupling between form, function, and behavior in design.

Research Problem

The lack of eco-friendly, self-sufficient, bio energy-generating systems by direct embedding of microorganisms in built environment (interior design & architectural elements). That achieves efficient electricity production, power management and maintaining safety measures and criteria.

Proposed objectives

This research aim is to achieve;

1. Methodology of embedding self-sufficient bio energy systems in architecture and interior design, including adaption of form, function and behavior coupling of these bio self-sufficient systems in the built environment.
2. Design of an integrated device that employs biodigital design in formalizing the embedding of bio- electricity generating systems in interior design elements; to achieve clustered self-sufficient interior systems.

Significance

Achieving renewable bioelectricity production in the form of self-sufficient clusters embedded in built environment.

Hypothesis

The potential of embedding renewable bioelectricity systems using microorganisms as biocatalyst in MFCs for electricity and bio-illumination, in interior design elements, as integrated clusters, and adhering to interior design criteria of functional, aesthetical and safety and health measures.

Methodology (material& methods)

Experimental methodology- Quantitative

-Experimental study:

- Microbiological survey on nonpathogenic strains for biocatalyst selection (isolation, culturing, identification, growth optimization & measuring).
- Bioelectricity generation test of MFC (reactor's design, materials, operation, performance).

-Computational simulation:

- Imagery study of microbial cultures growth behavior, biochemical polarization in MFC.

Statistical analyses of obtained data.

Thesis Structure

Since the current study is a basic scientific research, the author starts with a general review of current applications of embedding different microbial species in architecture, interior, furniture and urban design. This literature review was essential to set bases of this multidisciplinary research, including introducing all different related basic sciences that is mandatory to understand the principles and methods of embedding microbes in the built environment. The literature review was categorized into three chapters; the first chapter

introduced basic scientific fields employed in the methods and processes of embedding living organisms in design including: microbiology, biotechnology, bioinformatics, synthetic biology, biodigital design, and symbiotic design. These basic sciences were presented along with possible methods of application including bio composite materials, biosynthetic materials, bioreactors, and synthetic bio systems or synthetic biomaterials achieved by DNA manipulation.

The first chapter also included the state of art of currently existing manifesting projects in the different field presented in this chapter categorized by their ecological value through their function.

The following two chapters were specialized into presenting literature review in the field of harnessing energy from microbial activity; theses were divided into emitted light directly from bioluminescence activity of bioluminescent microbial species and the bioelectricity production from the bio catalytic activity of exoelectrogeneses of microbial strains. These two categories were presented in chapter 2, and chapter 3, respectively, along with the presentation of manifesting projects of their application in architecture, interior, furniture and urban design for each.

In chapter 2, the author exhibited the chemical bases of bioluminescence natural activity in different microbial species, including luciferin-luciferase enzymatic reaction, the fluorescence proteins, and the potential to synthesize this activity in non-naturally bioluminescent microbial strains through recombinant DNA technology. Chapter 2 also included different light spectrums and colors of emitted light of natural bioluminescence, in order to employ this activity in architectural, interior, and urban applications.

In chapter 3, the technology of microbial fuel cell was introduced to the community of designers and architects as a sort of bioreactors that are employed in producing bioelectricity. This introduction of the MFC device was essential in order to gain full understanding of the bioelectricity generation by microbial strains using MFCs. The chapter included definition, discerption, categorization, and went through identification of MFC architecture, parts, materials, performance and electricity production. Chapter 3 also included the basic methodology of using MFCs as systems embedded in built environment in architecture or interior design; this was exhibited in the manifesting projects.

Following the extensive scientific introduction and literature review, the author then exhibits the report of the experimental work done in this research, which is the pivotal part that corresponds to the identified hypothesis of this research. In chapter 4, the author exhibits full experimental stages of bioelectricity generation exploiting natural exoelectrogeneses of microbial strains, including proposing an enzymatic measure of Laccase; as a potent oxidoreductase enzyme that catalyzes the oxidation-reduction reaction of microbial cells oxidizing carbon source in culture medium that is the main electron producer, thus generating electrical current. This Laccase production survey was conducted among fungal strains isolated from soil. This was for two reasons, the first is the availability and persistency of these fungal strains that grow and live normally in soil, the second was based on literature review of previous research in the field of bioelectricity generation from different microbial species, reporting that fungal laccases are most potent in terms of electrical potential. The most potent fungal strain in producing Laccase was then molecularly identified by the author as *Aspergillus sydowii* NYKA 510 deposited in NCBI, then was further tested to optimize its growth conditions in order to increase its production of Laccase enzyme. The optimized growth conditions of the potent

fungal strain was then employed in the microbial fuel cell to produce bioelectricity. The best performance was obtained at 2000 Ω achieving 0.76 V, 380 mAm^{-2}, 160 mWm^{-2}, and 0.4 W. A project to design a self-sufficient lighting unit was implemented by employing a system of 2 sets of 4 MFCs each, connected in series, for electricity generation. In order to couple form and function in this design application, the fungal cells behavioral pattern in the oxidation-reduction reaction was imaged by the use of Scanning Electron Microscopy; images were then mathematically simulated and remodeled in a form finding process to generate the design form. The author proposed three methods (strategies) of employing the generated form, these were: the customized mass, the patterning, and the patterned customized mass which was chosen for application in a lighting unite used as an embedded cluster for domestic use.

Following this main experimental stage of bioelectricity generation, to fulfill functional aspect of self-sufficient clusters for renewable energy production. The author shifted to the second main aspect of designing these self-sufficient systems, which is the compatibility of the form and behavior with function. To achieve this it was essential to provide an extensive presentation of biological mathematical modeling bases, theories, methods and tools. In order to apply these mathematical modeling and simulation tools in designing the integration and coherency between form and function in the proposed scheme of self-sufficient bioelectricity producing clusters. For this purpose chapter 5, included a deep and detailed presentation of biological behavior patterns of microbial species, focusing on fungal colonies global behavior. This was to narrow the scope of search, as there are numerous biological behavioral patterns associated with physiological and physio-chemical processes in bioactive microorganisms, that is not included in the scope of this research and don't particularly serve its aim. Thus, chapter 5 included first the targeted behavioral patterns of microbial communities supported by the importance of these behaviors in contribution into the bio catalytic activity of microbial strains including nutrients search and up take strategies, spatial propagation through growth strategies, metabolic and signaling pathways, including chemotaxis. Following this section of microbial behavioral patterns, an extensive study of mathematical modeling bases and types was presented in order to interpret the previous exhibited biological patterns into mathematical equations that could be applied in design studies for form finding and generation or functional behavioral patterns creation in self-sufficient systems. These mathematical models included mainly cellular automata and agent based modeling methods. Chapter 5 also included a comparison between similar and contemporary design theories and trends distinguishing bioactive design in terms of methods, tools, and objectives.

Finally, and based on the previous study of literature review and experimental work, another experimental stage was conducted that tested mathematical modeling potentials of simulating fungal cells behavior of chemotaxis and oxidation-reduction reaction active sites, using combined mathematical models of cellular automata biased random walks and agent based modeling. The resulting model was applied in the design of interior design elements that is a network of self-sufficient clusters for producing bioelectricity. These clusters were also proposed for urban use. Thus proving the identified hypothesis of this study.

The study results were published in two phases, the first corresponding to the bio-electrochemical electricity generation by fungal strain *Aspergillus sydowii* NYKA 510 in the air-cathode, single chamber, microbial fuel cell, published in **Journal of Microbiology and Biotechnology, The Korean Society for Microbiology and Biotechnology**. The second phase

corresponds to the biological mathematical modeling of fungal cells behavior in chemotaxis and oxidation-reduction reaction active sites employing biased random walks and agent-based modeling published in Proceeding for 4th International Conference for Biodigital Architecture & Genetics.

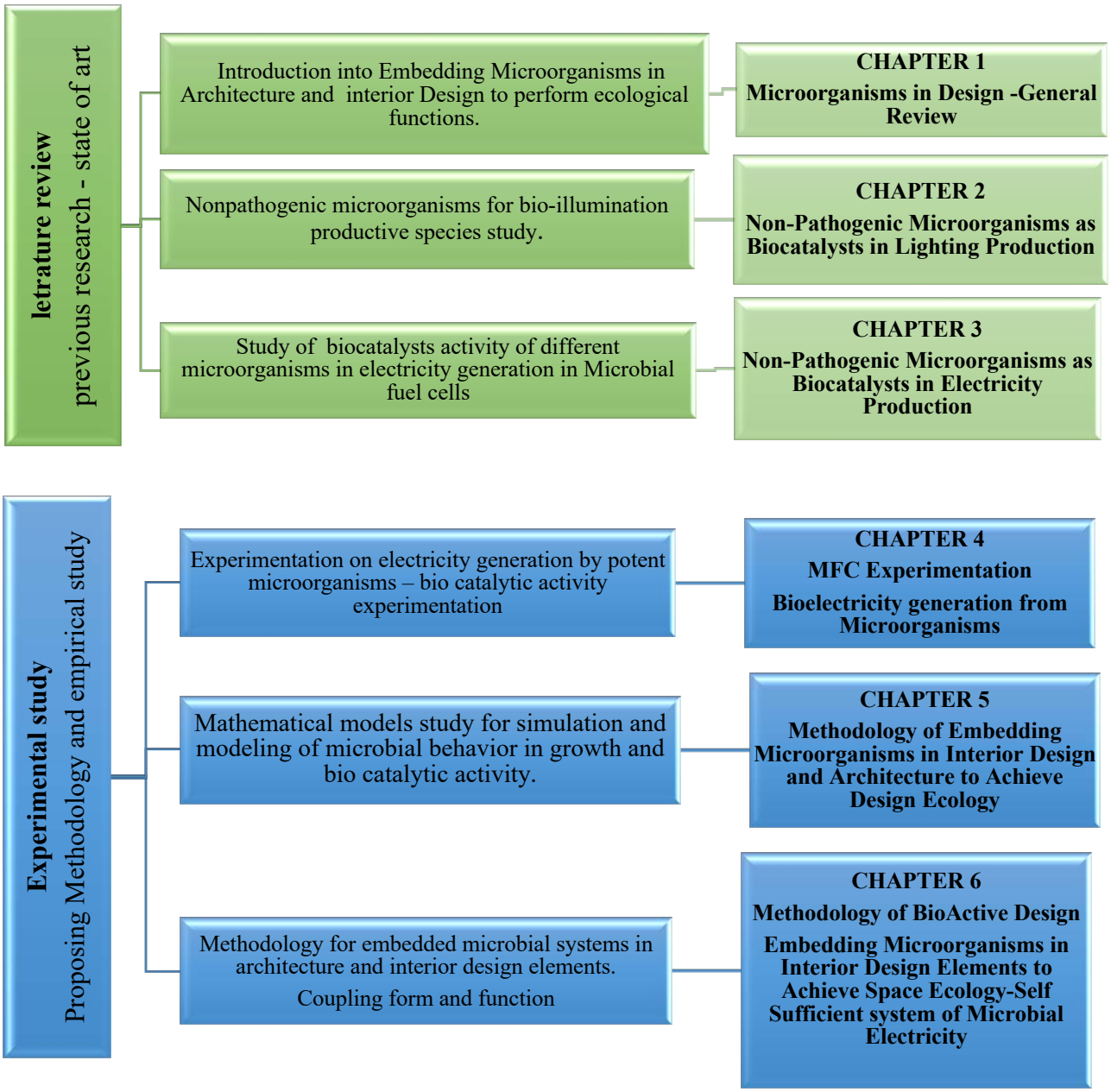


Diagram. [1]. Thesis structure. By author.

Due to the multidisciplinary nature of this thesis combining basic research with design theories, the thesis report followed a building blocks approach to exhibit its content, as the experimental work was capsulated in chapter 4 and chapter 6 each for its purpose respectively. Providing extensive discussion in each case. The author exhibited discussion this way in order to facilitate the understanding of each discipline of this multidisciplinary research to avoid confusion and redundancy and to provide a logic sequence and hierarchy of this research

different steps and disciplines. A conclusion chapter was included in the end of the thesis exhibiting; main findings and results of this work, and proposing possible future research advances based on it. Each chapter starts with a separate cover and an introduction explaining chapter's contents and the purpose of their display, and ends with a specific conclusion of this chapter, these partial conclusions were essential for building a concrete understand for experimental processes proposed and followed in this research.

Text style

The author used A4 paper size with moderate margins format of 0.75 inches on both sides. The text font selected is Times New Roman, Font 12, with 1.15 line spacing. The main headings followed a bold font 12 text of Times New Roman, with numbering stile of [1, 1.1, 1.1.1, etc.,].

The author used bold, italic, and sometimes-colored font to highlight the importance of some text in the content. Used colors followed color code in highlighting its importance from moderately to highly important as follows: black, green, blue, and red.

Diagrams, figures, and images numbering

The author created three groups of visual material, including diagrams, figures and images; each follows numbering of [1, 2, 3...], each visual material have a caption of font 10, Times New Roman, bold.

Reference Style

The author designed a style for references combining Vancouver (numbering) style with Chicago footnote style. Since the big amount of references (nearly 500 references) used in this study complying with its multidisciplinary nature, the author used the Vancouver style to make it easier to track references as organized by their place in the thesis. As well as shortening the text. The author then used the Chicago footnote style in order to make it more coherent and comfortable to look up references in each page due to the big volume of this thesis and the different fields that it contains.

Footnote style

First author, et al., Title, Journal, Volume, Pages, Date.

First author, et al., Title, Edition, Publisher, Country, Date.

References list style

[Nº] Authors, Title, Journal, Volume, Pages, Date.

[Nº] Authors, Title, Edition, Publisher, Country, Date.

CHAPTER 1
MICROORGANISMS IN DESIGN-GENERAL REVIEW

Image. [1]. Formal design of self-sufficient unite of bioelectricity generation, derived from *Aspergillus sydowii* NYKA 510 fungal strain. By the author.

This chapter is a general review focused on exhibiting the different aspects of embedding microorganisms in design and the main interdisciplinary sciences and definitions that cover these aspects. This chapter will include state of art of existing design and research projects as well to clarify the current and possible applications that guided the research along the experimental phase.

Chapter structure and content

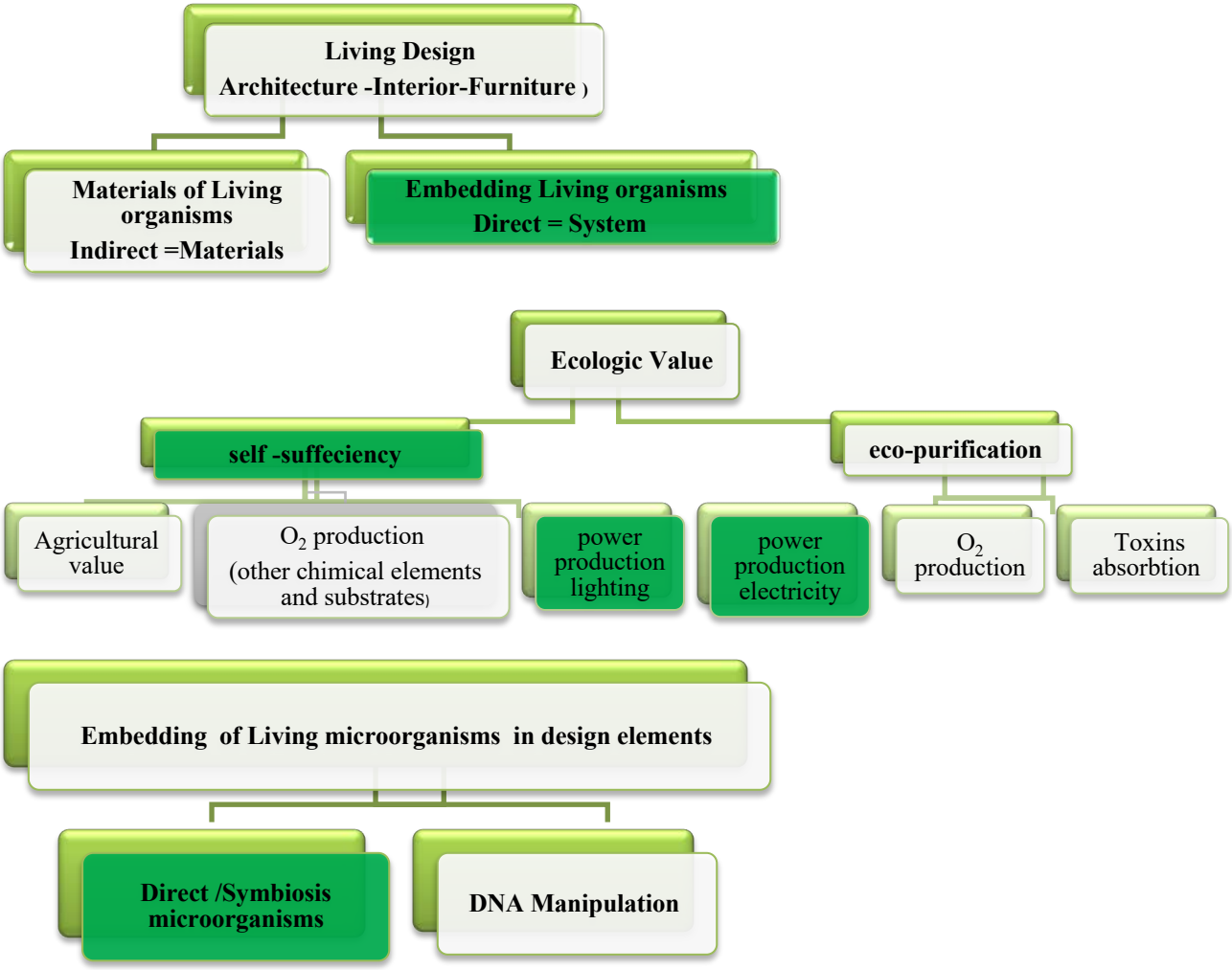


Diagram. [2]. Chapter 1. Microorganisms in design-general review, structure. By author.

Introduction

Biological systems exhibit a wide variety of forms and functions. Make highly efficient use of energy and other resources, and are capable of processes such as programmed self-assembly, adaptability and evolution. These processes are very difficult to achieve using traditional human engineered systems. [1]•. To this extent various trials emerged to employ these systems as a source and a physical component of the built environment, learning from its shapes and forms or simulating and encapsulating their intelligence and systems in eagerness to achieve maximum responsiveness, harmony, evolution and morphogenesis in built environment. Organicism, Bio mimicry, Bionic and other disciplines discussed mimicking forms, structures and systems to achieve specified limited ecological functions; extensive research have been carried on to try to approximate these functions of direct natural behavior in a living like manner through artificially mimicking its' systems, which resulted in machine intelligence sophistication, resources exhaustion, and industrial drawbacks.

To this end, a design multidisciplinary science emerged to employ these living systems by their original characteristics and natural intelligence. To perform its natural behavior in achieving the optimum ecological balance; this discipline mainly focuses on biotechnology and Synthetic Biology, enabling the development of complex material and systems, however, synthetic biology is still regarded as an emerging engineering discipline, and has yet to realize its full potential in terms of delivering complex synthetic biological systems.

In this case; more research on applications of synthetic biology in many aspects should be adopted, in order to highlight the main disciplines that this emerging science fuel. Architecture and interior design are the most dominating aspect on human everyday life. However, architecture emerged to provide shelter and safety for humans from natural extreme forces, it gone so far turning to isolate humans from their natural environment that they were designed to immerse in and evolve through. This thesis aim is to pave the road in searching potentials of naturalizing this built architectural barrier and how to turn it into natural container through emerging natural organisms that preform ecological balance; without hindering human safety and space functionality.

[1] Microorganisms

"A microorganism or microbe is a microscopic organism, which may exist in its single-celled form, or in a colony of cells" . The scientific study of microorganisms began with their observation under the microscope in the 1670s by **Antoine van Leeuwenhoek**. Microorganisms include all unicellular organisms, and so are extremely diverse. They were previously grouped together in the two-domain system as **Prokaryotes, Eukaryotes**. The third domain **Eukaryotes** includes all **multicellular organisms**, **and many unicellular protists and protozoans**. Many of the multicellular organisms are microscopic, namely micro-animals, are **fungi and algae** **[2]•**.They live in almost every habitat, some are adapted to extremes such as very hot or very cold conditions, others to high pressure and a few to high radiation environments.

•[1] M.D.-Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, ArchaID, Computer-Aided Design journal, 2014.

• [2] https://en.wikipedia.org/wiki/Microorganism#cite_note-1-28/1/2018.

[1.1] Bacteria –Fungus –Algae

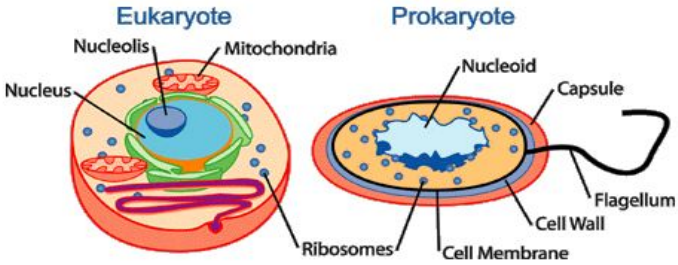
[1.1.1] Bacteria (prokaryotes)

Bacteria are **prokaryotic unicellular, and having no cell nucleus or other membrane-bound organelle**. Bacteria are microscopic, with a few extremely rare exceptions, [3]• Bacteria function and reproduce as individual cells, although they can often aggregate in multicellular colonies. [4]•. Some species can aggregate into complex swarming structures, operating as multicellular groups as part of their life cycle, [5]• or form clusters in bacterial colonies such as *E.coli*.

Their genome is usually a circular bacterial chromosome (**a single loop of DNA**, along with small pieces of DNA called plasmids). These plasmids can be transferred between cells through bacterial conjugation. **They reproduce by binary fission or sometimes by budding**. **However, many bacterial species can transfer DNA between individual cells by a horizontal gene transfer process referred to as natural transformation**. [6]•. Under optimal conditions, bacteria can grow extremely rapidly and their numbers can double as quickly as every 20 minutes. [7]•.

Eukaryotes

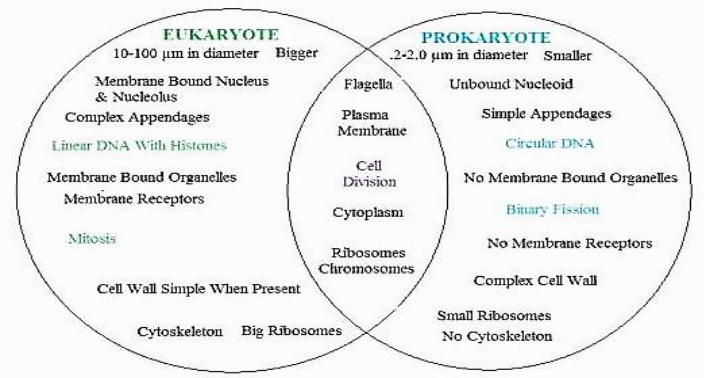
Most living things that are visible to the naked eye in their adult form are eukaryotes, including humans. However, a large number of eukaryotes are also microorganisms. Eukaryotes contain organelles such as the cell nucleus, the Golgi apparatus and mitochondria in their cells. **The nucleus contains the DNA that makes up a cell's genome**. **DNA** itself is arranged in complex chromosomes. [8• ; 9•]. Microbial eukaryotes can be either **haploid or diploid [10]•**, and some organisms have multiple cell nuclei, **unicellular eukaryotes usually reproduce asexually by mitosis under favorable conditions**. However, under stressful conditions such as nutrient limitations and other conditions associated with DNA damage, they tend to reproduce sexually by meiosis. [11]•.



[12]•

Figure. [1]. Eukaryotic cell and prokaryotic cell composition showing the deference of eukaryotic cell having nucleus and organelles that prokaryotic cell miss.

• [3] H. Schulz, B. Jorgensen, Big bacteria, Annual Review of Microbiology, 1, 55, pp. 105–37, 2001.
• [4] J.A. Shapiro, Thinking about bacterial populations as multicellular organisms, Annual Review of Microbiology, 1, 52, pp.81–104, 1998.
• [5] J. Muñoz-Dorado, et al., Myxobacteria: Moving, Killing, Feeding, and Surviving Together, Frontiers in Microbiology. 7: 781, 2016.
•[6] O. Johnsborg, et al., Natural genetic transformation: prevalence, mechanisms and function, Research in Microbiology, 10, 158, pp.767–78, 2007.
•[7] R. Eagon, Pseudomonas Natriegens, a Marine Bacterium with a Generation Time of Less Than 10 Minutes, Journal of Bacteriology, 4, 83, pp.736–7, 1962.
•[8] Eukaryota: More on Morphology. (Retrieved 10 October 2006)/30/1/2018.
•[9] S. Dyall, et al., Ancient invasions: from endosymbionts to organelles. Science. 5668, 304, pp.253–7, 2004.
• [10] https://www.biology-online.org/dictionary/Haploid-6-2-2018.
• [11] S. In Kimura, S. Shimizu, DNA repair as the primary adaptive function of sex in bacteria and eukaryotes, DNA Repair: New Research. Hauppauge, N.Y. Nova Sci. Publ, pp. 1–49, 2012.
•[12] https://theartofmed.wordpress.com/2015/06/03/types-of-pathogenic-microorganisms.29/1/2018.



[13]•

Figure. [2]. Comparison between eukaryotic cells and prokaryotic cells.

[1.1.2] Fungus

A fungus is eukaryotic that includes microorganisms such as yeasts, molds and mushrooms [14]•. These organisms are classified as a Eumycota kingdom, which is separate from the other eukaryotic kingdoms of plants and animals, because of the **chitin in their cell walls.** The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from unicellular aquatic chytrids to large mushrooms. ***Fungi are heterotrophs as they acquire their food by absorbing dissolved molecules, by secreting digestive enzymes into their environment*** *and they lack the ability to* ***photosynthesize.***

Moreover, **growth is their means of mobility**, except for spores (a few of which are flagellated), which may travel through the air or water. They also function as principal **decomposers in ecological systems**. Fungal species have been estimated at 2.2 million to 3.8 million species, of which only 120,000 have been described. 8000 of them are pathogenic for plants and 300 can be pathogenic to humans [15]•. **Fungi have been classified according to their morphology** (characteristics such as spore color or microscopic features) or physiology.

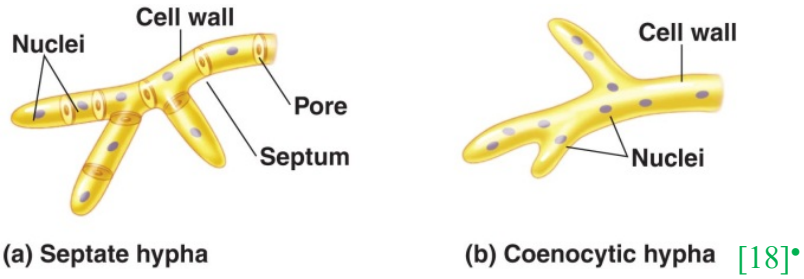
[1.1.2.1] Fungal morphology

Microscopic structures

Most fungi grow as **hyphae**, which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length. Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by a process called branching, or occasionally growing hyphal tips fork, giving rise to two parallel-growing hyphae. [16]•.

Hyphae sometimes fuse when they come into contact, a process called hyphal fusion (or anastamosis). These growth processes lead to the development of a **mycelium**, which is **an interconnected network of hyphae**. Hyphae can be either septate or coenocytic. Septate hyphae are divided into compartments separated by cross walls, with each compartment containing one or more nuclei; where coenocytic hyphae are not compartmentalized. [17]•.

• [13] https://theartofmed.wordpress.com/2015/06/03/types-of-pathogenic-microorganisms.8/2/2018.
•[14] "Fungus". Oxford Dictionaries. Retrieved 26 February 2011/8/2/2018.
• [15] https://www.nature.com/articles/nmicrobiol2017.120/8/2/2018.
• [16] S.D. Harris, Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems, Mycologia, 6, 100, pp.823–32,2008 .
•[17] J. W. Deacon, Fungal Biology, Wiley, pp. 267–276, 2013.



[18]•

Figure. [3]. Difference between septate hypha and coenocytic hypha.

Macroscopic structures

Sometimes fungal mycelia can be visible to the naked eye. They are commonly called molds. **Mycelia grown on solid agar media in laboratory petri dishes are referred to as colonies.** These colonies can exhibit growth shapes and colors that can be used as diagnostic features in the identification of species or groups. [19]•.

Fungi also contains an apothecium; which is a specialized structure important in sexual reproduction in the ascomycetes. A cup-shaped fruit body is often macroscopic and holds the hymenium. This hymenium is a layer of tissue containing the spore-bearing cells. Well known as mushrooms.

[1.1.2.2] Fungal growth

The growth of fungi as **hyphae** on liquid media or in solid substrates or as single cells is adapted for the efficient extraction of nutrients, as these growth forms have high surface area to volume ratios. [20]•. ***Hyphae are specifically adapted for growth on solid surfaces, and to invade substrates and tissues***. [21]•. ***They can exert large penetrative mechanical forces.***

Fungi also secrets hydrolytic enzymes into the environment to digest large organic molecules such as polysaccharides, proteins, and lipids into smaller molecules that may then be absorbed as nutrients. [22]•.

The vast majority of filamentous fungi grow in a **polar fashion** (extending in one direction) by elongation at the tip (apex) of the hypha. [23]•. Other forms of fungal growth include **intercalary extension** (longitudinal expansion of hyphal compartments that are below the apex) [24]•, and growth by **volume expansion** during the development of mushroom stipes and other large organs. [25]•.

•[18] http://www.medical-labs.net/fungi-hyphae-1522/forms-of-hyphae-septate-and-coenocytic-hyphae.

• [19] J. R. Hanson, Chemistry of fungi, pp. 127–141, 2008.

• [20] S.T. Moss, The Biology of Marine Fungi. Cambridge, UK: Cambridge University Press, pp. 76, 1986.

• [21] M.A. Peñalva, H.N. Arst, Regulation of gene expression by ambient pH in filamentous fungi and yeasts, Microbiology and Molecular Biology Reviews, 3, 66, pp. 426–46, 2002.

• [22] J.L. Pereira, et al., Novel insights in the use of hydrolytic enzymes secreted by fungi with biotechnological potential. Letters in Applied Microbiology. 6, 44, pp. 573–81, 2007.

• [23] R. Fischer, et al., Polarized growth in fungi--interplay between the cytoskeleton, positional markers and membrane domains, Molecular Microbiology, 4, 68, pp. 813–26, 2008.

•[24] M.J. Christensen, et al., Epichloë endophytes grow by intercalary hyphal extension in elongating grass leaves, Fungal Genetics and Biology, 2, 45, pp.84–93, 2008.

•[25] N. P. Money, Mushroom stem cells, BioEssays, 10, 24, pp.949–52, 2002.

Growth of fungi as multicellular structures consisting of **somatic and reproductive** cells has several functions [26]•, including the development of fruit bodies for propagation of sexual spores, and **biofilms for substrate colonization** and intercellular communication. [27]•.

Fungi have evolved a high degree of metabolic versatility that allows them to **use a diverse range of organic substrates for growth,** including simple compounds such as nitrate, ammonia, acetate, or ethanol. [28]•.

[1.1.2.3] Fungal reproduction

Asexual reproduction

This type of reproduction occurs via **vegetative spores (conidia)** or through **mycelial fragmentation**. Mycelial fragmentation occurs when a fungal mycelium separates into pieces, and each component grows into a separate mycelium. Mycelial fragmentation and vegetative spores maintain clonal populations adapted to a specific position, and allow more rapid dispersal than sexual reproduction. [29]•.

Sexual reproduction

This type of reproduction occurs through meiosis. [30]•. The major fungal groupings have initially been defined based on the morphology of their sexual structures and spores. [31]•.

Most fungi have both a haploid and a diploid stage in their life cycles. In sexually reproducing fungi, compatible individuals may combine by fusing their hyphae together into an interconnected network; this process, anastomosis, is required for the initiation of the sexual cycle. [31]•.

Spore dispersal

Both asexual and sexual spores are often actively dispersed by forcible ejection from their reproductive structures. This ejection ensures exit of the spores as well as traveling through the air over long distances. Specialized mechanical and physiological mechanisms, as well as spore surface structures enable efficient spore ejection. [32•; 33•]. The forcible discharge of single spores termed ballistospores involves formation of a small drop of water (Buller's drop), which upon contact with the spore leads to its projectile release with rapid initial acceleration. [34•; 35•].

[1.1.3] Algae

Algae is an informal term for a large, diverse group of photosynthetic organisms that are not necessarily closely related, and is thus polyphyletic. Included organisms range from unicellular microalgae genera, such as *Chlorella* and the diatoms, to multicellular forms, such as large brown alga. Most Algae are aquatic and autotrophic and lack many of the distinct cell and tissue types, which are found in land plants.

•[26] M. Willensdorfer, On the evolution of differentiated multicellularity, Evolution; International Journal of Organic Evolution, 2, 63, pp.306–23, 2009.

•[27] K.J. Daniels, et al., Opaque cells signal white cells to form biofilms in Candida albicans, The EMBO Journal, 10, 25, pp.2240–52, 2006.

• [28] G. A. Marzluf, Regulation of nitrogen metabolism and gene expression in fungi, Microbiological Reviews, 3, 45, pp.437–61, 1981.

•[29] J. Heitman, Sexual reproduction and the evolution of microbial pathogens, Current Biology, 17, 16, pp. R711–25, 2006.

• [30] D. Redecker, P. Raab, Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers, Mycologia, 6, 98, pp.885–95, 2006.

• [31] R.L. Metzenberg, N.L. Glass, Mating type and mating strategies in Neurospora, BioEssays, 2, 12, pp.53–9, 1990.

• Ibid

•[32] M.B. Linder, et al., Hydrophobins: the protein-amphiphiles of filamentous fungi, FEMS Microbiology Reviews, 5, 29, pp.877–96, 2005.

• [33] F. Trail, Fungal cannons: explosive spore discharge in the Ascomycota, FEMS Microbiology Letters, 1, 276, pp.12–8, 2007.

•[34] A. Pringle, et al., The captured launch of a ballistospore", Mycologia, 4, 97, pp. 866–71, 2005.

•[35] H.J. Brodie, The Bird's Nest Fungi, Toronto, Ontario: University of Toronto Press, p. 80, 1975.

Most algae are phototrophic, although some are mixotrophic, deriving energy both from photosynthesis and from uptake of organic carbon by either osmotrophy, myzotrophy, or phagotrophy. Algae have chlorophyll as their primary photosynthetic pigment. [36]•.

[1.1.3.1] Algae uses

Primarily algae is utilized as energy source through these forms; Algae fuel, Biological hydrogen production, Biodiesel, Ethanol fuel, Butanol fuel, Vegetable oil, and Biogas. Algae is directly related to the potential to produce more biomass per unit area in a year than any other form of biomass. [37]•. Algae is also employed in pollution control as sewage can be treated with algae, reducing the use of large amounts of toxic chemicals that would otherwise be needed.

[1.2] Pathogenic vs. non pathogenic

[1.2.1] Pathogenic microorganisms

Microorganisms like fungi, bacteria, viruses and parasites cause infectious diseases. An opportunistic infection is an infection caused by bacterial, viral or fungal pathogens that take advantage of a host with a trivial immune system. Mostly these pathogens do not cause disease in a healthy individual that has a normal immune system but in immuno-compromised patients. [38]•.

[1.2.2] Nonpathogenic microorganisms

Nonpathogenic organisms are those that do not cause disease to another organism. [39]•. Most bacteria are nonpathogenic. Some nonpathogenic microorganisms are commensals on and inside the body of animals and are called microbiota. Some of nonpathogenic microorganisms are opportunistic, that can be pathogenic if they enter an immune compromised body, and cause symptoms of infection. [40]•. A particular strain of microbe can be nonpathogenic in one species but pathogenic in another. [41]•. One species of microbe can have many different types or strains. One strain of a species can be nonpathogenic and another strain of the same can be pathogenic. [42]•.

[2] Bioactive Architecture and Design

[2.1] Bio-technology (Synthetic biology)

[2.1.1] Biotechnology definition

Biotechnology is defined as any technical application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use. As such, biotechnology has existed since the first used fermentation process to make bread, cheese and wine. According to European Federation of Biotechnology, “Biotechnology is the integrated use of **biochemistry, microbiology, and engineering sciences** in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells”. According to

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•[36] R. E. Lee, Phycology, Cambridge University Press, 2008.

•[37] Z. K. Yang, et al., Molecular and cellular mechanisms of neutral lipid accumulation in diatom following nitrogen deprivation, Biotechnology for biofuels, 1, 6, pp. 67, 2013.

•[38] https://www.scitechnol.com/scholarly/pathogenic-microorganisms-journals-articles-ppts-list.php.

• [39] "Nonpathogenic, Definition, meaning & more, Collins Dictionary, Retrieved 2016-06-30.

•[40] A. Zargar, et al., Bacterial Secretions of Nonpathogenic Escherichia coli Elicit Inflammatory Pathways: a Closer Investigation of Interkingdom Signaling, mBio, 2, 6, pp. e00025–15, 2015.

• [41] G.F. Rall, et al, Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump? PLOS Pathogens, 11, 11, 2015.

•[42] D. Liu, Molecular Approaches to the Identification of Pathogenic and Nonpathogenic Listeriae, Microbiology Insights, 59. 2013.

US National Science Foundation, "**Biotechnology** is the controlled use of biological agents, such as microorganisms or cellular components." [43]•.

Modern biotechnology refers to the understanding and application of genetic information of animal and plant species. Genetic engineering modifies the functioning of genes in the same species or moves genes across species resulting in Genetically Modified Organisms (GMOs). [44]•.

According to the current work purpose, the most potent identification of "**Biotechnology**, that it is combination of biology and technology; to use, modify or upgrade the part or whole of biological system for industrial and human welfare."[45]• (A.T. Estevez, 2014). By working with natural software (DNA), in living elements, with application of real genetic processes to architecture, for Automation as natural growth, genetic manipulation to obtain living elements, bioactive devices, and synthesizing living building materials.

Research is being conducted in the genetic control of growth to develop living cells that are converted into building materials and built environment that are directed by means of their specific genetic design. Thus producing architecture that is 100% ecological, recyclable and sustainable, with maximum energy saving throughout the construction process, is achievable as its growth is natural. [45]•.

[2.1.2] Biotechnology history

The term "biotechnology" was proposed by Hungarian engineer Karoly Ereky in 1919 to describe a technology based on converting raw materials into a more useful product. In 1953, JD Watson and FHC Crick cleared the mysteries around the DNA as a genetic material, by giving a structural model of DNA, popularly known as, ***'Double Helix Model of DNA'***. This model was able to explain various phenomena related to DNA replication, and its role in inheritance Using this technological advancement, other scientists were able to insert a foreign DNA into another host and were even able to monitor the transfer of a foreign DNA into the next generation. 1983 witnessed the conceiving of ***polymerase chain reaction (PCR)*** technique, which uses heat and enzymes to make unlimited copies of genes and gene fragments. In addition, the first artificial chromosome was synthesized. [46]•.

[2.1.3] Fields in biotechnology

[2.1.3.1] Genetic engineering

Also called genetic modification, is the direct manipulation of an organism's genome using biotechnology. Genes are the chemical blueprints that determine an organism's traits. Moving genes from one organism to another transfers those traits. Through genetic engineering, organisms can be given targeted combinations of new genes, and therefore new combinations of traits that do not occur in nature and, cannot be developed by natural means, are synthesized. [43]•.

[2.1.3.2] Tissue culture

Tissue culture is a method of biological research in which fragments of tissue from an animal or plant or microorganism are transferred to an artificial environment in which they

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• **[43] Z. Naz, INTRODUCTION TO BIOTECHNOLOGY, Research Gate, 2015.**
• **[44] R. Christensen, et al., BIOTECHNOLOGY: AN OVERVIEW, P.J. Industry & Services, European investment bank, 2002.**
• **[45] A. T. Estévez, The Future of Architecture: Bio digital Architecture and Genetics, ESARQ the Architecture School of the Universitat Internacional de Catalunya, Barcelona- Architecture Research, 4(1B), pp.13-20, 2014.**
• **Ibid**
• **[46] A. Bartoszek, et al., Managing innovations in biotechnology, European Project Semester, 2006.**
• **[43] Z. Naz, INTRODUCTION TO BIOTECHNOLOGY, Research Gate, 2015.**

can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ. ***Cells in culture may multiply; change size, form, or function; exhibit specialized activity or interact with other cells***. ***This method is of particular significance in the current work scope and methods of application in architecture and interior design***.

[2.1.3.3] Cloning

Cloning describes the processes used to create an exact genetic replica of another cell, tissue or organism. The copied material, which has the same genetic makeup as the original, is referred to as a clone. [43]•.

[2.1.4] Types of biotechnology

Biotechnology is divided into three main fields: ***green biotechnology, red biotechnology, and white biotechnology.***

[2.1.4.1] Green Biotechnology: Agricultural processes

The foundation of green biotech is crop improvement and production of novel products in plants, which is achieved by implanting foreign genes to plant species that is economically important. This contains:

- **Plant tissue culture**: It consists of producing -in laboratory conditions- a whole plant from part of it or even a single plant cell. Its advantage is rapid production of clean planting materials.
- **Plant genetic engineering**: provides genes to be implanted from one organism to another. This creates improved materials. [46]•.

[2.1.4.2] White Biotechnology: Industrial and environmental processes

White biotech uses molds, yeasts, bacteria and enzymes to produce goods and services or parts of products. It offers a wide range of bio-products. Most of the white biotech processes results in the saving of water, energy, chemicals and in the reduction of waste compared to traditional methods. For example: **biomass,** this term stands for renewable raw materials such as; starch, cellulose, vegetable oils and agricultural waste that are used to produce chemicals, biodegradable plastics, new fibers and bio fuels. The process of manufacturing them requires the use of enzymes as well. [46]•.

White biotechnology ecological effect

White biotechnology enjoys a positive social acceptance because of its environmental friendly features. However, there is an issue of white biotech with a harmful impact on the biological diversity: genetically modified organisms are used and these may be released into the environment. Thus raising ethical issues by interfering with the genetic code of plant and animal, including human, species. As such, it may be perceived as unnatural or violating. [44]•. In order to avoid it, industrialists using them in factories, bioreactors and greenhouses are required to apply strict **bio-safety regulations.**

[2.1.5] Biotechnology advantages

Biotechnology have multiple advantages as it reduces pollution and waste, and combats environmental pollution by developing the use of biosensors for early detection of pollutants. It

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• **Ibid**
• **[46] A. Bartoszek, et al., Managing innovations in biotechnology, European Project Semester, 2006.**
• **Ibid**
• **[44] R. Christensen, et al., BIOTECHNOLOGY: AN OVERVIEW, P.J. Industry & Services, European investment bank, 2002.**

also decrease the use of energy, raw materials and water and creates new materials and bio-fuels from waste. It provides an alternative to some chemical processes as well.

[2.1.6] Biotechnology disadvantages

Although the important role that biotechnology has in preserving ecology, it may have some drawbacks of its processes, these are; ethics-related concerns include cloning, stem cell research, and genetic modification of organisms. Along with the uncertainty in its long-term effects. As they may affect directly or indirectly the future in unforeseen ways; such as that, genetically modified species can damage the natural ecosystem [43•; 47•]. In addition, the value of biotech products is often miscalculated with failure to include the factors of risk and product development periods, which can lower the return on profit.

[2.1.7] Biotechnology and synthetic biology (SB)

Synthetic biology combines various disciplines, such as biotechnology, genetic engineering, molecular biology, molecular engineering, systems biology, biophysics, electrical engineering, computer engineering, control engineering and evolutionary biology. Synthetic biology applies these disciplines to build artificial biological systems for research, engineering, medical and design and industrial applications. Synthetic biology is defined as "**an emerging discipline that uses engineering principles to design and assemble biological components**" [48]•. Or "**Designing and constructing biological modules, biological systems, and biological machines for useful purposes**" as well.[49•; 50•; 51•] .and recently, synthetic biology has been defined as the artificial design and engineering of biological systems and living organisms for purposes of improving applications for industry or biological research.[52]•.

The purpose of SB, according to the Royal Academy of Engineering, is ‘‘**to design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems’’** SB **is associated with molecular level of manipulation** of simple organisms through genetic modification.[1]•. Hence, SB is a more specialized branch of the big tree of biotechnology. [53]•. The goal of synthetic biology is to extend or modify the behavior of organisms and engineer them to perform new tasks. It is worth mentioning that the discovery of mathematical logic in gene regulation in the 1960s and the early achievements in genetic engineering that took place in the 1970s, paved the way for today’s synthetic biology. [53]•.

However, SB is still an emerging discipline that undergo extensive investigations about its drawbacks, for example; the inability to fully predict the functions of even simple devices in engineered cells and construct systems that perform complex tasks with precision and reliability. That stems from several sources of uncertainty, some of which signify the incompleteness of available information about inherent cellular characteristics. The effects of gene expression noise,

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• **[43] Z. Naz, INTRODUCTION TO BIOTECHNOLOGY, Research Gate, 2015.**
• **[47] ACGT: Alliance for Cancer Gene Therapy, https://acgtfoundation.org.**
• **[48] Intellectual Property and the Commons in Synthetic Biology: Strategies to Facilitate an Emerging Tec. W97 binnenwerk-8, Rathenau Constructing Life. IEEE, 2006.**
•**[49] T. Nakano, Molecular Communication, Cambridge, 2013.**
• **[50] Registry of Standard Biological Parts. Retrieved 2014-09-11. http://parts.igem.org.**
•**[51] E.C. Hayden, Synthetic-biology firms shift focus, Nature, 7485, 505, pp. 598, 2014.**
• **[52] A.E. Osbourn, et al., Synthetic biology, New Phytologist, 3, 196, pp. 671–677, 2012.**
• **[1] M.D. Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60. pp.28-39, 2015.**
•**[53] E. Andrianantoandro, et al., Synthetic biology: new engineering rules for an emerging discipline, Molecular Systems Biology, 2006.**
• **Ibid**

mutation, cell death, undefined and changing extracellular environments, and interactions with cellular context. [53]•.

The process of genetic engineering offers a broader definition of SB, which is associated with the **practices of copying DNA sequences from the genome of organisms and, through recombinant DNA, importing the sequence from one organism to another, so that the host organism exhibits some characteristic of the donor.** These practices are now routinely used within **molecular biology** laboratories and there are already wide applications of genetically modified organisms. There is, however, a stricter definition of SB being proposed, which is based on a more formal conceptualization of genetic modification as an engineering design process. In this context; biological systems, are considered to be akin to electrical systems with biological circuits. [1]•.

Systems Biology offers a detailed descriptions of the mechanisms through which DNA is transcribed into messenger Ribonucleic acid (mRNA) to guide the production of protein molecules in protein factories (ribosomes). These proteins act as machines that perform all the functions of living cells. Recently it became possible to map entire genomes for individual organisms, which make it possible to make associations between specific DNA expression and the characteristics and behaviors of individual organisms. While DNA is described as the blueprint of life, the relationship between DNA sequences, the expression of proteins and the characteristics of biological systems are significantly more complex than this analogy implies. [1]•.

While there are instances of isolated gene sequences resulting in clearly defined characteristics in an organism, much of what is understood in terms of the morphology and behavior of biological systems is derived from groups of different genes being expressed through the more complex proteome which is the entire population of proteins produced by a cell or organism, at particular growth stages or in particular environments. [1]•.

[2.1.7.1] Synthetic biology in science and engineering

A highly prominent approach to SB is to abstract biological systems through design of more traditional forms of engineering. This emphasizes on simplifying the process of designing biological systems through:

- ***Engaging within an engineering design cycle****, this includes a clear set of requirements, design, implementation, testing, verification and refinement, emphasizing on extensive simulation and modeling throughout the process.*
- ***Describing DNA sequences and their products as standardized clusters****; which are interchangeable and can be used to construct genetic circuits for different functions.*
- ***Trading complex lab-based practices of recombinant DNA for using synthesized DNA*** (DNA, which has been coded, and 'printed out' from a computer). [1]•.

• **Ibid**

•**[1] M.D. Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60. pp.28-39, 2015.**

• **Ibid**

• **Ibid**

• **Ibid**

These computerized processes of conceptualizing SB as systems' and devices' engineering implies a wide reliance on bio informatics, that propose an invading tool to survey and compute an enormous amount of biological information; and also propose computation to simulate and mimic its' characteristics.

However, this approach to biological systems' design is argued. Some suggest that complex biological networks cannot be reduced and partitioned into discrete clusters, which leads to informal and hidden SB practices. This debate about the nature of biological processes stems from a long-standing argument between Elementalism (studying systems through their reduction into parts**) and Organicism (the study of the system as an irreducible whole by recognizing the role of emergent complexity**). In SB, an elementalist model emphasis DNA as the material of design manipulation. On contrary to the organicist view which the current work comply with; that requires new designs to be considered from multiple possible perspectives and involve the manipulation of chemical, physical and cellular environments in concert with possible design outcomes which are emergent and difficult to describe with reference to the functional parts alone .[1]•.

[2.1.7.2] Synthetic biology in architectural design

It have been seen that in the context of science and engineering, SB understands engineering biological systems similarly to engineering mechanical or electronic systems with clear definitions of parts and their assembly, which can be expressed through functional hierarchies and enable abstraction and top-down specification. This is not the case when in it comes to architecture and interior design. Architecture is a nonlinear inter/multidisciplinary context that implies a bottom-up methodology when it comes to naturalizing its whole and parts; a recent approach that adopt this view presents the concept of ***material ecologies***; according to *Oxman, 2007;*

"*Material Ecology is an emerging field in design denoting informed relations between products, buildings, systems, and their environment. Defined as the study and design of products and processes integrating environmentally aware computational form-generation and digital fabrication, the field operates at the intersection of Biology, Materials Science and Engineering, and Computer Science with emphasis on environmentally informed digital design and fabrication. With the advent of digital fabrication techniques and technologies, digital material representations have come to represent material ingredients, In other words, designers are now able to compute material properties and behavior built-in to form-generation procedures. Such unity - like that found in natural bone, a bird's nest, might promote a truly ecological design paradigm, facilitating formal expression constrained by, and supportive of its hosting environment."* [54]•.

This identifies that **material ecologies**, aims to understand engineered systems in nonlinear manner through the design process, which considers factors such as **material performance**, **design intent**, **fabrication and environment** as reciprocal and interrelated parameters.

A material model, which is based on observations of real biological systems, admits that material organization in living systems is not designed but, rather, emerges from constraints inherent in the materials themselves, and in their interaction with the environment. This approach has been taken even further in a design paradigm, which suggests a '***literal biological***

• **Ibid**

• **[54] http://www.materialecology.com- http://2012.acadia.org.**

paradigm', suggesting that the designer should '***go beyond using shallow biological metaphors or a superficial biomorphic formal repertoire*' *and, through architectures of synthetic life, understand the built environment as 'a synthetic life-form embedded within dynamic and generative ecological relations'***.[55]•. Furthermore, academic architectural designers have suggested that a literal biological paradigm changes the relationship between visualization and designed object [56]•, **enabling a design process similar to cultivation than engineering. [1]•.**

From the engineering approaches described above, emerges a new potential to extend the current understanding of SB through different methods, which utilizes bottom-up, and top-down methodologies to develop material systems these systems are bio-based (biomaterials).

[2.2] Bio-material science

Biomaterial science involves elements of medicine, biology, chemistry, tissue engineering and materials science. [57]•. "**A biomaterial is any material, natural or man-made, that comprises whole or part of a living structure or a device which performs, augments, or replaces a natural function**".[58]•.

[2.2.1] Bio-based material

A bio-based material is a material made from substances derived from living (or once-living) organisms. [59]•. Bio-based materials or biomaterials fall under the broader category of bio-products or bio-based products, which includes materials, chemicals and energy, derived from renewable biological resources**.** Bio-based materials are often biodegradable. [57]•.

Biodegradation (bioremediation) is the fragmentation of materials by bacteria, fungi, or other biological decomposers. [60]•. The term is commonly associated with environmentally friendly products, capable of decomposing back into natural elements. Decomposition of biodegradable substances may include both biological and abiotic steps. Biodegradable matter is generally organic material that provides a nutrient for microorganisms. These are so numerous and diverse that a huge range of compounds can be biodegraded. Microorganisms secrete bio extracellular surfactant, to enhance this process.

In practice, almost all chemical compounds and materials are subject to biodegradation processes. The significance depends on relative rates of such processes. A number of factors determine the rate at which this degradation of organic compounds occurs. [61]•. Main factors include light, water and oxygen. Temperature is also important as chemical reactions proceed more quickly at higher temperatures. The degradation rate of many organic compounds is limited by their bioavailability. Compounds must be released into solution before organisms can degrade them. [62]•.

• **[55] M. Hensel, (Synthetic) life architectures: ramifications and potentials of a literal biological paradigm for architectural design, Architecture Studies, 76, pp.18–25, 2006.**

• **[56] B.M. Cruz, S. Pike, Neoplasmatic design. Architecture Studies, 76, pp.6–7, 2008.**

• •**[1] M.D. Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60. pp.28-39, 2015**

• **[57] A. Tathe, et al., A BRIEF REVIEW: BIOMATERIALS AND THEIR APLLICATION, International Journal of Pharmacy and Pharmaceutical Sciences, 2010.**

•**[58] B. D. Ratner, et al., Biomaterials Science: An Evolving, Multidisciplinary Endeavor, In book: Biomaterials Science, 2012.**

• **[59] https://en.wikipedia.org/wiki/Bio-based_material-30-1-2018**

• **[57]**

• **[60] M. Vert, et al., "Terminology for biorelated polymers and applications , Pure and Applied Chemistry, 2, 84, pp. 377–410. 2012.**

• **[61] G. K. Sims, A.M. Cupples, Factors controlling degradation of pesticides in soil. Pesticide Science, 55, pp.598–601, 1999.**

•**[62] G.K. Sims, The effects of sorption on the bioavailability of pesticides. London: Springer Verlag. pp. 119–137, 1991.**

Biodegradability can be measured in a number of ways. Respirometry tests can be used for aerobic microbes, as a solid waste sample is placed in a container with microorganisms and soil, and mixture aerated. Over the course of several days, microorganisms digest the sample bit by bit and produce carbon dioxide, the resulting amount of CO_2 serves as an indicator of degradation. [63]•.

1.Project discipline	**Bio materials, Bio-based materials**	
Ecological value	Fire-resistant building material.	
Project application	Building material (Architecture).	
Project information	Name	Mycotecture project.
	Time	2012 AIA SF.
	Place	San Francisco. USA.
	Designer	Mycologist Philip Ross.

Microorganism Fungi (prokaryotes), Mushroom mycelium, living. Fungus ***Ganoderma lucidum***.

Image. [2]. The grown mushroom *Ganoderma lucidum*. By Philip Ross

Concept

The concept of this project focused on experimentation on building bricks made of fungal mycelium and the measuring of their potential to adhere to each other and achieve strength and rigidity. In this project, Mycelia was cultivated and dried into forms that are lightweight and resistant to fire, mold and water. [64]•. The Fungus was grown into bricks and stacked into an arch. A variety of different brick forms, assembles, arrangements and different lacquers and finishes were applied to the outer layer of the bricks to seal them and give them a glossy finish. [65]•.

Description

Image [3, 4]. The built prototype of the Mycotecture project pavilion exhibiting strong cohesion in the structure. By Philip Ross

•[63] Measuring Biodegradability, The University of Waikato, June 19, 2008.

• [64]https://www.treehugger.com/green-architecture/mycotecture-mushroom-bricks-philip-ross.html/23/2/2018.

•[65] https://inhabitat.com/phillip-ross-molds-fast-growing-fungi-into-mushroom-building-bricks-that-are-stronger-than-concrete/30-1-2018.


Image [5, 6]. Details in the living material made of mushroom mycelium.


Image [7, 8]. Left, the single block made of the living mushroom mycelium and right, prototype blocks.


Image [9, 10]. Different assemblies of the mushroom blocks for various structural forms potentials.

2.Project discipline	**Bio materials, Bio-based materials**	
Project ecological value	Biodegradable, Lightweight building material.	
Project application	Furniture design.	
Project information	Name	3D-Printed Mycelium Chair.
	Time	2013.
	Place	The University of Aachen of Applied Science , Germany
	Designer	Eric Klarenbeek.

Microorganism: Mushroom mycelium –living.

Concept

Mycelium Chair is mushroom-sprouting seat that fuses organic materials with modern 3D printing technology. The designer developed a way to 3D print with living cells. The sculptural fungus chair is sowed with mushroom spores that flourish over time, to represent organic technology.

Description

The project explores combining modern 3D printing technology with the biological building materials of fungi. In order to create a flexible material, the designer extracted mycelium from fungus and used the thread-like material as a base. Then mixed the mycelium

with a compound of organic straw and water, creating a substance that could be fed into a 3D printer.

[66]•

Image [11, 12]. Left. Research samples of 3D printed container tissue. Right, the Scale model.

Image [13, 14]. left. The 1:1 chair mockup with the complete growth of mushroom. Right, 3D-printed segment of bioplastic shell.

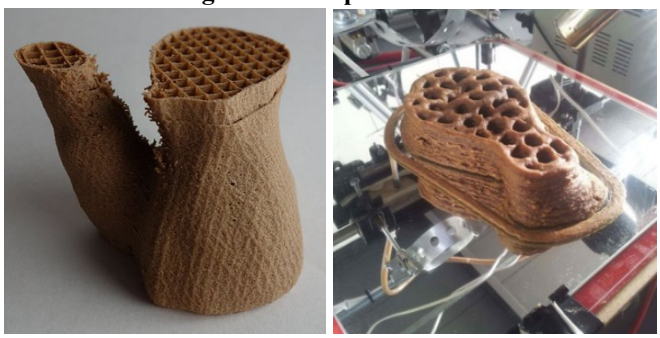

Image [15, 16] Left, 3D-printed segment of straw core. Right, 3D-printing straw substrate.

The new substance was then printed into a sculptural chair inspired by the natural growth of fungus. Once printed, the mycelium is still living, and continues to grow. For design purposes, the designer dried the chair out and covered it with a layer of bioplastic in order to cease the mycelium's growing process and to preserve the delicate shape. Living mushrooms were added to retain the chair's living element, and reinforce the durability of the chair, as they grow thicker. [67]•.

[2.2.2] Bio-composite materials

A composite material is defined as a combination of two or more materials that results in better properties than its individual components when used alone. Each material retains its

• **[66]https://www.dezeen.com/2013/10/20/mycelium-chair-by-eric-klarenbeek-is-3d-printed-with-living-fungus/30-1-2018.**
• **[67] https://inhabitat.com/3d-printed-mycelium-chair-sprouts-living-ushrooms/eric_klarenbeek_1.**

separate chemical, physical, and mechanical properties, which remain separate and distinct on a macroscopic level within the finished structure, i.e. straw reinforced mud-brick [68•; 69•]. The main advantages of composite materials are their high strength and stiffness, combined with low density, when compared with bulk materials, allowing for a weight reduction.

Designing Bio composites: the process of designing a bio composite material is to program living matter to get a composite material made of organic components showing amplified or shifted features, different from the ones the components show separately. These materials can be reinserted in a metabolic active life cycle after being used. [70]•.

Composites are a subclass of anisotropic materials, which have different material properties in all directions at a point in the body [69]•, which are classified as orthotropic. Orthotropic materials have properties that are different in three mutually perpendicular directions. They have three mutually perpendicular axes of symmetry, and a load applied parallel to these axes produces normal strains.

Disadvantages of composites include high raw material costs and usually high fabrication and assembly costs; adverse effects of both temperature and moisture; poor strength in the out-of-plane direction where the matrix carries the primary load, susceptibility to impact damage and delamination or separations; and greater difficulty in repairing them. [69]•.

Currently it is not available to achieve a competitive, long-life, 100% bio based, renewable composite material. The main obstacles remain to be the depreciative strength and persistence of water absorption with increased renewable content as natural fibers have tendency to absorb water, causing de-lamination of the reinforcement from the matrix.

Natural fibers have exceptional strength to weight characteristics, even outperforming carbon fibers. ***Engineered Natural /Bio-fibers*** are defined as a blend of surface treated baste and leaf fibers whose composition is based on the correct blend that achieves an optimum balance of mechanical properties. [71]•. With natural fibers, these agents differ because of the chemical make-up of the fiber itself. Technological advances in the lack of these bonding agents in tandem with additives to reduce the absorptivity and swelling of natural fibers will lead to increased use of natural fibers and overall improve bio-based composites. [68]•.

A number of research projects was conducted to develop 100% biodegradable materials by embedding microorganisms along with other natural fibers (agricultural wastes) in composite materials. One of these researches adopted material growth by disinfecting molds with hydrogen peroxide, filling them with previously boiled agricultural waste, spreading mushroom seeds on

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• [68] J.B. Cleveland, The potential application of Natural Fiber Reinforced Bio-polymer (NFRbP Composites in Architecture), Faculty of The School of Architecture, University of Southern California, Master of Building Science, 2008.

• [69] F.C. Campbell, Introduction to Composite Materials, Sructural Composite Materials, Material Information Society, 2010.

• [70] E. M. González, et al., Ecovative Design-growing architecture through mycelium and agricultural waste, AAR, Columbia University, GSAPP, 2010.

• Op.cit.

• Ibid

• [71] L. Drzal, R. Inc., Sustainable Bio-based Structural Composite materials for Housing and Infrastructure Applications: Opportunities and Challenges.

•[68] J.B. Cleveland, The potential application of Natural Fiber Reinforced Bio-polymer (NFRbP Composites in Architecture), Faculty of The School of Architecture, University of Southern California, Master of Building Science, 2008.

them, placing the molds inside plastic bags, and letting the mycelium grow to put all the components together. Once it is ready, the molds are removed and the outcome is dried. [70]•.

17  18,19  20,21 

22, 23 

From left to right, Image [17]. Greensulate panel (Ecovative design), Image [18, 19]. Greensulate material with seeds of mushroom on it, Image [20, 21]. Greensulate material with seeds inside the bags, Image [22, 23]. Grass growing on greensulate materials [70]•.

3.Project discipline	**Bio materials, Bio-composite materials**	
Project ecological value	• Biodegradability.	
Project application	Architecture –building material.	
Project information	Name	The Living Hy-Fi Mushroom Tower.
	Time	2014.
	Place	MoMA PS1's 2014.
	Designer	David Benjamin.

Microorganism: Mushroom mycelium, nonliving.

Concept

 

Image [24, 25]. Hy-fi tower project visualization on the MoMA PS1's 2014.

The Hy-Fi towers are made of organic and reflective bricks: the organic bricks make up most of the towers' structures while the reflective bricks sit atop them. This arrangement bounce light down on the towers and the ground, reflecting light effects on the interior walls.

Instead of being thick and dense at the bottom, the grown construction material is thin and porous at the bottom to create a gravity-defying effect. These natural building walls establish

•[70] E. M. González, et al., Ecovative Design-growing architecture through mycelium and agricultural waste, AAR, Columbia University, GSAPP, 2010.

• Ibid

a more comfortable microclimate in the summer by letting in cool air from the bottom and pushing hot air out of the top.

The bricks are produced using a combination of corn stalks and a developed living root structures made of mycelium (mushroom roots) by Ecovative. The designer grow bricks inside of custom-made molds using 3M's daylight mirror film. [72]•.

Description

The resulting bio-bricks are made using almost no energy or carbon emissions, and can be decomposed at the end of their life cycle. [73]•.

Image [26, 27]. The complete built Hy-fi towers proving the potentials of mycelium composite panels as a temporal building materials.

[2.2.3] Biofilm structure

According to Kolter and Greenberg, (2006), the term biofilm refers *to* ***complex heterogeneous structures comprising different populations of microorganisms that attach and form a community on inert or organic surfaces***. ***These adherent cells become embedded within a slimy extracellular matrix via van der Waals forces and hydrophobic effects***. [74]•.

This matrix is composed of extracellular polymeric substances (EPS) (A large proportion of the EPS is strongly hydrated, almost 97% water to protect cells from desiccation, however, hydrophobic EPS also occur; e.g. cellulose, which is produced by a range of microorganisms).

This EPS matrix encases the cells within it and mediates surface adhesion, ***cell-to-cell communication through biochemical signals as well as gene exchange and self-organization within the biofilm***, as well as structural integrity, and nutrient acquisition. [78]•. ***The EPS matrix also traps extracellular enzymes and keeps them in close proximity to the cells. Thus, the matrix represents an external digestion system and allows for stable synergistic micro groups of different species***. The cells within the biofilm produce the EPS components, which are a polymeric composite of extracellular polysaccharides, proteins and DNA. The properties of the surface, such as charge, hydrophobicity and roughness, determine initial microbial attachment. [75•; 76•; 77•].

• **[72]https://inhabitat.com/nyc/the-livings-100-organic-hy-fi-towers-win-moma-ps1s-2014-yap-competition/30-1-2018.**

•**[73] https://inhabitat.com/nyc/hy-fi-tower-made-of-self-assembling-mushroom-root-bricks-rises-at-moma-ps1.**

• **[74] P. Watnick, R. Kolter, Biofilm, city of microbes, Journal of Bacteriology, 10, 182, pp.2675–2679, 2000.**

• **[78] M.D. Robertson, et al., Architects of nature: growing buildings with bacterial biofilms, Microbial Biotechnology, 5, 10, pp.1157–1163, 2017.**

•**[75] C.D. Nadell, et al., The sociobiology of biofilms, FEMS Microbiology Reviews, 1, 33, pp.206–224, 2009.**

•**[76] D. López, et al., Biofilms, Cold Spring Harbor Perspectives in Biology, 7, 2, pp.a000398, 2010.**

•**[77] L. Hall-Stoodley, et al., Bacterial biofilms: from the natural environment to infectious diseases, Nature Reviews Microbiology,2, 2, pp.95–108, 2004.**

During surface colonization different microorganisms, e.g. bacterial cells are able to communicate using ***quorum sensing (QS)*** products such as N-acyl homoserine lactone (AHL). Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including minerals, soil particles. The final stage of biofilm formation is dispersion, in which the biofilm is established and may only change in shape and size. [78]•.

The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium. [79]•. Microbes form a biofilm in response to various different factors, [80]• that may include cellular recognition of specific or non-specific attachment sites on surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics. [81]•. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated. [82]•.

A biofilm may also be considered a ***hydrogel, which is a complex polymer containing many times its dry weight in water***. Biofilms are biological systems; the microbes organize themselves into a coordinated functional community. [83]•.

Biofilms can be harnessed for constructive purposes. For example, **many sewage treatment plants include a secondary treatment stage in which wastewater passes over biofilms grown on filters**, which extract and digest organic compounds. In such biofilms, bacteria are mainly responsible for removal of organic matter (BOD), including pathogens and other microorganisms.

Biofilms can help eliminate petroleum oil from contaminated systems.by the hydrocarbon-degrading activities of microbial communities. [84•; 85•]. ***Biofilms are also used in microbial fuel cells (MFCs) to generate electricity from a variety of starting materials, including complex organic waste and renewable biomass***. [86•; 87•].

• [78] M.D. Robertson, et al., Architects of nature: growing buildings with bacterial biofilms, Microbial Biotechnology, 5, 10, pp.1157–1163, 2017.

• [79] G. A, R. Kolter, Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis, Molecular Microbiology, 3, 28, pp. 449–461, 1998.

• [80] G. A. O'Toole, R. Kolter, Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development, Molecular Microbiology, 2, 30, pp.295–304, 1998.

• [81] E. Karatan, P. Watnick, Signals, regulatory networks, and materials that build and break bacterial biofilms, Microbiology and Molecular Biology Reviews, 2, 73, pp. 310–47, 2009.

•[82] D. An, M.R. Parsek, The promise and peril of transcriptional profiling in biofilm communities, Current Opinion in Microbiology, 3, 10, pp. 292, 2007.

• [83] C. Case, B. et al., Microbiology An Introduction, (10th eds) Creative Pultrusions, Pultex Global Design Manual, 2000.

•[84] V.A. Martins dos Santos, et al., Genomic Insights into Oil Biodegradation in Marine Systems, In book: Microbial Biodegradation: Genomics and Molecular Biology, Caister Academic Press, UK, 2008.

•[85] E. In Díaz, Microbial Biodegradation: Genomics and Molecular Biology, Horizon Scientific Press, p. 1971, 2008.

•[86] S.L. Chua, et al., A stable synergistic microbial consortium for simultaneous azo dye removal and bioelectricity generation, Bioresource Technology, 155, pp. 71–76, 2014.

• [87] R.Y. Zhang, et al., The Biofilm Lifestyle of Acidophilic Metal/Sulfur-Oxidizing Microorganisms, In: Biotechnology of Extremophiles: Advances and Challenges. Rampelotto Pabulo H (Ed.), Springer International Publishing, Cham, Switzerland, pp. 177-213, 2016.

Eukaryotic biofilm

Along with bacteria, [88•; 89•; 90•]. Biofilms are often initiated and produced by Eukaryotes. [91]•. Both **fungi and microalgae** are known to form biofilms in such a way. [91•;92•; 93•].In the environment, **fungal biofilms** are an area of ongoing research. For example, in the soil, plant associated fungi have been shown to decompose organic matter, protect plants from bacterial pathogens. [94•; 95•; 96•]. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic (visible to the naked eye). [75]•.

[2.2.3.1] Biofilm applications in architecture

Biofilms recently have been participating strongly in biomaterials development for built environment applications, one of the subsidiary topics is Bio mineralization, which is the process that living organisms use to consolidate materials by harnessing mineralization, inducing the formation of mineral crystals and influencing their distribution, morphology and growth. Bio materials research objected to building materials already makes extensive use of the products of bio mineralization notably in the form of calcium carbonate. Sedimentary rocks, including limestone and marble, which are products of bio mineralization, and this is refined into lime to be used as binding agent in concrete. [97•; 98•].

Bacterially induced calcium carbonate forms as the result of urease production by bacteria cells, which in turn alters the alkalinity of the bacteria environment such that calcium will tend to bind with carbon. [99]•. A number of bacteria strains, notably ***Bacillus pasteurii*** and ***Bacillus megaterium***, are known to change the conditions of a calcium rich environment, such that calcium carbonate is crystallized. [97]•.

Bacterially induced calcium carbonate precipitation is proposed as an environmentally friendly method to protect decayed ornamental stone. Which relies on the bacterially induced formation of a calcium carbonate precipitate on limestone. The carbonate cement promoted by bacteria is highly coherent, and this technique has been employed for the improvement of the durability of cementitious materials. Calcium carbonate bio-deposition technologies have been used for consolidation of sand columns and for repair and remediation of cracks in concrete.

Bacterially induced calcium carbonate precipitation was also used to produce '**self-healing**' concrete. ***Bacillus megaterium*** spores and suitable dried nutrients are mixed and applied to

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•[88] T. Abee, et al., Biofilm formation and dispersal in Gram-positive bacteria, Current Opinion in Biotechnology, 2, 22, pp.172–9, 2011.

• [89] F. Rossi, R. De Philippis, Role of Cyanobacterial Exopolysaccharides in Phototrophic Biofilms and in Complex Microbial Mats, Meeks JC, Haselkorn R, eds. Life. 2, 5, pp.:1218-1238, 2015.

• [90] T. Danhorn, C. Fuqua, Biofilm formation by plant-associated bacteria, Annual Review of Microbiology, 61, pp. 401–22, 2007.

• [91] L. M. Joubert, et al., Microbial Exopolymers Link Predator and Prey in a Model Yeast Biofilm System, Microbial Ecology, 2, 52, pp.187–197, 2006.

• Op.cit.

• [92] A. Orell, et al., Biofilm Lifestyle of Thermophile and Acidophile Archaea. In: Witzany G (ed). Biocommunication of Archaea. Springer, Switzerland. pp 133-146, 2017.

• [93] C. Van Colen, et al., Ecology of intertidal microbial biofilms: Mechanisms, patterns and future research needs, Journal of Sea Research, 92. 2 – 5, 2014.

• [94] M. Burmølle, et al., Microbial Biofilms: Current Research and Applications, Caister Academic Press. pp. 228, 2012.

• [95] J. W. Costerton, et al., Microbial biofilms, Annu Rev Microbiol, 49:711-45, 1995.

• [96] M. E. Davey, G. A. O'toole, Microbial Biofilms: from Ecology to Molecular Genetics, Microbiology and Molecular Biology Reviews, p. 847–867, 2000.

•[75]

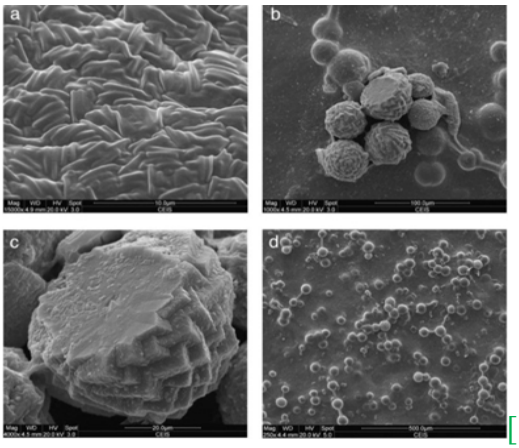
• [97]

• [98] http://www.ncl.ac.uk/press.office/press.release/item/cracks-in-your-concrete-you-need-bacillafilla#.UY5Lib9Z-8 [11.02.2014].31-1-2018.

• [99] S. S.Fisher, et al., Microbiological precipitation of CaCO3, Soil Biol, 31, pp.1563–1571, 1999.

•[97] M. D. Robertson, et al., Radical Vernacular: Bacterial Architecture on Mars, Journal of the British Interplanetary Society 67(JBIS):213-217, 2014.

steel-reinforced concrete. When the concrete cracks, water ingress dissolves the nutrients and the bacteria germinate triggering calcium carbonate precipitation, [78]• resealing the crack and protecting the steel reinforcement from corrosion. This process can also be used to manufacture new hard materials, such as bio-cement.



[1]•

Image [28]. Electron microscope images of *Bacillus pasteurii* growing in agar. (a) Shows bacteria cells without the presence of calcium. (b) Shows spherical faceted crystals of calcium carbonate induced by the *Bacillus pasteurii* bacteria. (c) Shows a close-up image of a crystal whose surface is fractured, revealing bacteria cells embedded inside the crystal structure. (d) Shows the network of fine filaments, which connect the crystals together. [1]•.

Another application of bacterial cultures and biofilms, the branching patterns of bacterial colonies that could be utilized directly in design form generation with direct embedding of bacterial strains in translucent containers to exhibit its natural morphologies. As by altering physical and chemical conditions on a series of agar plates, including the level of nutrients, density of the hard medium and the shape of the physical container, bacterial colonies respond by growing into branching and fractal like patterns. [100•; 101•]. In different physical conditions, the colonies respond by branching into areas with high concentrations of nutrients showing directionality where their physical space is constrained and moving along channels and almost thin surface topographies of the agar. [97]• .

•[78]

•[1] M.D. Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60. pp.28-39, 2015.

• Ibid

• [100] E. B. Jacob, Bacterial Complexity: More is Different on All Levels, in Systems Biology: The Challenge of Complexity, ed. S. Nakanishi, R. Kageyama, and D. Watanabe, Springer, Tokyo, pp.25–35, 2009.

• [101] E. B. Jacob, From snowflake formation to growth of bacterial colonies II: Cooperative formation of complex colonial patterns, Contemp. Phys, 38, pp.205-241, 1997.

• [97]

Image [29]. Left (top) *Bacillus subtilis* experiments showing development of a branching, fractal pattern. (Bottom) shows three inoculated points. Image [30].Right (top) *Paenibacillus dentritiformis*-V in a labyrinth-like container. (Bottom) Cross-shaped container with *P. dendritiformis-C* inoculated in four points. Independent colonies have grown to the extent of merging into one. [97]•.

One of the most promising types of bacterial biofilms is the ***cellulose biofilms*** produced by bacterial cellulose, ***Acetobacter*** bacteria is a non-photosynthetic organism that procure glucose, sugar, glycerol, or other organic substrates and convert them into pure cellulose.

Acetobacter xylinum is one of the most potent cellulose-producing bacterium. Cellulose produced by this strain is almost the only biopolymer that is synthesized, completely biodegradable and recyclable with a great mechanical strength, and high tensile strength consistent dimensional stability, light weight, remarkable capacity to hold water, selective porosity, and high wet strength. [102]•.

[103]•

The production of biofilm-based lamps by bacterial biofilms; left to right, Image [31]. Symbiotic colony of bacteria and yeast that grows on the surface of the medium is the bacterial cellulose and have leather-like texture when dried. It grows on surface and takes the shape of the container. During bio-based production process, microbes like *Acetobacter* are used to convert glucose into cellulose within a fermentation process, producing a gel-like textile surface with thickness of up to 400 mm. Image [32]. The bacterial biofilm in gel –like phase, Image [33]. The application of the bacterial biofilm as a material in lighting unite design.

• **Ibid**

• **[102] R. M. Brown, Jr. Microbial Cellulose: A New Resource for Wood, Paper, Textiles, Food and Specialty Products, Department of Botany, The University of Texas at Austin, Austin, Texas 78713-764.**

• **[103] M. Faidi, Feasability of Bacterial Cellulose in Furniture Design, thesis, Aalto University School of Arts, Design and Architecture Interior architecture, 2017.**

4.Project discipline	**Bio materials, Biofilm material project**	
Project ecological value	• Energy production (by photosynthesis). • Toxins filtration (co_2 absorption), o_2 production. • Passive cooling & shading. • Food production.	
Project application	Architecture and urban agriculture.	
Project information	Name	Algaetecture.
	Time	2015.
	Place	Future Food District for Expo Milano 2015.
	Designer	Ecologic Studio.

Microorganism: Micro algae, living.

Concept

In this project Micro-algae is used to change the design of architectural surfaces. By utilizing the large areas used as facades and roofs as photosynthetic surfaces in order to respond to the current state of global warming.

Micro-algae is an important photosynthetic organism which absorbs large amounts of carbon dioxide and produce oxygen, acting as a second skin of buildings, boosting passive cooling and increasing shading. In addition, micro-algae systems can be used as an innovative energy and food production system, growing into a biomass, which can be processed for energy.

Description

Image [34]. The single micro algae biofilm unite. Right the cladding system. Ecologic Studio

The micro-algae have been integrated within a custom designed four-layered ETFE cladding system, whilst the flows of energy, water and CO2 are controlled and regulated in real-time and made to respond and adjust to weather patterns and visitors' movements.[104]•.

•[104] http://www.isplora.com/news/algaetecture-1/9/2018

5.Project discipline	**Biomaterials, Biofilm.**	
Project ecological value	Biodegradability.	
Project application	Furniture design.	
Project information	Name	Xylinum.
	Time	2012.
	Place	Jenpolymers in Jena, Jena, Germany.
	Designer	Jannis Hülsen.

Microorganism. Bacteria ***Acetobacter xylinum***, bacteria cellulose.

Concept

This project utilizes bacterial-cellulose produced by ***Acetobacter xylinum***. As the bacterial strain within a nutrition, liquid transforms sugar into a cellulose-fiber structure. The designer developed the technique to shape the material during the production process inside the liquid.

Description

The properties of the cellulose fleece are characterized by their three-dimensional nano grid structure, which leads to excellent mechanical properties, as its stability in a wet condition is to steel. Moreover, it is non-allergic and fully recyclable, and the cellulose material shows similarities to materials such as leather. [105]•.

Image [35]. Left, the perspective view of *xylinum* upholster chair, Image [36]. Top view shows the thin leather like layer of *Acetobacter xylinum*-bacterial cellulose.

Designer upholstered the stool by using bacteria to grow a cellulose skin over its surface. The furniture is immersed in a tank while the bacteria consumes sugar and builds a cellulose fiber structure. Once finished it can be dried out to form a material that is 100% biodegradable.

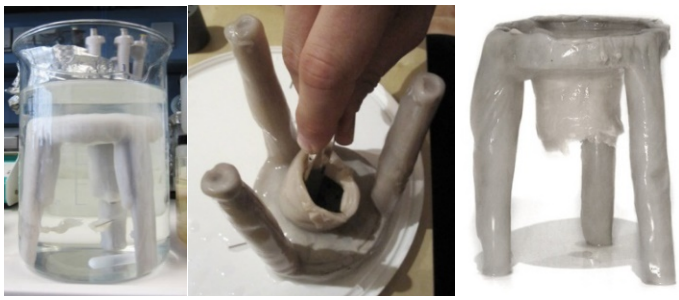


Image. [37, 38, 39]. Prototyping production process of Xylinum upholster chair.

• **[105] https://www.dezeen.com/2012/03/06/xylinum-by-jannis-hulsen**

[2.2.3.2] Biofilms in electricity production

6.Project discipline	**Bio materials, Biofilm material project**	
Project ecological value	• In energy applications; batteries. • Coating biofilms with enzymes that catalyze the breakdown of cellulose, which is useful for converting agricultural waste to biofuels.	
Project application	Energy production –biofuel.	
Project information	Name	Living materials.
	Time	March 23, 2014.
	Place	MIT –USA -Massachusetts Institute of Technology.
	Designer	A. Chen, Z. Deng, A. Billings, U. Seker, B. Zakeri, M. Lu, R. Citorik.

Microorganism: Bacteria - bacterium E. coli.

Concept

MIT engineers have coaxed bacterial cells to produce biofilms that can incorporate nonliving materials, mainly gold nanoparticles and quantum dots. These "living materials" combine the advantages of live cells, which respond to their environment, produce complex biological molecules, and span multiple length scales, with the benefits of nonliving materials, which add functions such as conducting electricity or emitting light.

Description

The bacterium ***E. coli*** produces biofilms that contain "curli fibers" which are **amyloid proteins that help *E. coli* attach to surfaces**. Each curli fiber is made from a repeating chain of identical protein subunits called CsgA, which can be modified by adding protein fragments called peptides. **These peptides can capture nonliving materials such as gold nanoparticles, incorporating them into the biofilms**. [106]•.

By programming cells to produce different types of curli fibers under certain conditions, the researchers were able to control the biofilms' properties and create gold nanowires; They also engineered the cells so they could communicate with each other and change the composition of the biofilm over time.

MIT team disabled the bacterial cells' natural ability to produce CsgA, then replaced it with an engineered genetic circuit that produces CsgA but only when a molecule called AHL is present. To control curli fiber production, when AHL is present; the cells secrete CsgA, which forms curli fibers that join into a biofilm, coating the surface where the bacteria are growing.

After that, the researchers engineered E. coli cells to produce CsgA tagged with peptides composed of clusters of the amino acid histidine, only when a molecule called aTc is present. The two types of engineered cells grown together in a colony, allowing researchers to control the material composition of the biofilm by varying the amounts of AHL and aTc in the environment. If both are present, the film will contain a mix of tagged and untagged fibers. If gold nanoparticles are added to the environment, the histidine tags will grab onto them, creating rows of gold nanowires, and a network that conducts electricity.

• **[106] http://news.mit.edu/2014/engineers-design-living-materials-1/30/2018**

[2.3] Bio digital design

As exhibited previously, numerous application of biotechnology, synthetic biology, biomaterials, in industrial and design fields, it is important to gain insight on the actual and proposed future practices of bio-based architecture with its different disciplines and applications.

Bio-architecture is the architecture that includes living beings, as the prefix "**bio", should exclusively be reserved for what really integrates real life amongst its architectural elements.** [107]•, this type of architecture is a special case than the consumed terms of ; environment, ecology, sustainability, etc. The Bio digital architecture /design term was originally introduced by **Alberto T. Estévez**, [107]• along with other terms that defined this interdisciplinary field; such as **(Biolearning), (Bio manufacturing)** and **Genetic architecture** to describe the process of bio digital design:

***The bio digital design is an interdisciplinary field that employs computational (algorithmic) tools and natural sciences (micro, molecular biology, and genetic design and engineering) by embedding natural microorganisms / genetic manipulation / enzymes synthesizing, etc. as part of architectural element weather as a material or a device; for achieving a living architecture that operates special ecological values**, these values are as shown previously; productivity (lighting, electricity, food, etc.) or purification. **As stated by Alberto T. Estévez "it is no longer about building 'in' nature, but building 'with' nature, and even building nature itself"** [107]•.

The first manifestations of experimentation in this field was in urban design application; the **Genetic Barcelona Project which was the genetic creation of bioluminescent plants for urban and domestic use** by Alberto T. Estévez. This project was one of the vanguard manifesting projects that employed genetic manipulation to employ nature for urban design functionality, the project aimed "for illuminating architectural spaces" getting trees to work as "lamps" through bio manufacturing for illuminating streets, plants illuminating homes, and vegetation illuminating the roadsides without electricity. The project included three phases:

1. The creation of plants with natural light by genetic transformation for urban and domestic use. In 2005, the first seven lemon trees with luminescent leaves, provided by GFP **green fluorescent protein** gene were produced. The transformed lemon trees got their **green fluorescent protein** through the expression of the GFP gene. The trees were made with the objective of being of architectonical and urban use ;they can be also multiplied by planting their branches, becoming non-manufactured or bio manufactured lamps.
2. The second phase of the project, **"Bio-lamps":** in 2007/2008, searched bacterial **bioluminescence** for urban and domestic use, focusing on how to achieve bioluminescent plants with a bacterial genes group that is the responsible for bioluminescence. "Bio-lamps" were created as a kind of "batteries" with bioluminescent bacteria that are originally found **in abyssal fish**. They were utilized to light the first systematically fully illuminated living light apartment

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• **[107] A. T. Estévez, Learning from Nature: architecture and design in the first biodigital age- "Aprendiendo de la naturaleza", 2nd International Conference of Biodigital Architecture & Genetics, ESARQ (UIC), Barcelona, 2014.**

• **Ibid**

• **Ibid**

without electricity .The second phase was very effective for lighting, but too problematic in terms of durability: as every 10 days, the "bio batteries" needed to be changed. In addition, a "lamp" that could guarantee the required air-tightness, oxygen, and food was too complicated to manufacture compared with a simple bioluminescent plant or tree. Therefore, a third phase was designed, trying to introduce the genes responsible for bioluminescence in ornamental plants. [107]•.

- **Bio digital vs. Biomimetic**

Along the same trends, the biomimetic term is, as a primitive imitation of nature, the term biomimetic, does not seem entirely appropriate or accurate in its application, as it is too broad and diverse. "**In reality no biomimetic, a mimesis of life, can copy or imitate nature without further upheaval**. **This term is being used when in fact it is merely a formalist inspiration**.

It is an inspiration, not an imitation. The same word is also used when talking about a previous observation of a living being, which leads to synthesizing a characteristic that can be of interest for its application in different fields, eventually followed by its proper application, which is not imitative either. **There is thus no such thing as a mimesis, but rather a learning process, learning from nature** or **biolearning,** which is different to **biomimetic** (mimesis, imitation, copy of nature), or what could be described as **bio inspiring."** [107]•.

In order to gain a full insight into the knowhow of application of bio digital design to architecture, the most tangible part of the idea of a living creature would be its DNA: in each cell, its entire appearance and even in its most remote corners. It resounds in all its parts, configures the whole and controls its constant evolution (emerging system). The DNA of the building needs to be clear for its creator, with an alive system, which will make it grow on its own. In pursuit of coherence between the architectural genotype and phenotype, between the internal and conceptual engine and its harmonious final and constructive implementation. (A. T. Estévez, 2014). [107]•.

[2.3.1] Genetic architecture in architecture

*Genetic Architecture term could be differentially defined by two main disciplines. **The original one refers to genetic manipulation as a branch of red biotechnology**. **The other emerged when parametric and algorithmic design tools used the rules of Darwinian Theory of emergence and evolution applied to genetic optimization engines that help designers find a 100% perfect solution for a functional or formal generative equation in their design** solution. Combining both methods results in the third, which is, what we are discussing in the scope of this research; and the two main definitions are exhibited as follows:

Genetic architecture in biotechnology: refers to the underlying genetic basis of a phenotypic trait and its variation properties. [108]• . Phenotypic variation for quantitative traits is, at the most basic level, the result of the segregation of alleles at quantitative trait loci (QTL- is a section of DNA (the locus) which correlates with variation in a phenotype (the quantitative

• **Ibid**
• **Ibid**
• **Ibid**
•**[108] T. F. Hansen, "The Evolution of Genetic Architecture". Annual Review of Ecology, Evolution, and Systematics. 37 (1): 123–157. 2006.**

trait)). [109]• . Usually the QTL is linked to, or contains, the genes, which control that phenotype. [110]• . Environmental factors and other external influences can also play a role in phenotypic variation. Genetic architecture is a broad term that describe any information regarding gene and allele number, the distribution of allelic and mutational effects, and patterns of pleiotropic, dominance, and epistasis.

Genetic design in algorithmic design are derived from genetic algorithms which are **class of highly parallel evolutionary, and adaptive searching methods**. [111]. Their key characteristic is "**a string-like structure equivalent to the chromosomes of nature**," **to which the rules of reproduction, gene crossover, and mutation are applied**. Various parameters are encoded into the "a string-like structure" and their values changed during the generative process.

A number of similar forms, pseudo-organisms, are generated, which are then selected from the generated populations based on predefined fitness criteria. The selected organisms, and the corresponding parameter values, are then crossbred, with the accompanying gene crossovers and mutations, thus passing beneficial and survival enhancing traits to new generations.

Optimum solutions are obtained by small incremental changes over several generations. [111]•.To gain a better understanding of the bio digital architecture; the following manifesting projects will exhibit bio digital procedures, although not presenting the genetic manipulation in genetic architecture with its full capacity.

7.Project discipline	**Embedding microorganisms in Architecture**	
Project ecological value	• Bioreactor. • Oxygen production.	
Project application	Landscape	
Project information	Name	Alga(e)zebo.
	Time	London Olympic 2012 Event.
	Place	Euston Square Gardens, London.
	Designer	Design team: Marco sandmarjan, London. Manufacturer: Formstaal GmbH & Co.KG, Stralsund Germany. Photo bioreactor: Richard Beckett - DMC London; UCL Algal Biotechnology, London UK.

Microorganism

Symbiosis; Microalgae: Algal Strains: ***Chlorella vulgaris*** and bacteria.

Concept

This project is an installation that consists of a large decorative canopy-structure. The complex patterns of the surface create a unique ornamental structure that evokes an ever-changing effect of light and shadows.

•[109] C. Miles, M. Wayne, "Quantitative trait locus (QTL) analysis". Nature Education.2008.

• [110] T.F.C. Mackay, "The Genetic Architecture of Quantitative Traits". Annual Review of Genetics. 35 (1): 303–339.2001.

• [111] B. Kolarevic, Digital Architectures , University of Pennsylvania, USA- ACADIA 2000: Eternity, Infinity and Virtuality, page 254

The installation internal space functions as a sitting facility for visitors to rest and gather, the Gazebo also initiates the recyclable materiality and biotechnological augmentation of the structure. [112]•.

The irregular outline of the Gazebo design allows for trees or taller bushes to grow in between the structure. The multifaceted patterns create a scaffold for smaller vegetation to grow into it as a pergola. The vertical columns incorporate algae tubes with different strains of locally bred algae that vary in texture and color.

Description

The design, with its complex geometry and perforated motifs, is originated from the implementation of sophisticated digital media processes. Custom-made algorithmic and parametric scripting programs allow for the optimization and design efficiency of two-dimensional processes (nesting, scripting) and structural integrity in three dimensions (topological projections). [113]• .

Image. [40, 41]. Digital fabrication process of the bioreactor metal container inspired by trees and employing branching algorithms (L-systems). By Marco sandmarjan

Photo Bioreactors: the separate columns consist of varying volume bioreactors and contain a range of different microorganisms. From carbon dioxide consuming strains of algae to localized bacteria from surrounding gardens.

Image. [42, 43]. The bioreactor fixed inside the container. By Marco sandmarjan

•[112] http://syndebio.com/algaezebo/SyndebioAlga(e)zebo by marcosandmarjan with richard beckett » -1/9/2018

• [113] https://www.ucl.ac.uk/bartlett/architecture/research/algaezebo/23/2/2018

Image. [44, 45]. Testing of algae insertion into different thicknesses of growth medium, Image. [46]. The lighting emission from the bioreactor.

Image. [47]. Carbon-impregnation of 3D prints, which work as a scaffold that attracts the growth of algae.

8. Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	• Oxygen production. • Biofuel.	
Project application	Interior design –architecture	
Project information	Name	BIO. tech HUT.
	Time	2017.
	Place	EXPO 2017 Astana (Kazakhstan)
	Designer	Ecologic Studio: Marco Poletto and Claudia Pasquero (London, UK).

Microorganism: **Micro algae.**

Concept

The BIO. Tech HUT is an interactive bio-exhibition project composed of three fluidly interconnected environments that loosely embody the fundamental programs of a living space.

- The Lab, where new species of microorganisms are tamed and engineered into artificial cultivation environments, growth patterns and material assemblies, as shown in Image. [48, 49].

Image. [48, 49]. The lab sector of the exhibition. Ecologic Studio

- The Living Hut, is divided into three rooms:
    1. Bio. Light Room: is a dark and calm space in which the only visible light is emitted by bioluminescent bacteria when shaken and oxygenated by the air handling system.
    2. H.O.R.T.U.S Room.
    3. The Algae Photo-Bioreactor Room is filled with growing phototropic microorganisms that use photosynthesis to generate biomass and oxygen, while absorbing carbon dioxide.

At the core of the Garden Hut is a harvest area for the processing and transformation of biomass into food and electricity.

Description

The *photo-bio reactive cladding is developed from a system that uses high-speed airflow to lift the living medium into lab grade glass tubes. The air stream creates swirls on the fluid inside the tubes, and generates a stirring effect that catalyzes the desired O2 / CO2 exchange. The fluid then descends by gravity to complete the loop***.** Multiple glass tubes are looped around the BIO. tech HUT, and become architectural elements.

Structurally, the loops of glass tubes are supported by a series of sectional frames in high-performance honeycombed polycarbonate. The resulting structure is lightweight, fully recyclable and has the unique effect of scattering and enhancing the penetration of solar radiation deep into the BIO. tech HUT. [114]•, as shown in Image [50, 51].

Image. [50, 51]. The two rooms of the photo bioreactor the HORTUS room, showing photo bio reactive cladding of glass tubes and, showing ranging colors of tubes according to algal growth. Ecologic Studio

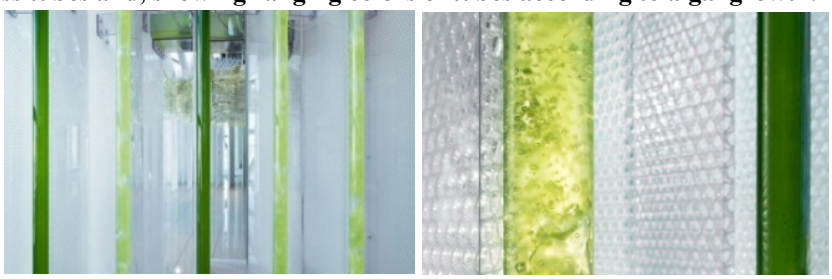

Image. [52]. Left, the glass tubes containing the algae culture. Image. [53]. Right, the sectional frames in high-performance honeycombed polycarbonate that scatter solar radiation into the Bio. Hut. Ecologic Studio

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• **[114]** http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=162-2-2-2018.

9.Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	• Oxygen production.	
Project application	Interior design –architecture.	
Project information	Name	HORTUS Astana: Fibrous Structures.
	Time	June 10, 2017.
	Place	The biotech hut exhibition, pavilion of Astana EXPO 2017.
	Designer	Ecologic Studio. Marco Poletto and Claudia Pasquero (London, UK).

Microorganism: Microalgae- cyanobacteria.

Concept

HORTUS (Hydro Organisms Responsive to Urban Stimuli) is a bio exhibition. The project design starts with a three-dimensional distribution of wide spectrum artificial light sources. The flows of energy emitted as wide spectrum radiation are digitally mapped in space to visualize their intensive field.

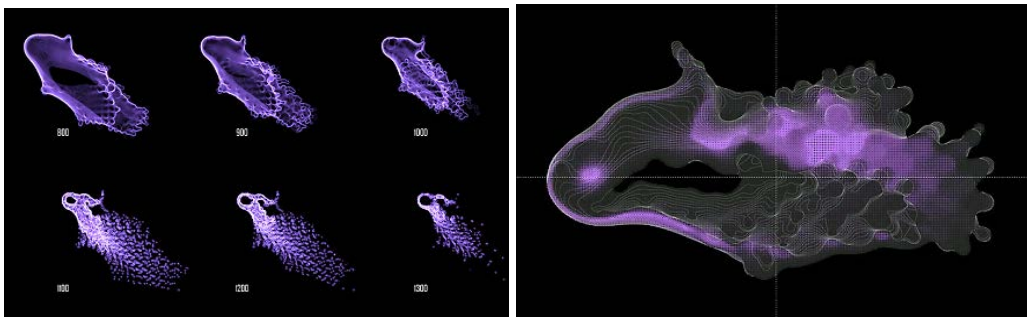

Figure. [4]. Digital mapping of wide spectrum radiation of emitted flows of energy of artificial light sources. Ecologic Studio

After that, *Cyanobacteria* are introduced, their metabolic features deployed to convert latent radiation into actual processes of photosynthesis, oxygenation and energy. Their articulation in space is digitally mediated to arrange the photosynthetic organisms along iso-surfaces of optimal incoming radiation; vectors sample the fields at discrete locations establishing a cloud of extensive structural points. [115]•, as shown in figure. [5].

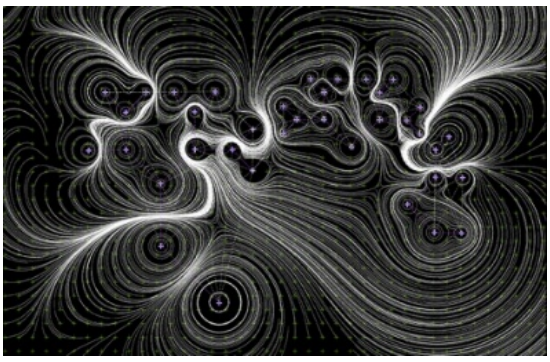

Figure. [5]. The digital visualization of cyanobacteria articulation in space to arrange the photosynthetic organisms along isosurfaces of optimal incoming radiation.

•[115] http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=20&idproj=163-2-2-2018

The project design computes the network of connecting paths, bringing nutrients and CO2 to the photosynthetic nodes hosting living cyanobacteria (actualized into PVC pipes wrapping the substratum).

Visitors are active part of the system, feeding it of CO2 with their exhale and consume the released oxygen as it spreads in the surrounding atmosphere.

Description

The design container artwork is hanging from the ceiling of the 4th floor and has the form of a cloud, with different levels depending on the physical light. HORTUS is divided in 4 clusters, which operate as an integral unit. Each part is made of a core structure of laser cut aluminum sections, acrylic holders and PVC pipes creating the surface of the cloud. It also integrates wide spectrum lights and a glass container for the microalgae and a little pump for fluid circulation.

Visitors are to move freely around and below in the installation, to integrate themselves within it and view the internal landscapes and algae flows, and feed CO2 to the cyanobacteria thus liberating the oxygen produced during the loop. [115]•.

Image. [54, 55]. Different views of the fibrous structure of the cloud. Ecologic Studio

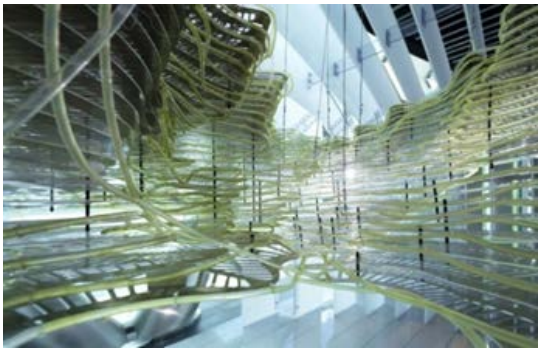

Image. [56]. Detail in the system composed from tubes that host the micro-algae.

• **Ibid**

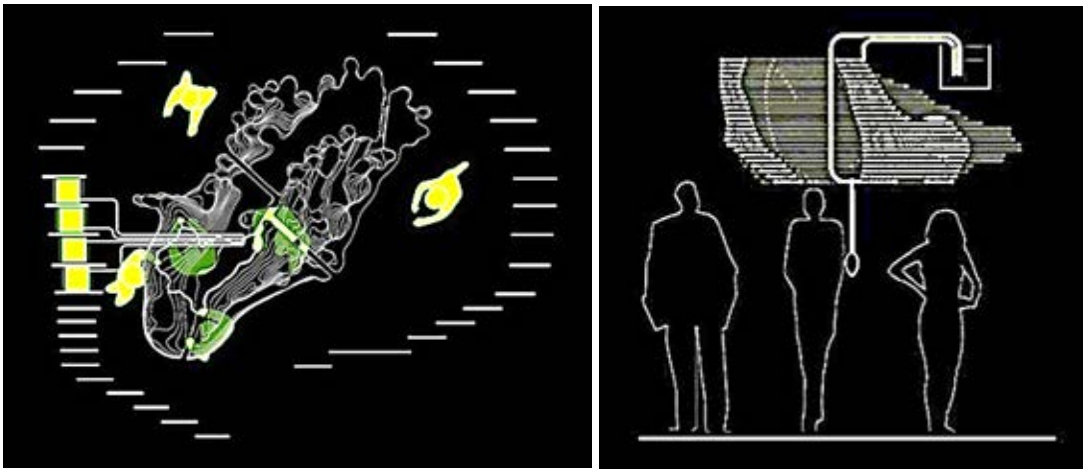

Figure. [6, 7]. Left, plan of the microalgae system showing the assembly of tubes in plan and section. Right, the respiration interactive system that result in algae growth. Ecologic Studio

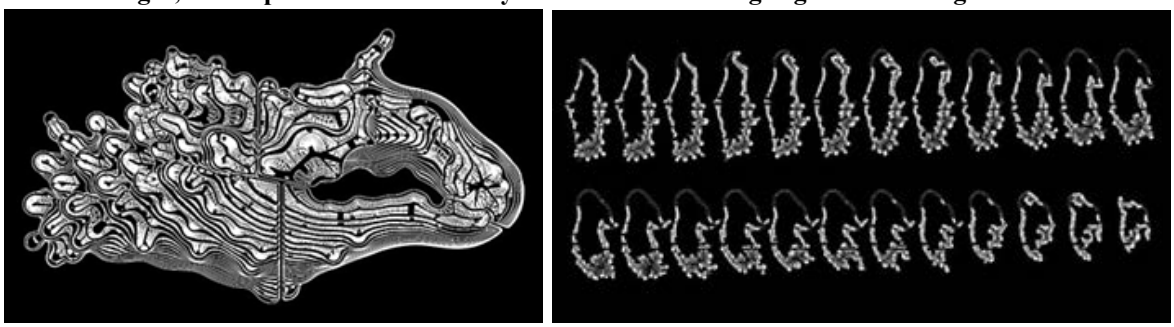

Figure. [8, 9]. The digital designed profiles assembled in contoured fashion that compose the cloud. Ecologic Studio

10.Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	• Oxygen production. • Environmental responsiveness in real time to sun exposure.	
Project application	Architecture –urban design.	
Project information	Name	Algae Folly v2.0.
	Time	November 17, 2015.
	Place	Praça da República, Braga.
	Designer	Ecologic Studio: Claudia Pasquero, Marco Poletto (London, UK) - INL International Iberian Institute for Nanotechnologies.

Microorganism: Microalgae - ***Chlorella vulgaris***

Concept

Urban Algae Folly is a living edible architecture integrating micro-algal cultures and real time digital cultivation protocols within a soft ETFE skin. This vision is embodied in a photosynthetic, nurturing folly. The properties of microalgae are enhanced by their cultivation within a custom designed soft ETFE cladding system. A special CNC welding

technology enables design and controls the morphology of the cushions under stress as well as the fluid dynamic behavior of the nutritious medium as it travels through it. [116]•.

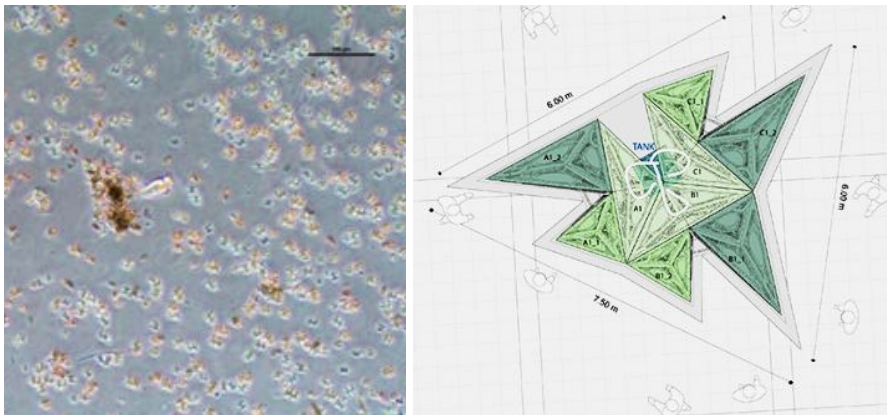

Figure. [10, 11]. Left, the growth of microalgae culture in microns. Right, the device plan with total dimension of 6 m x 6 m x 7.5 m , and circulation manipulation in urban design. Ecologic Studio.

Image. [57, 58]. Two different exterior views of the device. Ecologic Studio.

Image. [59, 60]. Left, interior view of the device showing the assembly of tubes and its relation with structural design using perforated metal to allow natural lighting for the container fixed inside the perforated panels ETFE cladding system of the device. Right, the micro algae container containing the nutrient medium. Ecologic Studio.

Description

The flows of solar energy, water and oxygen are regulated to respond and adjust to weather patterns and visitor's movements in real-time. As the sun shines algae, would photo synthesize and grow thus reducing the transparency of the Folly and changing its appearance. [116]•.

• [116] http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=148-2-2-2018
• Ibid

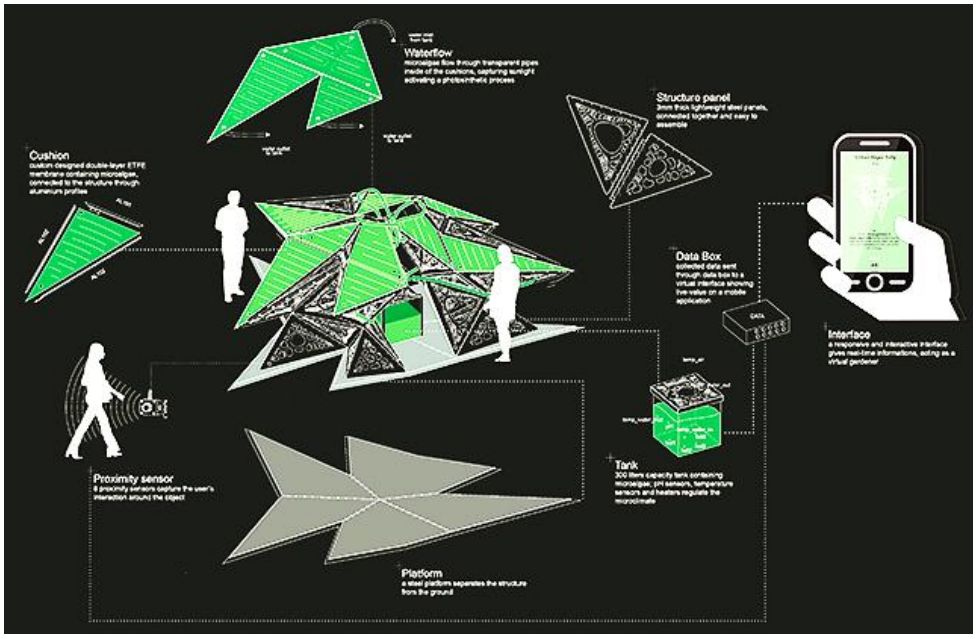

Figure. [12]. The interactive system that the device is adjusted to, showing in bottom left, motion sensors adjusted to respond to visitor's movement in real time. in the top left shows the panels form and assembly, with perforated extra skin of ETFE, in the bottom right shows the bio container that production of o_2 that the folly have produced and that would be monitored by mobile phones. [116][•]. Ecologic Studio.

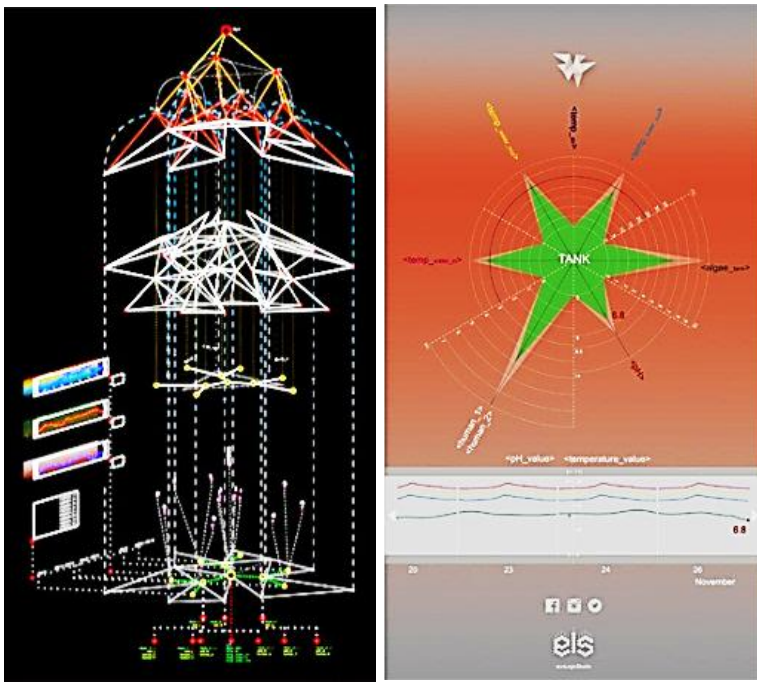

Figure. [13, 14]. Left to right, real time interaction network, showing sensors spots and connections to the real time responsive system, that changes panels openness according to the collected data of visitors motion and weather constrains, right shows real time sun exposure analysis. Ecologic Studio.

Since this process is driven by, the biology of micro-algae is inherently responsive and adaptive; visitors will benefit from natural shading property while being able to interact with it in real-time; *their presence will trigger electro valves to alter the speed of*

[•] Ibid

algal flow through the folly provoking an emergent differentiation across the space. In any moment in time, the actual transparency and color of the folly will be the product of this complex set of relationships among climate, micro-algae, visitors and digital control systems .[116]•.

The Urban Algae Folly will produce 35 g of ***Chlorella*** every day. In term of protein, this is the equivalent of 750 g of Meat per day. The Urban Algae Folly is also adsorbing 1.5 Kg of CO2 per day, produce 750 g of Oxygen per day.

12.Project discipline	**Embedding microorganisms in Architecture, Bio digital**	
Project ecological value	• Oxygen production. • Environmental responsiveness in real time to sun exposure.	
Project application	Architecture –urban design.	
Project information	Name	Urban Algae Folly.
	Time	2015.
	Place	The Future Food District - Milan EXPO 2015.
	Designer	Ecologic Studio.

Microorganism: Microalgae, ***Spirulina.***

Concept

The Urban Algae Folly is an interactive pavilion integrating living micro-algal cultures, a built example of bio digital architecture utilizing microalgae, ***Spirulina***. ***Spirulina*** are exceptional photosynthetic machines; they contain nutrients that are fundamental to the human body, such as minerals and vegetable proteins; as well as oxygenating the air and absorbing CO2 from the urban atmosphere ten times more effectively than large trees.

The architecture of the Algae Folly stems from ETFE architectural skin system; it has the ability to provide the ideal habitat both to stimulate ***Spirulina's*** growth and to guarantee visitors' comfort.

Image. [61, 62]. The assembled algae folly device. Ecologic Studio

• Ibid

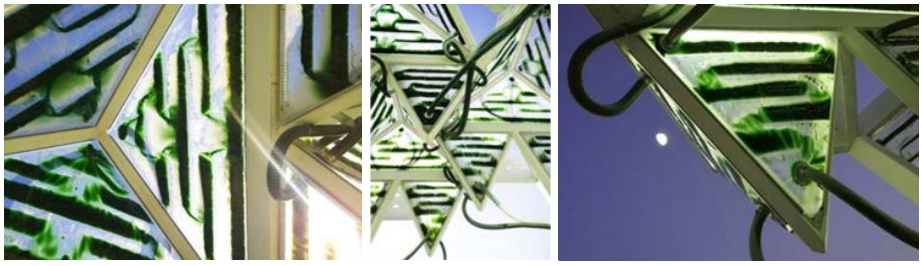

Image. [63, 64]. Panels' assembly plugged in with water circulation to allow algae to grow by photosynthesis to change the panels' transparency. Ecologic Studio

Description

During sunny days, the microalgae grow rapidly thus increasing the shading potential of the architectural skin and improving human comfort, visitors, with their presence, will in turn activate the digital regulation system which will stimulate algal oxygenation, solar insolation and growth. At any time the effective translucency, the color, the reflectivity, the sound and productivity of the Urban Algae Folly are the result of the symbiotic relationship of climate, microalgae, humans and digital control systems. [116][•].

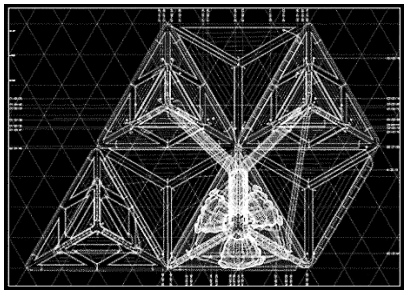

Figure. [15]. Watering system distribution over the algae panels , showing the chosen form of triangular panels to achieve highest level of form manipulation and assembly with adjacent panels and connection with water circulation system. Ecologic Studio

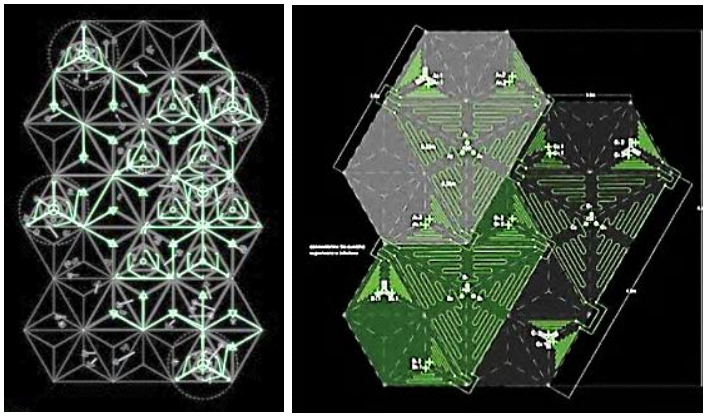

Figure. [16, 17]. Left, panels' assembly, conjunction points and circulation inlets direction. Right, The composition of the triangular panels with inner zigzag shaped bars to maintain algae by maximizing surface area for algae attachment. Ecologic Studio.

[•] **Ibid**

13.Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	• Oxygen production.	
Project application	Architecture –urban design.	
Project information	Name	STEM cloud v 2.0.
	Time	2008.
	Place	BIENNALE 2008.spain.
	Designer	Ecologic studio.

Microorganism: Microalgae.

Concept

STEM cloud v2.0 is a project that operate as a cultivating ground for micro-ecologies found in the local river of Seville, the Guadalquivir, as well as involving the public in the cultivation process.

The transparency and porosity of the architectural system allows the process to be visually and materially monitored and interfered with the microclimate of the gallery. The public feeds the microbial colonies present in the river water with nutrients, light and CO2 and as a result oxygenate the gallery space; the growth process is triggered by patterns of interaction with the public and in turn affect these patterns with its visual effects. Multiple feedback cycles are provoked within the components of the system, with the gallery environment and within the city itself. The cybernetic loops which are the basic cybernetic set for the Seville experiment includes 3 components:

- The urban environments (the river ecology and the gallery space),
- The architectural machine (STEM cloud).
- Human behavior (the visitors).

Description

Simulation & analysis of feedback cycles :

1. The first feedback cycle is composed of machinic feedback cycle1 and organic growth in relationship to radiation field added to a wide spectrum light that is positioned strategically to generate a radiation field that is kept constant in time. Algae growth is stimulated by the field and responds to it; feedback arises while each block develops its own internal equilibrium.
2. The second feedback cycle is composed of machinic feedback cycle 2 plus organic growth in relationship to nutrients concentration added to nutrients that are inserted into the system to initiate its starting condition. Blocks that are more active will consume more nutrients and grow faster. Overgrown blocks will be more opaque to light affecting the radiation field. The coordination between nutrient and radiation will emphasize on the light and texture variation. [117]•.
3. The third cycle is of machinic feedback cycle 3+ oxygenation according to the frequency of use plus photosynthetic activity that is monitored live and visually fed back to the user. The more active block will signal the need to be fed with CO2 provided by the user. Users respond to visual clues (LED intensity) and trigger modifications with their action. [117]•.

• Ibid
• Ibid

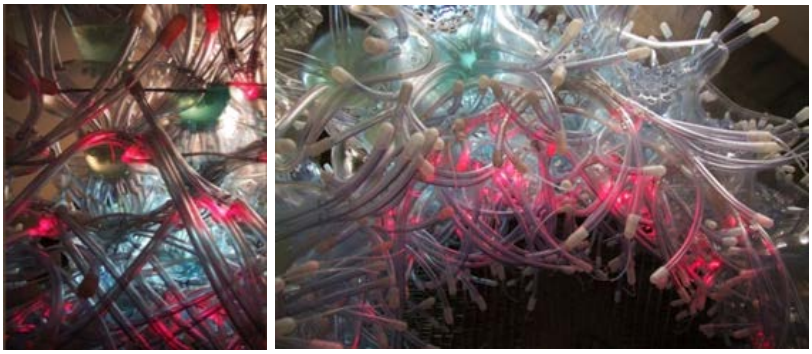

Image. [65, 66]. The STEM cloud assembled in exhibition showing LED that form visual clues to attract visitors to respond to it [117][•]. Ecologic Studio.

Image. [67]. showing the cloud respiration tubes to blow co2 into the algal cultures to provoke growth through photosynthesis [117][•]. Ecologic Studio.

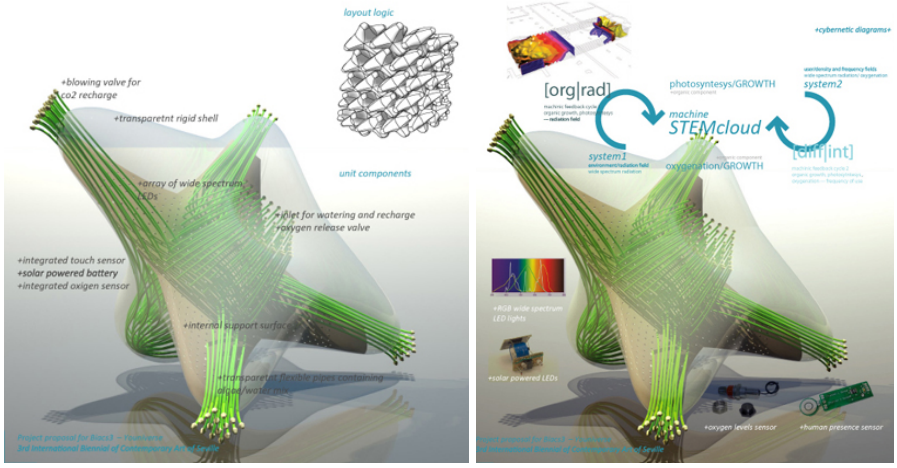

Figure. [18]. Left, the STEM cloud unite showing blowing valves of co2 at 3 corners, the forth corner transparent flexible pipes containing algae and water; the tubes are maintained inside transparent rigid shell, on top of shell; inlets of watering and oxygen recharge valves. The unite is integrated with array of wide spectrum LED lights, touch sensor, oxygen sensor and solar powered battery, top right shows layout logic of unites assembly. [117][•].

Figure. [19]. Right, left bottom, solar powered LED, right bottom, shows oxygen level sensor, human presence sensor integrated in the cloud unite. [117]. Ecologic Studio.

[•] **Ibid**
[•] **Ibid**
[•] **Ibid**

14.Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	• Oxygen production.	
Project application	Architecture / cyber-Gardens / landscape.	
Project information	Name	Alive.
	Time	Presented at Alive: New Design Frontiers, 2013.
	Place	Espace Fondation EDF, Paris, France.
	Designer	Ecologic Studio.

Microorganism: Micro -algal organisms (***chlorella vulgaris***).

Concept

The project 'HORTUS.PARIS' by ecologic studio is an interactive living environment installed at the Alive: New Design Frontiers exhibition in Paris. The design is inspired from the architectural element of the column; imagined as a living and responsive organism, by employing it as a photo bioreactor of microalgae, that connects floor to ceiling. The project hosts micro-algal organisms ***chlorella vulgaris*** and is fitted with ambient light, sensing technologies and a custom-designed virtual interface.

The flows of energy expressed in artificial light radiation, matter represented in biomass and carbon dioxide and information (tweets) are triggered during the four-month exhibition period. As algal organisms require carbon dioxide to grow, visitors contribute to the growth of the installation by blowing inside an air pump system inside the photo-bioreactors, as a result of biochemical reactions of algal growth, oxygen is released, after that the algae growth moves onto a filtering surface on the ground. [118]•.

Description

Image. [68, 69]. Left, the bioreactor column design of Alive exhibition showing connection between interior design elements, floor, ceiling and column. Right, suspended algal cultures containers with tubes of co_2 respiration to boost algal growth. [118]•. Ecologic Studio.

• [118]http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=71&idproj=127-2-2-2018

•Ibid

Image. [70, 71]. Left, the algae container unite plugged with respiration tubes to provide it with co_2 for algal growth. Right, the bottom part of the column design. [118]•. Ecologic Studio.

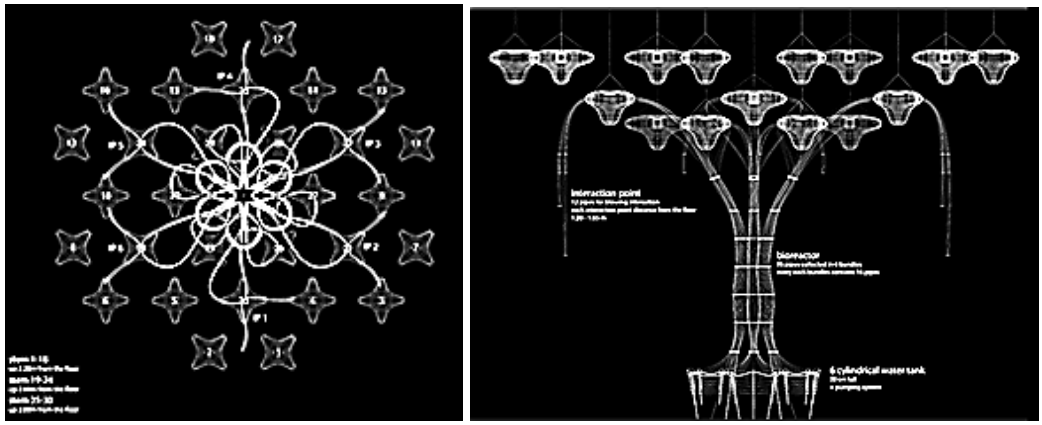

Figure. [20]. Left, Alive plan shows disruption of for star shaped unites that contain the algae and the arrangement of their connections of respirational tubes. Figure. [21]. Right, Alive section showing the tree shaped column. Ecologic Studio.

[2.4] Bioinformatics

Bioinformatics is conceptualizing biology in terms of molecules (in the sense of physical chemistry) and applying "informatics techniques" (derived from disciplines such as applied math, computer science and statistics) to understand and organize the information associated with these molecules, *on a large scale. Bioinformatics is a management information system for molecular biology.* [119]•.

The main goals of bioinformatics are as follows:

1. Bioinformatics organizes data in a way that allows researchers to access existing information and to submit new entries as they are produced.
2. Develop tools and resources that aid in the analysis of data.
3. Use these tools to analyze the data and interpret the results in a biologically meaningful manner.

Bioinformatics depends on its main sources of information resembled in ***raw DNA sequences (strings of the four base-letters comprising genes), protein sequences (comprising strings of 20 amino acid-letters), macromolecular structures, genome sequences, Gene expression, and metabolic pathways.***

The genome is an important element of information in bio informatics, the most important aspect of complete genomes is the distinction between coding regions and non-coding regions

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• **Ibid**

• **[119] N.M. Luscombe, et al., Review -What is bioinformatics? An introduction and overview, USA, Yearbook of Medical Informatics, 2001.**

which is considered junk' repetitive sequences making up the bulk of base sequences especially in eukaryotes. This to identify which regions to clone for desired function or behavior; thus bioinformatics play crucial role in employing the identification of these regions in specific cloning for DNA manipulation processes that could form a key role in adding desired traits in engineered bio materials or bio devices in architecture and design.

DNA manipulation techniques does not only depend on the cloning of a desired gene, it needs to be translated and expressed through key elements (proteins). That is why we need to measure the gene expression level. Gene expression level measurements are made under different environmental conditions; different stages of the cell cycle and different cell types in multi-cellular organisms. Other genomic-scale data used to test this expression level include ***biochemical information on metabolic pathways, regulatory networks, protein-protein interaction*** data, and systematic knockouts of individual genes to test the sustainability of an organism.

* Recently, bioinformatics application in architectural design was part of specialized biolearning processes mainly focusing on extracting organisms' molecular and genetic behavior and what they include of biochemical and biological reactions. Employing this aspect of bioinformatics in architecture and interior design is still an emerging research discipline due to the interdisciplinary nature of this field; and mainly focused on structural design than biosynthesized devices and materials.

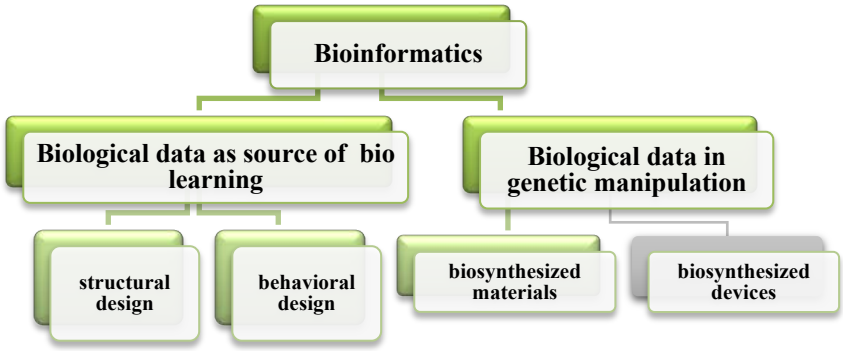

Diagram. [3]. Bioinformatics application in design process. By author.

[2.4.1] Bioinformatics in synthesizing materials and devices

Employing bioinformatics in architectural and interior design focuses on one important aspect, which is synthesizing materials. This is quite presented in **Oxman and Rosenberg,** (2007) definition of ***Material Based Design Computation (MBDC)***, defined as '*the process of computationally enabled form-finding, informed by material properties*'. The MBDC process makes use of computational modelling of materials with simulations of, e.g. finite element analysis being used to generate a form for manufacture. Employing 3D printers capable of printing in more than one material and varying the density of each material that gives the designer tight control over the structure and use of materials only where needed. [78]• . The key potential of this bio informatics based technique is to directly integrate those systems in design and manufacture. Which enable to embed computational design *in vivo* in the material itself.

Bioinformatics also plays role in synthetic biology as a synthetic biologist/designer uses a ***diverse library of biological devices to assemble complex pathways that function like***

• **[78] M.D. Robertson, et al., Architects of nature: growing buildings with bacterial biofilms, Microbial Biotechnology, 5, 10, pp.1157–1163, 2017.**

integrated circuits. The connection of these modules to each other and their integration into host cells allows the synthetic biologist/designer to extend or modify the behavior of cells in a programmatic fashion. Although independently operating engineered cells can perform tasks of varying complexity, more sophisticated coordinated tasks are achievable using populations of communicating cells, much like the case with computer networks. [53]•.

As understood from MBDC definition, Genetic programing enables computation to be embedded into the material elements themselves. [1]• . ***Proposing a tight coupling between computational simulation, material properties and the manufacture of material form. The design methods developed in the context of material ecologies opens new applications in creating biological materials through modifying forms; these applications would be manufactured directly by living cells***. [1]•. This cellular approach involves selecting and modifying the appropriate genes that the information would be held within them, accordingly the cells would structure themselves into a desired form, such that a scaffold is formed on which the mineral crystals can be patterned. [1]•.

On the other hand, manipulating biological agents directly through biological devices process inputs to produce outputs by regulating information flow, performing metabolic and biosynthetic functions, and binding with other devices and their environments. Biological devices represent sets of one or more biochemical reactions that occurs naturally indicated by the presence of biological agents including transcription, translation, protein phosphorylation, allosteric regulation, ligand/receptor binding, and enzymatic reactions. [53]•.

The combination of both bio engineered materials and devices was presented in the first synthetic biological devices that controlled transcription by modifying promoter sequences to bind novel transcriptional activators and repressors in prokaryotes and eukaryotes. Recent researches focused on non-transcriptional control, which depends on non-coding RNAs that can activate silence gene expression by regulating translation events in prokaryotes. [53]•.

Rational redesign based on mathematical modeling and ***directed evolution of devices*** would help matching biosynthetic modules so that they function properly together; this is based on the fact that a module is a compartmentalized set of devices with interconnected functions that performs complex tasks. Similarly, in the cell, modules are specific pathways, such as a metabolic pathway or a signal transduction pathway. [120]•.

For changing the global behavior of a system, rational mutations of devices (evolution) are particularly useful when the properties of these devices are fairly well known. *In order to generate devices that enable a module to fit specific required criteria, there are multiple methods including; modifying the kinetics of transcription and translation, operator binding, attraction, and binding cooperatively of transcription factors*. ***The requisite computational tools use abstractions of biochemical reactions to model devices and typically require the rate constants of those reactions***. Although this direct determination of rate constants in vivo is still

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• [53] E. Andrianantoandro, et al., Synthetic biology: new engineering rules for an emerging discipline, Molecular Systems Biology, 2006.
• [1] M.D. Robertson, et al., Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60. pp.28-39, 2015.
• Ibid
• Ibid
• [53] E. Andrianantoandro, et al., Synthetic biology: new engineering rules for an emerging discipline, Molecular Systems Biology, 2006.
• Ibid
• [120] S. Kalantari, et al., GROWMORPH: Bacteria Growth Algorithm and Design, Conference: Proceedings of the 22nd CAADRIA Conference. (CAADRIA) 2017, 479-488, 2017.

inaccurate, it could be overcome by the technique of parameter estimation in biological networks. [120]•.

Although computational models exhibits correlation with the observed characteristics of various biological morphologies, this relation is not completely proven. ***However, it has been proposed to design and program cells to reproduce behaviors that comply with patterns found in Turing equations through much simpler cellular circuits*****,** to approximate this correlation between cellular complexity and observed morphological patterns. One example of this approach conducted by ***Davies that has presented some initial experiments to program a Turing pattern to occur in cell culture based on the genetic manipulation of cells such that they produce promoter and inhibitor molecules.*** This was supposed to be achieved by altering the chemical gradients across a plate of connected cells, that would cause a gene responsible for producing a luminescent chemical to be turned on and off in a phased pattern. [120]•.

A research project conducted by Araya, et al. (2012) exhibits another manifestation example; this project explored novel methods of design and fabrication through the coupling of digital tools and technologies with living biological systems in a controlled environment to induce specific biological functions and material production processes. Another project by Derme, et al. (2016) ***similarly developed bio-fabrication and scaffolding techniques to control the 3D membranes and morphologies of bacterial cellulose, to propose an application in biomaterials, digital fabrication, and material-informed computational modeling.*** [120]•.

[2.4.2] Bioinformatics as a source of biolearning

Bio informatics exploits algorithmic technology in order to incorporate biological functional behavioral patterns into design and fabrication processes, or as an actual component in design and fabrication processes. [120]•.

One of the most manifesting areas of development in nature-learned morphogenesis includes projects that analyze natural growth and configuration/adaptation over time. ***Moving beyond static configurations to dynamism in final design solutions, based on advances in soft robotics and nano-engineering, these approaches aim for modeling the natural processes that achieve effective designs within their local environment.*** [120]•.

15.Project discipline	**Bio informatics, Biological data as source of biolearning .**	
Project ecological value	Optimized structure, environmental fitness.	
Project application	Architecture –urban design.	
Project information	Name	Grow Morph.
	Time	2017.
	Place	Computer-Aided Architectural Design Research in Asia (CAADRIA).
	Designer	Saleh Kalantari, Mohammed H. S. Tabari.

• Ibid
• Ibid
• Ibid
• Ibid
• Ibid

Microorganism Bacteria ***Acetobacters xylinus*** and ***Synechococcus elongatus.***

Concept

Grow Morph is a research project in biolearning architectural design; it analyzes the logic of bacterial cellular growth. This is achieved through examining various patterns of bacterial growth, including their parametric logic, their use **of responsive membranes and scaffolding structures**, and their environmental fitness, with the use of computational tools and methods to simulate and model biological processes. [120]•.

This project focuses on studying two strains of bacteria, ***Acetobacters xylinus*** and ***Synechococcus elongatus***, simulations were ran using programming language processing to model the parametric environment and morphology of the bacteria's growth. The resulting models were applied to design and fabrication processes. In advanced modeling stages, the bacterial growth structures were reconstructed in 3D digital space, and physical models were designed and fabricated to illustrate the potential of this bacteria-based architectural logic.

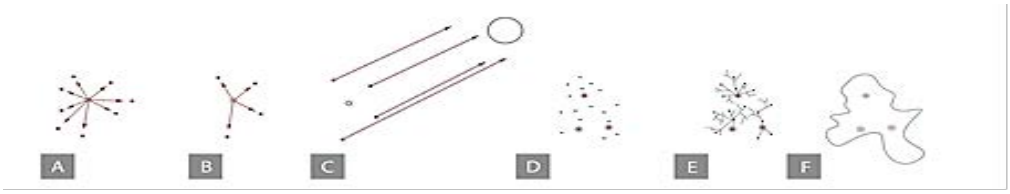


Figure. [22]. Bacterial growth behavior in the presence of light stimulation. [120]•.

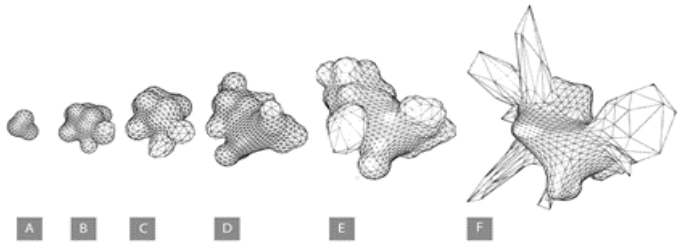


Figure. [23]. Mesh production based on the "marching cubes" algorithm: (a) cube numbering, and (b) triangulated cubes (Lorensen and Cline 1987). [120]•.

Description

This project analyzed the growth patterns of ***Acetobacters xylinus*** and ***Synechococcus elongatus*** as a potential basis for computer-mediated architectural assemblies. ***The resulting simulations were used to understand the structural morphogenesis of bacterial cellulose for developing applications of controlled-growth models, in addition to analyzing the model as a model for man-maid structure that achieves environmentally responsiveness***. Three versions of simulations were operated, incorporating different parametric conditions based on observed bacterial behavior.

To gain novel insights into nature's methods in dealing with physical dynamics, environmental parameters, and feedback within cell and tissue structures, code driven models which abstracts biological growth patterns are utilized as an essential tool.

Applications presents novel designs for responsive surfaces, new fabrication processes, and unique spatial structures in future architectural practices. [120]•.

• **Ibid**
• **Ibid**
• **Ibid**
• **Ibid**

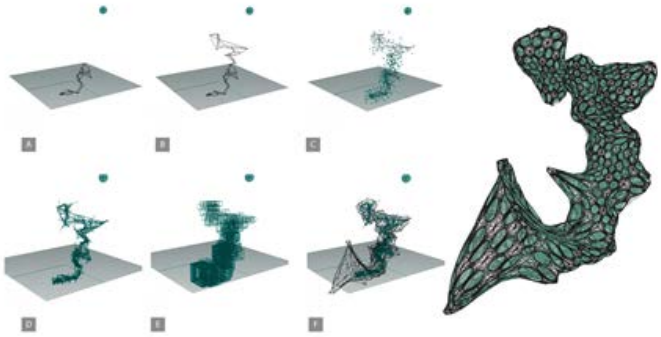


Figure. [24]. Simulation based on first parametric condition; The parameters of the model were adjusted to investigate structural forms resulting from various kinds of observed bacterial behavior. The first parametric condition was the base model. It simulates the behavior of *A. xylinum* bacteria when stimulated by a UV light source, each cell in the model is programmed to identify eight random nearby locations, eliminate six of the locations based on distance and light levels, and then extrude a mesh toward the remaining two points. [120]•.

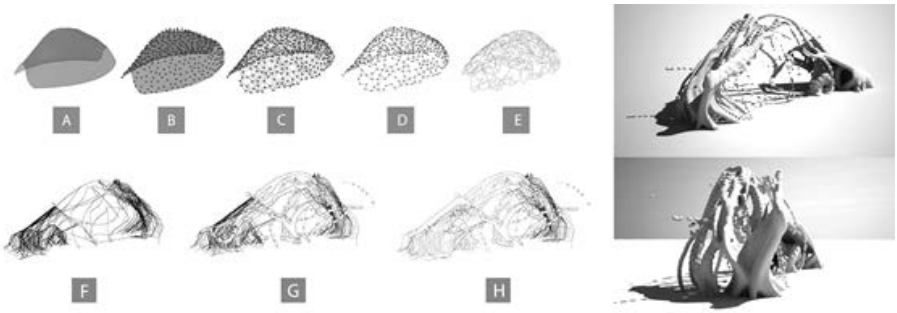


Figure. [25]. Simulation based on the second parametric condition; the growth pattern was adjusted to describe the more cohesive and aggressive behavior of *A. xylinum* in conditions of abundant nutrients, under the stimulation of a UV light source. Each cell selects eight random nearby locations. Then the algorithm defines a polygon based on those locations, and the cell selects six new points on the surface of the polygon, choosing the points on the surface that are closest to the origin cell. This process is repeated, defining a new, smaller polygon based on these six points, and then choosing the three nearest points on the new polygon. Of these three, the one with the lowest light intensity is discarded, and a mesh is extruded toward the other two points. [120]•.

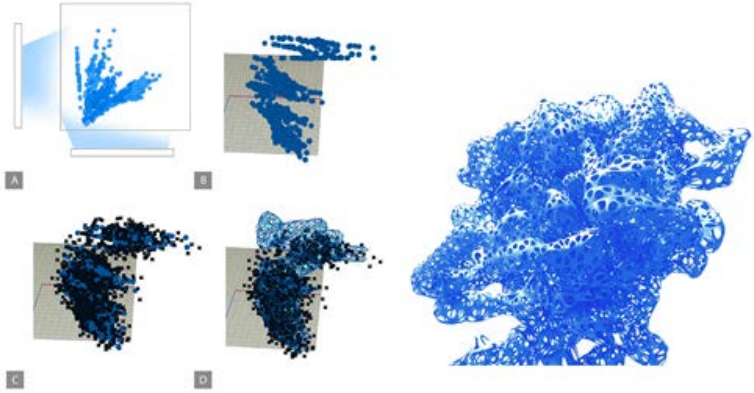


Figure. [26]. Simulation based on third parametric condition; based on the symbiotic combination of *Synechococcus elongatus* with *Acetobacter xylinum*. The notable feature of this addition is that the *S. elongatus* cells, forms hexagonal space groups that interact with their surroundings *A. xylinus* growth patterns, under the influence of a UV light source. [120]•.

• Ibid
• Ibid
• Ibid

16.Project discipline	**Embedding microorganisms in Architecture, Bio digital design**	
Project ecological value	Biodegradable –structural resistant material.	
Project application	Architecture.	
Project information	Name	Greensulate.
	Time	2009/2010.
	Place	Columbia University, GSAPP.
	Designer	Ecovative Design, Eduardo Mayoral González.

Microorganism Fungus –mushroom.

Concept

The project explores the possibilities in informing and re informing the geometry and morphology of a single module, which produces complex outcomes through aggregation processes. This module is made of Greensulate, which is a low-tech bio composite; and 100% compostable and biodegradable material made of mushroom roots (using mycelium as a resin), seed husks and agricultural waste. Greensulate performs like an Expanded Polystyrene in thermal insulation. It consumes about ten times less energy and produces eight times less CO_2 emissions, a Greensulate panel has better structural properties than a foam one, achieving a very low flammability and it resists longer than foam against fire. It does not emit toxic gases when it burns, it can be broken down into pieces and spread on a garden after used, being part of a biological metabolic active life cycle. [70]•.

Image. [72, 73]. Left, the greensulate material panel. Right, the material after genetic modification to add the luminescence property to the panel. [70]•.

The module has a relatively small size and can be mass-produced growing the material inside molds. Several populations of modules with a higher order of complexity can be tested through software simulations to define usable spaces according to the feedback obtained. It is possible to produce a set of 100% disposable biodegradable, compostable outcomes, such as ***3D usable structures, inhabitable spaces, insulating façades. This material can also be combined with other organic elements like branches, so that the outcome performs***

• **[70] E. M. González, et al., Ecovative Design-GROWING ARCHITECTURE THROUGH MYCELIUM AND AGRICULTURAL WASTE, AAR, Columbia University, GSAPP, 2010.**

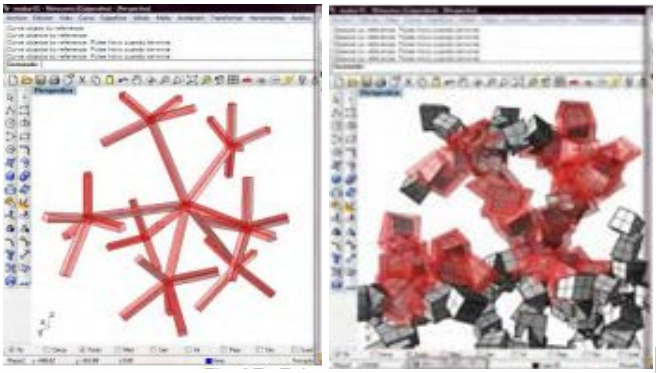
• **[70]**

structural features besides thermal insulating properties. Thus making it is possible to grow architecture instead of building it, or a mixture of the two. [70]•.

The application includes two approaches; ***a modular 3D usable structure conformed by an aggregation process, and the second one, an inhabitable growing dwelling.*** [70]•.

Description

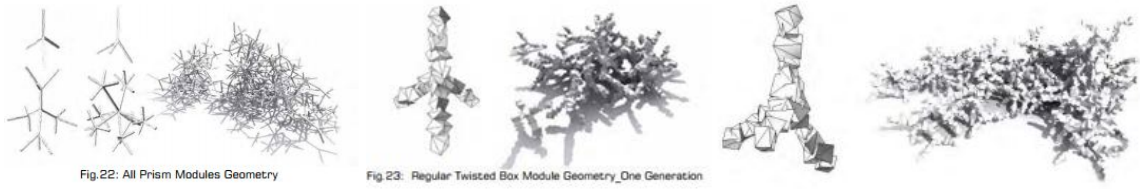
The module is provided with two different topologies that can be parametrically modified. These different topologies show two different possibilities to determine the shape and the size of the branches. One generates a square prism around each branch and controls the length and the width of the rectangle base. The other one generates twisted boxes along each branch and controls their size, their number, and the scale. The variations on the topology of the module determine the behavior of the whole outcome. [70]•.



[70]•.

Figure. [27]. Left, prism topology. Right, twisted box topology.

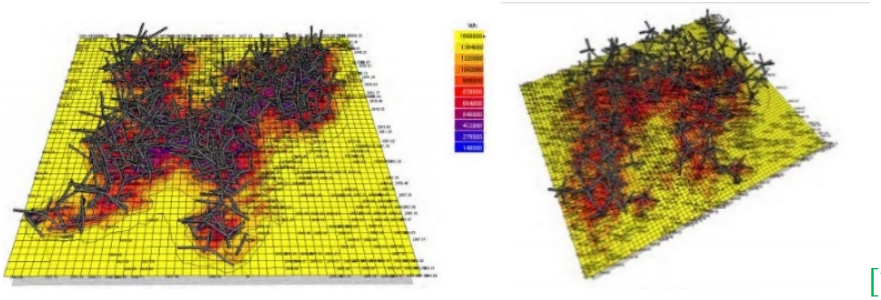
Modifying these parameters including; the variation of porosity, density and friction properties that determine the morphology of the overall structure, affects the shape and the size of the module, These variations establish different conditions for lighting, solar access, thermal insulation, accessibility, populations are tested running software simulations for solar access and lighting, and were re-informed according to the feedback obtained. [70]•.



[70]•.

Figure. [28, 29, 30]. three iterations of the two different topologies aggregation; left, prism module population, middle, the regular twisted box population, right, the irregular twisted box population.

• **Ibid**
• **Ibid**
• **Ibid**
• **[70]**
• **Ibid**
• **[70]**



[70]•.

Figure. [31, 32]. Left, one generations of solar insulation simulation of irregular prism topology population. Right, two generations of solar insulation of prism topology.

The solar access test shows that the one generation of the Irregular Prism model performs a very good solar insulation, reaching very low solar access values. In addition to homogeneous low solar access peaks. The two-generation model of the Irregular Prism does not achieve very low solar access values although it has a uniform distribution of insulation and average values. Very few low peaks are detected for this kind of geometry and the difference between them and the average is significant. [70]•.

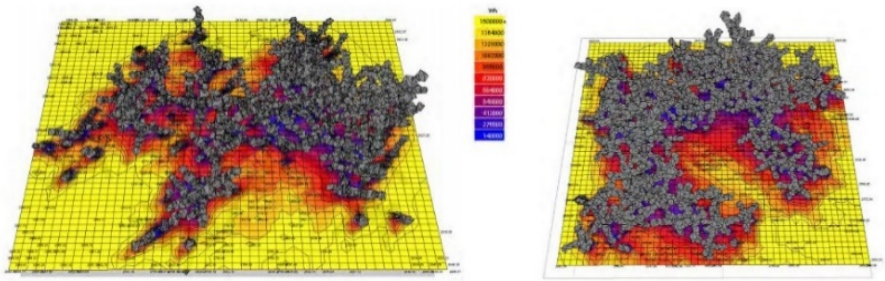


Figure. [33, 34]. Left, the simulation of solar insulation for the one generation of irregular twisted box topology model. Right, the simulation of the solar insulation for the two generations of irregular twisted box topology model, The Irregular Twisted Box One Generation model achieves the lowest solar access values distributed in a concentrated area. It also shows a medium-low range of average values surrounding the structure. It has very significant low peaks and there is a remarkable difference between them and the average values. The Irregular Twisted Box Two Generations model performs low values and shows a very well distributed insulation pattern, which makes it the best solution for solar insulation. [70]•.

Image. [74]. left to right, the 3D container model of the irregular prism and irregular twisted box, to be filled with the greensulate material seeds. Image. [75, 76]. Left, the central connection part of the irregular prism. Right, the central connection part of irregular twisted box (the joints).

• **[70] E. M. González, et al., Ecovative Design-GROWING ARCHITECTURE THROUGH MYCELIUM AND AGRICULTURAL WASTE, AAR, Columbia University, GSAPP, 2010.**

• **Ibid**

• **Ibid**

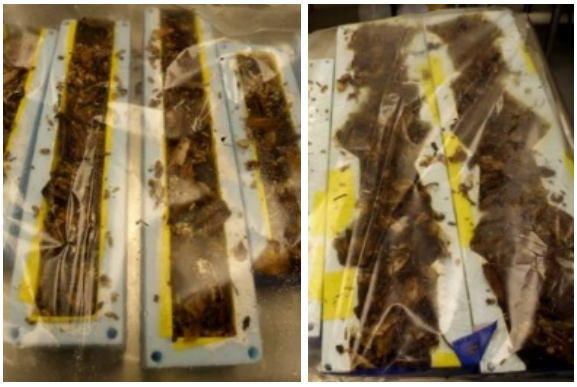


Image. [77, 78]. Left to right, the irregular prism and irregular twisted box branches filled with the greensulate seeds. [70]

In this project, the molds were prepared to be filled with Greensulate to achieve a slippery finishing layer. Then they were disinfected with hydrogen peroxide and filled with previously boiled agricultural waste. In final stage, mushroom seeds were spread on them and they were put inside bags in a moistly environment letting the mycelium grow to cement all the elements. Before the growing process was finished, the molds were assembled to grow together. [70]•.

Image. [79, 80]. Left, the twisted box topology module connected parts of greensulate, right, the irregular prism topology module connected parts made of greensulate. [70]•.

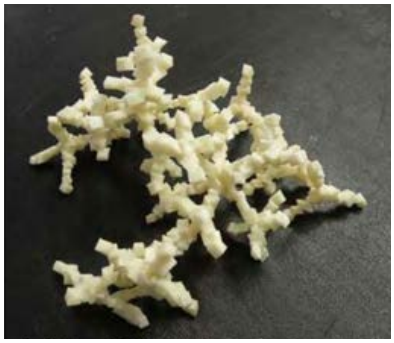


Image. [81]. The full population of twisted box topology model in 3D printed prototype. [70]•.

In this project, two different modules were fabricated 1:1 scale to test the possibilities of the proposed material and check its physical and mechanical behavior in reality adjusted to fit theses specific geometries. The first mold was prepared for the prism geometry and the second one for the twisted box. The molds for the modules were digitally designed and 3D printed for the central part and milled for the parts corresponding to the branches. [70]•.

• **Ibid**
• **Ibid**
• **Ibid**
• **Ibid**

These structures are proposed as public furniture pieces, devices to divide spaces, or supports for 3D-vertical gardens as plants can grow on them. It also could be embedded with genetically manipulated plants to glow or natural glowing mushrooms, the structure will emit certain amount of light in the dark. [70].

Figure. [35, 36]. Left, a visualization of proposal for application in urban design using the irregular prism module as a shading wall made from greensulate. Right, shows irregular prism green wall after growing plants on the greensulate material. [70].

[2.5] Symbiotic design

Symbiosis refers to the interrelationship between two or more forms of life, which are mutually beneficial or dependent on each other in a direct, indirect, visible or interpretive way.

These benefits or dependencies may be metaphorical, physical in nature or philosophical interpretations of how two systems within the environment depend on one another in order to survive. [121]•.

Symbiotic design is the next step to conceiving an architecture that provides veritably sustainable and ecologically conscious design elements, providing the opportunity for mutual reinforcement and a coupling between the urban and architecture space and the biological elements wither naturally and directly embedded in bio devices or bio synthesized or engineered. [121]•.

The symbiosis of various elements in the built environment allows for continuously changing dynamic balance between the two opposing elements, permitting sudden mutations and a living architecture cycles and growth as it responds and interacts with the world around it.

According to Kurokawa, (2006), future cities should be designed with the goal is to couple and reinforce architecture and urban space with the natural environment, fusing each into one homogenous entity. This implies symbiotic, mutually beneficial relationship between the opposing two elements. [121]•.

Moreover, as learned from biology, coupling occurs between connective elements only when there is an element of variety. Each “molecule” or architectural element acts as a catalyst for these reactions to occur, however, some elements may act only as an intermediate connecter to catalyze an interaction between two other components, not as the final reaction partner. Thus, the variety of elements increases the chance of potential reactions that may occur. These reactions would be subjective to who or what is interacting with each other, which can in turn

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• **[121] P. W. Murdoch, Mutually Reinforcing Design & Symbiosis, Architectural Design, Master of Architecture, thesis, 2012.**
• **Ibid**
• **Ibid**

form symbiotic, mutually benefiting relationships. [121] the pairing of two elements is dependent on both their position and formal qualities. The elements may be physically connected, or paired by each other's function. This proposed coupling can be formed between a pair of elements through different methods; visual, structural, functional and geometrical. [121].

[3] Conclusion

1. Employment of living organisms in interior design and architecture depends on multidisciplinary sciences of biotechnology, bio digital architecture, bio based materials, bio informatics and symbiotic design.
2. The embedding of microorganisms in interior design and architecture aims to employ these organisms' characteristics in achieving ecological balance through main aspects:
   - Environmental purification.
   - Ecological chemical balance by production of certain elements.
   - Food production.
   - Power production (lighting and electricity) as biocatalysts.
3. Microorganisms embedding in architectural, interior and furniture design could be categorized in two aspects:
   - Materials development: Bio **composite materials, Bio films, Biodegradable materials, genetically modified materials**.
   - Systems development: which imply direct embedding of the living microorganism to benefit from physiological processes performed by it:
     - **Photosynthesis**; o_2 production, co_2 reduction (bacteria, algae).
     - **Bio mineralization**; improve vegetation production (food production) (bacteria, fungi).
     - **Bioluminescence**; emitting light (chapter 2) (bacteria, fungi).
     - **Bio degradation**; wastes reduction, ecological balance. (Bacteria, fungi, algae).
     - **Growth activities**; biocatalysts in energy production through chemical reactions (chapter 3) (bacteria, fungi, algae).
4. Embedding microorganisms in architectural and interior design achieves direct morphogenesis through hybridizing living organism's natural physiological characteristics with materials and devices achieving ecological responsiveness, emergence and optimization in real time and through the design's living cycle, enabling for a living architecture and interior design.
5. Embedding microorganisms in architectural, and interior design elements includes three levels of emersion:
   - **Direct embedding**; through embedding the living organisms as a culture to maintain and grow the microorganism to exploit physiological processes performed by the organism.
   - **Bio symbiosis processing level;** by synthesizing natural symbiosis between two microorganisms in special environment (the design container) to exploit a certain symbiosis product.

- **Genetic processing**; through employing genetic modification to certain species of organisms to achieve certain characteristics employed in building materials.

6. Bio digital design is an interdisciplinary design science based on employing computational design and fabrication with biological and genetic sciences to produce living architecture with natural biological traits through embedding living organisms, microorganisms in two fields:
    - Material synthesizing; through embedding of microorganisms or genetic manipulation in order to achieve special properties in materials.
    - Systems: preform specific tasks through employing biological processes (e.g. bioreactors).
7. Bio informatics is the main tool that enables the processes of synthetic biology through three branches:
    - Biological data organization; through providing existing molecular biology and genetic data; as a tool to add more entities; and by providing a tool to analyze and process these data in order to synthesize new systems.
    - Synthetic biology tool for synthesizing new materials that have specific physiological traits through the aid of genetic manipulation and the notion of **DNA sequences**, **protein sequences**, **macromolecular structures**, **genome sequences, Gene expression**, Metabolic pathways, **Raw DNA sequences**, **protein sequences.**
    - Synthetic biology tool for synthesizing new devices that mimic the natural biochemical and physical reactions to develop cellular circuits that perform specific tasks.
8. Bioinformatics is a highly interconnected discipline with design and it could be employed through two main aspects:
    - Biolearning as a source of inspiration of forms and behaviors: by employing and analyzing biological data on molecular and genetic level in their own physiological traits in natural biological processes. In addition, their response to various stimuli including environmental simulation and behavioral simulation and use the feedback as patterns in form or behavior design (interactive or responsive ecological systems).
    - Biosynthesizing of new materials and devices that will act as a part of natural ecological system.

CHAPTER 2

Non-Pathogenic Microorganisms as Biocatalysts in Lighting Production

Image. [82]. Visualization of Natural Bioluminescence system used as lighting unites in urban design. Design form is based on microbial behavior using biased random walk model. Bioluminescent strain *Alivibrio fischeri*. By the author.

Introduction

Humankind has always made trivial use of bioluminescent organisms [122]•, due to the difficulty in handling and controlling these organisms. Recently, genetic engineering has managed to harness their advantages in many fields. Trans-genesis techniques have enabled extraction of bioluminescent proteins like GFP (Green Fluorescent Protein) from living organisms and merging them in DNA of other organisms. Originally, these bioluminescence recombinant DNA techniques have been harnessed to identify cells or favor image display. [123]•. Recently, biotechnology guides applications of bioluminescence in variant fields such as: applications for zero-energy lighting systems.[124]•. in this chapter the author highlights the bioluminescence phenomenon in nature the genetic basis of naturally bioluminescent creatures as well as artificial genetic manipulation potentials that enabled bioluminescence in non-naturally bioluminescent organisms, this chapter will also exhibit case studies where bioluminescence were employed as an ecological lighting source in architecture and interior design.

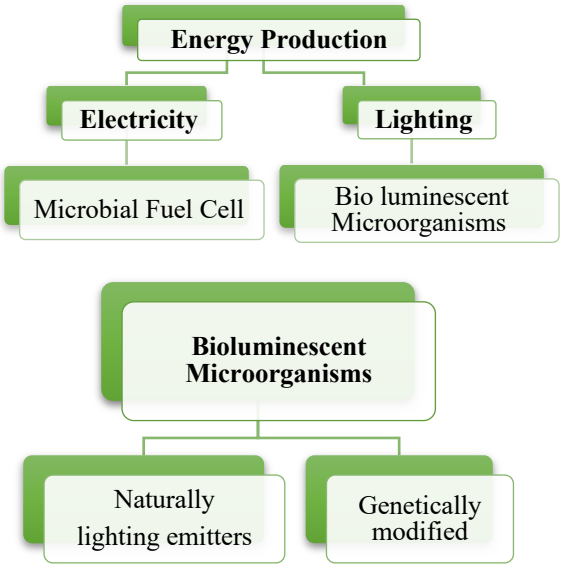


Diagram. [4]. Renewable bio energy production from microbes. By author.

[1] Lighting by Bioluminescent Microorganisms

[1.1] Bioluminescence

Bioluminescence is a Greek word (“Bios” for living and Latin word “lumen” for light) which literally means a living light. This type of chemiluminescent phenomenon have been monitored in many types of organisms. Luminescent species are found among marine and terrestrial bacteria, annelids or segmented worms (e.g., fire worms), beetles (e.g. Fireflies, click beetles, railroad worms), algae (e.g., dinoflagellates), crustaceans (e.g., ostracod), mollusks(e.g. Squid, clams), coelenterates (e.g., jellyfish), bony fish (e.g., flashlight fish), and cartilaginous fish (e.g., sharks). [125]•.

• [122] T. Wilson, J. W. Hastings, Bioluminescence, Annual Review of Cell and Developmental Biology, 14, 197-230, 1998.

•[123] M. Zimmer, Glowing genes: A revolution in biotechnology, Prometheus Books, Amherst New York, 2005.

•[124] I. G. Diez, et al., Design of prototype biosynthetic material for use as construction material, Seville University.

• [125] G. Parashar, et al, Isolation and Characterisation of Bioluminescent Bacteria, Department of Pharmaceutics, RajarshiShahu College of Pharmacy& Research, Tathwade Pune. 33. IJSART. Volume 2 Issue 5, 2016.

Scientifically, Bioluminescence is identified as the emission of visible light by biological systems, which arises from enzyme-catalyzed reaction (luciferase) of molecular oxygen with a substrate (luciferin). [126]•. ***In the Bioluminescence process, the chemical energy of an exergonic reaction is converted into light energy via a mechanism in which one of the components of the system is raised to an electronically excited state. The process of light emission could be continuous (bacteria, fungi) or may occur in brief flashes (firefly and dinoflagellates) and it may be intracellular (bacteria, fungi, firefly) or be extra-cellular with the chemical compounds in the medium in which the reaction occurs.*** [127]•.

Biochemically, all known luciferases are oxygenases that oxidize their corresponding substrates (generically named luciferin, and literally meaning "light-bearing" molecules) in the presence of oxygen. The spectra properties of this bioluminescent emission are dependent on various parameters, such as;

- Luciferase/luciferin structures,
- Organism habitat,
- Optical biological filters,
- Accessory lumipores [e.g. green fluorescent protein (GFP) and yellow fluorescent protein (YFP)]. That indicates that the different emitted colors can be attributed to the different enzyme conformations under in vivo and in vitro conditions [128]•.

The color of the bioluminescent signal is strongly dependent on an organism's habitat. Thus, deep-sea species have blue emissions (450–490 nm), the bioluminescence of coastal marine species is green (490–520 nm), whereas terrestrial and fresh water species are red-shifted to 550–580 nm [129•; 130•].

In higher organisms such as fish and squids, evidence suggests that they emit their own light or have an ***endosymbiosis with luminous organisms to perform a phenomenon known as counter illumination***. This strategy is performed by the squid ***"Euprymna scolopes"***, which uses luminous bacteria contained in his gut to erase the shadow that is cast by the moon in the oceans bottom that is detected by predator when the squid swims at night [131]•.

The first studies with luciferase were conducted to analyze the reaction mechanism with the luciferin [132]•. The luciferase used in this study was monomeric and was isolated from firefly by William McElroy. [133•; 134•]. When a physical stimuli or stress is perceived by the eukaryotic cell a sudden drop in pH occurs that detaches a protein called luciferase binding protein (LBP), this way the Dinoflagellate protein is released to perform the reaction. [135•; 136•].

•[126] H. J. Weitz, Naturally bioluminescent fungi, Mycologist, Volume 18, Cambridge University Pres, UK, 2004.

•[127] S. C. Sabharwal, et al., A new bioluminescent fungal system J. Biosci, Vol.5, Number 1, pp. 53–62, 1983.

• [128] J. G .Morin, J .Hastings, Energy transfer in a bioluminescent system. J Cell Physiol 77 (3):313–318, 1971.

• [129] K. Jia, R. E. Ionescu, Measurement of Bacterial Bioluminescence Intensity and Spectrum: Current Physical Techniques and Principles, Adv Biochem Eng Biotechnol, 154: 19–45, Springer International Publishing Switzerland, 2015.

•[130] J. Hastings, J. Morin, Bioluminescence. In: Prosser CL (ed) Neural and integrative animal physiology. Wiley, New York, NY. pp 131–170, 1991.

•[131] A. K. Campbell, Living light: biochemistry, function and biomedical applications, Essays Biochem, 24:41-76, 1989.

•[132] W. D. McElroy, M. DeLuca, Firefly luminescence, In Chemi- and Bioluminescence, ed. JG Burr. New York: Dekker, pp. 387–99, 1985.

•[133] W. D. McElroy, H. H. Seliger, Origin and evolution of bioluminescence. In Horizons in Biochemistry, ed. MKasha & B Pullman. New York: Academic, pp. 91–101, 1962.

•[134] V. C.Bode, J. W. Hastings. The purification and properties of the bioluminescent system in Gonyaulax polyedra. Arch. Biochem. Biophys. 103:488. 4, 1963.

•[135] J. B. Buck, et al., Control of flashing in fireflies, III. Peripheral excitation, Biol. Bull. 125:234, 1963.

•[136] J. Malave-Orengo, et al., Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico, Microbial Biotechnology and Bioprospecting laboratory, University of Puerto Rico, Puerto Rico.

Accordingly, until now there are five basic luciferin-luciferase systems: ***bacterial luciferin, dinoflagellate luciferin, vargula luciferin, coelenterazine and fire fly luciferin,*** which have been found to be similar to the luciferin isolated from ***Mycena citricolor*** cultivated mycelium. [137]•.

The function of bioluminescence may vary from one organism to the other, such as for defense against predators, for predation or for communication with their mates. [138•; 136]•. ***The explanation of luciferase genes regulation permitted the discovery of intercellular communication among bacteria. This, in turn, has led to a better understanding of bacterial pathogenesis and the associations of microorganisms in the environment.*** [139]•.

[1.2] Bioluminescence in Bacteria

Luminous bacteria are the most widely distributed light emitting organisms with the majority existing in seawater and the rest living in the terrestrial or fresh water environment, while most species of luminescent bacteria are capable of living free, the majority are found in nature associated in ***symbiosis with other organisms***. [140]•.

Each species of luminous bacteria differs in a number of properties, including the specific growing conditions (nutritional requirements, and growth temperature) as well as, the reaction kinetics of the luciferase involved in light generation; ***however all luminous bacteria are rod shaped, gram-negative microorganisms with flagella facilitating motion***. Luminous bacteria are also facultative anaerobes capable of growth when the supply of molecular oxygen is limited. Bacterial bioluminescence occurs naturally in eleven bacterial species from four genera ***(Vibrio, Photobacterium, Shewanella, and Photorhabdus).***

The DNA sequences coding the proteins in the luminescent system are termed the ***"Lux genes"*** , bacterial luciferase is a heterodimer, composed of two different polypeptides, designated Alpha, and Beta and encoded the ***Lux A and the Lux B Genes***, respectively, the active site is located within the subunit. [140]•.

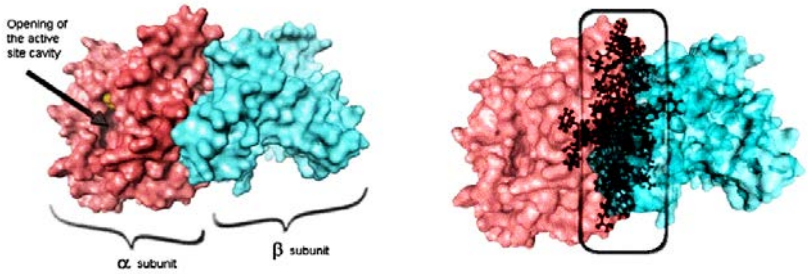


Figure. [37, 38]. Left, bacterial luciferase structure. Right, the internetwork of extensive subunit interactions, highlighted in the rectangular box, involving ionic attractions, hydrogen bonds and hydrophobic contacts, that is responsible for assembly of functional bacterial luciferase. [140]•.

•[137] E. M. Ilondu, A. A. Okiti, Bioluminescence in Mushroom and its Application Potentials, Nigerian Journal of Science and Environment, Vol. 14, Nigeria-p134, 2016.

• [138] J. W. Hastings, et al., Biochemistry and physiology of bioluminescent bacteria, Ado. Microbiot Physiol. 26, 235-291, 1985.

• Ibid

•[139] V. Nunes-Halldorson, N. Duran, Bioluminescent Bacteria: *LUX* Genes as Environmental Biosensors, Brazil, 2USDA-ARS US Water Conservation Laboratory, Phoenix, U.S.A.

• [140] L. Y. C. Len, E. Meighen, Bacterial bioluminescence, biochemistry and molecular biology, progen Biotech inc Richmond, Canada, 20-3-2018.

• Ibid

• Ibid

In the absence of Beta subunit, the Alpha subunit alone functions inefficiently, with a poor light yield, as the crystal structure of luciferase reveals extensive interactions and complex binding patterns between several chains and backbone amides of alpha and beta subunits.

Generally, this bacterial bioluminescence reaction of **luciferase-catalyzed bio reaction involves three substrates: oxygen, reduced riboflavin 5'-phosphate ($FMNH_2$), and long-chain aliphatic aldehyde**. The excess energy which is liberated from the oxidation of $FMNH_2$ and aldehyde associated with the reduction of molecular oxygen released as blue-green (490 nm) light. This process gives bacteria an important ecological role. [140•; 141•; 142•].

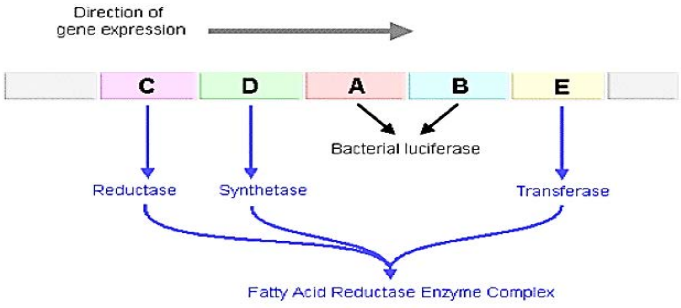
Equation: [1] The net chemical equation of the bacterial luciferase catalyzed reaction

FMNH2 + RCHO + O2 ----> FMN + RCOOH + H2O + light (490nm). [140]•.

The characteristic color of the emitted light in bioluminescence activity indicates the energy level of the photon that was produced when the excited electron on the Flavin chromophore returns to the ground state. Researchers have discovered that Flavin analogs with exchanged atoms in the chromophore moiety resulted in different luciferase emission colors. [140]•.

As mentioned previously, the presence of luminous proteins affect the color of the emitted light, some luminous bacteria carry florescent proteins to modulate the emission color, distinguishing themselves from other strains. [140]•.

A constant light emission in luminous bacteria must be maintained by several different enzymes, continuously generating the substrates for the bacterial luciferase for bioluminescence reaction. Those enzymes that replenish the aldehyde substrate are coded on the ***lux operon***; in particular the fatty acid **reductase**, a multi enzyme complex whose lux genes (Lux C, Lux D, and Lux E) and can be recycled after each reaction. The three proteins that form the enzymatic complex are **reductase, synthetase and transferase** that were identified through radioactive labeling, immediately beside the lux A and Lux B genes of luciferase.



[140]•

Figure. [39]. Arrangement of Lux CDABE open reading frames.

Luminous proteins involved in the reaction are only present in certain species of bacteria. For example, **lumazine and yellow fluorescent protein (YFP)** are two different proteins present in ***Photobacterium, phosphoreum*** **and in some strains** ***Vibrio fisheri*** respectively. Both are called

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• **[140] L. Y. C. Len, E. Meighen, Bacterial bioluminescence, biochemistry and molecular biology, progen Biotech inc Richmond. Canada, 20-3-2018.**

• **[141] C. Miyamoto, et al., Comparison of the lux systems in Vibrio harveyi and Vibrio fischeri. J. Biolumin. Chemilumin. 3:193-199, 1989.**

•**[142] C. Miyamoto, et al., Transcriptional regulation of lux genes transferred into Vibrio harveyi. J. Bacteriol. 172:2046-2054, 1990.**

• **Ibid**

• **Ibid**

• **Ibid**

• **Ibid**

accessory proteins because they are not crucial for the reaction to take place. The first one (lumazine) shifts the color of light to shorter wavelengths, therefore increasing the energy of the emission. The second protein shifts the light produced to such a degree that the color changes completely to yellow (540nm) [143•; 136•].

It is known that the emission spectrum of natural bacterial bioluminescence has a wide peak, ranging from 400 to 650 nm with a maximum absorption at 490 nm. However, bacterial bioluminescent color can present fluctuations. For instance, the bacterial bioluminescent peak can be Red-shifted to 530 nm, or Blue-shifted to 470 nm. [144]•. The majority of bacteria cells emit ***blue-green light*** with a single peak located around 490 nm, however, experimentally, two bioluminescent peaks have been observed using a sensitive photodetector. [145]•. ***The first peak appeared at 490 nm when detected by a conventional spectrophotometer based on photomultipliers. The second peak was visualized in the yellow range of 585–595 nm when using a more sensitive Raman CCD camera***. [146]•. This changing of read peaks values according to the used device inspires the manipulation of bioluminescent bacterial agents embedded in interior design elements for lighting purposes.

Thouand et al. [145]• claimed that the second peak in the yellow range could be attributed to the presence of an accessory emitter protein inside bacterial cells and/or the auto fluorescence of the luciferase enzyme. "The YFP", was found to be responsible for the appearance of a second peak and was temperature sensitive (+4 °C) both in vivo and in vitro, with changes in the color of bioluminescent emissions and a reaction rate that increased up to tenfold. [147•; 129•].

Another method to affect the bacterial lighting spectrum is by using **random mutation** or **site-directed mutagenesis.** [148•; 149•]. However, the bacterial activity was greatly reduced and only a small shift of bioluminescent spectrum was obtained by these methods. Thus, under in vivo and in vitro conditions, bacteria bioluminescence can be affected by the formation of a non-covalent complex between luciferase and specific fluorescent proteins (e.g. lumazine protein from ***Photobacterium phosphoreum.*** [150]•, yellow and blue fluorescent proteins from ***Alivibrio fischeri*** strain *Y1*). [151•; 152•].

•[143] T. O. Baldwin, M. M. Ziegler, The biochemistry and molecular biology of bacterial bioluminescence. Chem. Biochem. Flavoenzymes 3, 467-524, 1992.

•[136] J. Malave-Orengo, et al., Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico, Microbial Biotechnology and Bioprospecting laboratory, University of Puerto Rico, Puerto Rico.

• [144] G. Mitchell, J. W. Hastings, The effect of flavin isomers and analogues upon the color of bacterial bioluminescence, J Biol Chem 244(10):2572–2576, 1969.

•[145] G. Thouand, et al., Comparison of the spectral emission of lux recombinant and bioluminescent marine bacteria. Luminescence 18(3):145–155, 2003.

•[146] E. G. Ruby, K. H. Nealson, A luminous bacterium that emits yellow light, Science 196 (4288):432–434, 1977.

•Ibid

•[147] J. Hastings, Chemistries and colors of bioluminescent reactions: a review, Gene 173 (1):5–11, 1996.

•[129] K. Jia, R. E. Ionescu, Measurement of Bacterial Bioluminescence Intensity and Spectrum: Current Physical Techniques and Principles, Adv Biochem Eng Biotechnol, 154: 19–45, Springer International Publishing Switzerland, 2015.

•[148] T. W Cline, J. W Hastings, Mutated luciferases with altered bioluminescence emission spectra, J Biol Chem 249(14):4668–4669, 1974.

•[149] L.Y Lin, et al., Changes in the kinetics and emission spectrum on mutation of the chromophore-binding platform in Vibrio harveyi luciferase, Biochemistry 43(11):3183–3194, 2004.

• [150] J. Lee, et al., Bioluminescence spectral and fluorescence dynamics study of the interaction of lumazine protein with the intermediates of bacterial luciferase bioluminescence, Biochemistry 28(10):4263–4271, 1989.

• [151] H. Karatani, J. Hastings, Two active forms of the accessory yellow fluorescence protein of the luminous bacterium *vibrio fischeri* strain Y1. J Photochem Photobiol B: Biol 18 (2):227–232, 1993.

• [152] H. Karatani, et al., A blue fluorescent protein from a yellow-emitting luminous bacterium, Photochem Photobiol 55(2):293–299, 1992.

Due to the limited formation of luciferase-bacterial fluorescent protein complex and *its non-covalent nature* [153]•, *the bioluminescent spectrum is very sensitive to external perturbation factors, such as* ***temperature and concentration***. [154]•. The bioluminescent intensity reflexes the overall health of the microorganisms and the bioluminescence reaction with reflex reactions sensitive to a wide variety of toxic substances. Some bacterial strains are more persistent in terms of bioluminescence activity such as marine bacteria that have the ability to survive at very low temperature and high salinity. Such as ***psychrophiles*** and ***halophiles*** respectively. Marine bacteria are also characterized by their pressure tolerance, especially those at depths. [155•; 156•].

[1.2.1] Bacterial Strains that exhibits bioluminescence

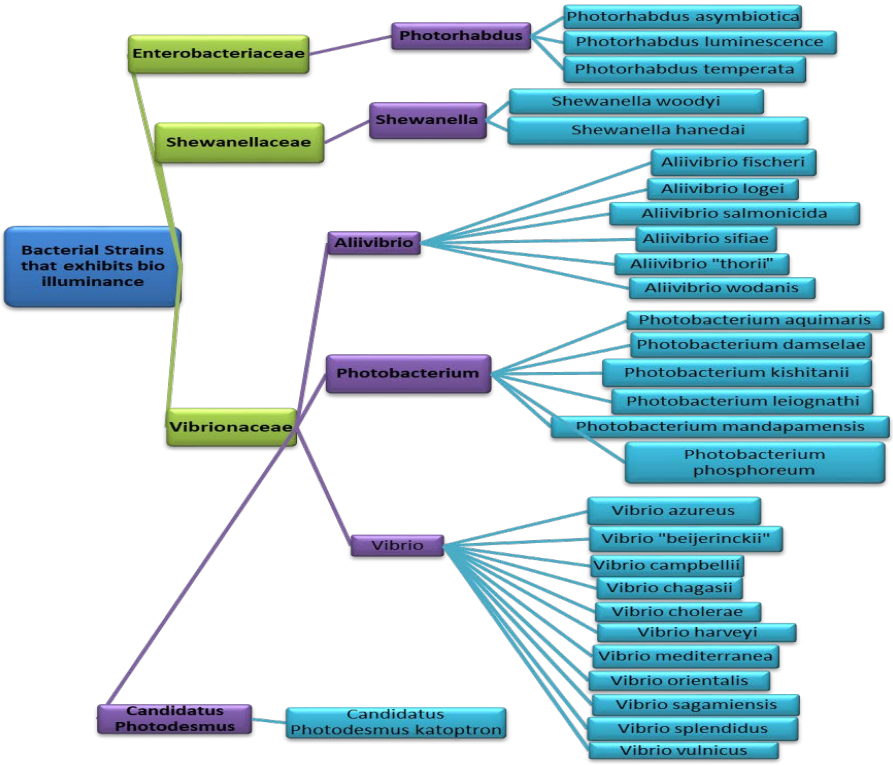

Diagram. [5]. Naturally bioluminescent strains in Bacteria. By author.

[1.2.2] Stimulating bioluminescence in bacteria

- **The Optimization of Bacterial Culture Medium**: numerous researchers have found that bacteria cultivated in a medium with acetate exhibited the highest sensitivity. [157]•. Bioluminescent substrate should have strong adhesion ability for the bacterial cells, have no chemical interaction with the target compound(s), and be transparent and biocompatible.

• **[153] V. N. Petushkov, et al., Interaction of photobacterium leiognathi and *vibrio fischeri* Y1 luciferases with fluorescent (antenna) proteins: bioluminecence effects of the aliphatic additive, Biochemistry 35(37):12086–12093, 1996.**

• **[154] D. Ke, S. C. Tu, Activities, kinetics and emission spectra of bacterial luciferase-fluorescent protein fusion enzymes, Photochem Photobiol 87(6):1346–1353, 2011.**

• **[155] A. Eberhard, et al., Structural identification of autoinducer of Photobacterium fischer*i* luciferase, *J.Biochemistry*, 20, 2444-2449, 1981.**

• **[156] M. Kannahi, S. Sivasankari, Isolation and Identification of Bioluminescent Bacteria from Marine Water at Nagapattinam Sea Shore Area, *International Journal of Pharmaceutical Sciences Review and Research*, 26(2), Article No. 59, Pages: 346-351, 2014.**

•**[157] T. Charrier, et al., A multi-channel bioluminescent bacterial biosensor for the on-line detection of metals and toxicity. Part I: design and optimization of bioluminescent bacterial strains, Anal Bioanal Chem 400(4):1051–1060, 2011.**

- **Quorum sensing**

In order for the light emission to occur, luminous bacteria have to grow in a confined, nutrition rich environment, as bioluminescence in bacteria can be regulated through a phenomenon known as auto induction. ***Auto induction or quorum sensing*** was first discovered in ***Vibrio fischeri: it is cell-to-cell communication that ties gene expression to bacterial cell density. Quorum sensing involves the self-production of a diffusible pheromone called an auto inducer (AI), which serves as an extra cellular signal molecule that accumulates in the medium and evokes a characteristic response from cells. In bioluminescence, once the concentration of the AI reaches a specific threshold (above 10^7 cells mL^{-1}), it triggers the energetically costly synthesis of luciferase and other enzymes involved in luminescence***. Thus, by sensing the level of AI, the cells are able to estimate their density and ensure that the luminescent product will be sufficiently high to cause an impact in the environment. [139•; 140•].

Many bacteria regulate their set of lux genes by the mechanism of quorum sensing. As the production of the luminose enzyme increases, the light generation increases accordingly. As well known; ***general metabolism, operon expression, bacterial growth, cultural condition affects the luminescence activity***; from which it is articulated that controlling bioluminescent organisms to emit the desired quantum of light is a very much sensitive process to many parameters including; e.g. physical, chemical, radiological, etc.[158]•.

- **Genetic manipulation: (**Regulation of the *lux* Operon).

The conversion of non-luminous bacterium such as *E.coli* to a light emitter requires only ***the insertion of the lux CDABE genes. Encoding the bacterial luciferase and the fatty acid reductase complex into the cell indicates that $FMNH_2$ is readily provided from the electron transport chain in the bacteria. Although the biosynthesis of riboflavin and FMN in luminesce bacteria is carried out in multiple steps by enzymes that are not encoded by the lux gene system, these enzymes are generally present in the majority of bacterial strains.*** [140]•.

The characterization of the *lux* operon was determined through the cloning of a 9 kb DNA fragment obtained from ***V.fisheri*** that was transferred into *E. coli*. Successful expression was exhibited when *E. coli* was able to emit light. ***Mutational analysis showed five crucial genes required for the reaction (lux AB and lux CDE). The genetic organization was determined to be arranged as an operon, a diverging with a left and right side containing two different promoters that are transcribed in opposite directions.*** [159•; 160•].

The first gene***, lux I at the start of the right side of the operon produces a small peptide that works as an auto inductor that triggers the synthesis of the structural genes***, *lux CDABE*, in that same order as shown in figure. [40].

• [139] V. Nunes-Halldorson, N. Duran, Bioluminescent Bacteria: *LUX* Genes as Environmental Biosensors, Brazil, 2USDA-ARS US Water Conservation Laboratory, Phoenix, U.S.A.

•[140] L. Y. C. Len, E. Meighen, Bacterial bioluminescence, biochemistry and molecular biology, progen Biotech inc Richmond. Canada, 2018.

•[158] V. S. Kulkarni, B. S. Kulkarni, Isolation of Bioluminescent Bacteria and Their Application in Toxicity Testing of Chromium in Water, International Journal of current microbiology & applied science, 4(10)23-32, 2015.

• Op.cit.

• [159] P. V. Dunlap, E. P. Greenberg, Control of Vibrio fischeri lux gene transcription by a cyclic AMP receptor protein LuxR protein regulatory circuit. J. Bacteriol, 170:4040-4046, 1988.

• [160] P. V. Dunlap, E. P. Greenberg. Control of *Vibrio fischeri* luminescence gene expression in *Escherichia coli* by cyclic AMP and cyclic AMP receptor protein, J. Bacteriol, 164:45-50, 1985.

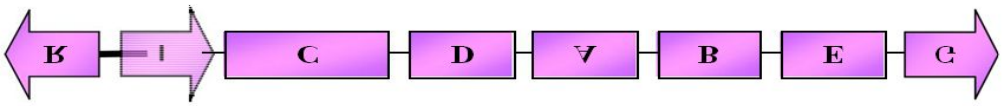

Figure. [40]. Bioluminescence in bacteria is produced by the expression of *lux* genes. The *Vibrio fischeri lux* operon is organized in two different arrangements: from *lux I* to *lux G* are expressed downstream and opposite to *lux R* [161•; 140•].

To the left side of the operon a gene is present that codes for another regulatory protein, a repressor with dual function in all bioluminescent strains. This repressor has one site that interacts with the DNA and a second site that binds to the small peptide (lux I). When attached to the auto inducer, it forms a complex that promotes transcriptional activation in the right border through positive regulation. On the other hand, when the auto inducer concentration is not high enough, the repressor continues to be produced. Therefore, inhibiting the transcription of structural genes by binding to operator located in the control region. [159•; 136•].

The ongoing research in lux-based systems has been pushed from ***the original prokaryotic bacterial lux CDABE gene case further to eukaryotic cells. The first breakthrough of expressing lux genes in eukaryotic hosts used autonomous bioluminescence from Saccharomyces cerevisiae yeast*** [162]•, since this discovery, the expression of bacterial lux genes in eukaryotic cells has been under investigation. The development of a robust bioassay based on luminescent yeast, with the potential for lux gene expression in human cell lines [163•; 164•], has been reported. Thus, many bacterial cells can be genetically engineered with lux reporter genes to induce the production of light, dramatically enlarging the spectrum of in vivo bacterial investigations with this non-aggressive and easy to perform technique [129]•.

[1.2.3] Bacterial bioluminescence ecological value

Knowledge of the *lux* gene organization has stimulated the use of bioluminescence genes for the development of ***whole cell biosensors*** that have a broad range of environmental applications. These applications include construction of biosensors for detection of contaminants, and measurement of pollutant toxicity. [139]•.

An example is the Microtox system that has been used to assess the impact of chemicals in the environment. This is an available toxicity bioassay that uses the naturally bioluminescent bacteria, ***Photobacterium phosphoreum***, and recently, ***Vibrio fischeri***. Light emission in ***P. phosphoreum*** depends on functional metabolism. Consequently, toxic agents that affect the metabolism or compromise bacterial viability cause a reduction in light output that is proportional to the toxicity of

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• [161] J. Engebrecht, et al., Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri. Cell 32:773-781, 1983.
•[140] L. Y. C. Len, E. Meighen, Bacterial bioluminescence, biochemistry and molecular biology, progen Biotech inc Richmond, Canada, , 2018.
•[159] P. V. Dunlap, E. P. Greenberg, Control of Vibrio fischeri lux gene transcription by a cyclic AMP receptor protein LuxR protein regulatory circuit. J. Bacteriol. 170:4040-4046, 1988.
• [136] J. Malave-Orengo, et al., Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico.
• [162] R. K. Gupta, et al., Expression of the Photorhabdus luminescens lux genes (lux A, B, C, D, and E) in Saccharomyces cerevisiae, FEMS Yeast Res 4(3):305–313, 2003.
•[163] D. Close, et al., The evolution of the bacterial luciferase gene cassette (lux) as a real-time bio reporter, Sensors 12 (1):732–752, 2012.
• [164] D. M. Close, et al., Autonomous bioluminescent expression of the bacterial luciferase gene cassette (lux) in a mammalian cell line, PLoS One 5(8): e1244, 2010.
•[129] K. Jia, R. E. Ionescu, Measurement of Bacterial Bioluminescence Intensity and Spectrum: Current Physical Techniques and Principles, Adv Biochem Eng Biotechnol, 154: 19–45, Springer International Publishing Switzerland, 2015.
• [139] V. Nunes-Halldorson, N. Duran, Bioluminescent Bacteria: *LUX* Genes as Environmental Biosensors, Brazil, 2USDA-ARS US Water Conservation Laboratory, Phoenix, U.S.A.

the sample. The results are expressed as effective concentration (EC50) values at which there is a 50% decrease in light emission. [139]•.

[1.2.4] Bacterial luminescence alternatives

The inherent limitation of bacterial luciferase enzymes has prompted attention to other bioluminescence systems such as ***the green fluorescent protein (GFP)*** of the jellyfish ***Aequorea victoria***. Some advantages of the GFP fluorescent protein are: it is very stable, does not require the addition of an aldehyde substrate, more efficient and less energy costly than bacterial luciferases. [139]•.

[1.3] Bioluminescence in Fungi

Currently there are more than 40 species of bioluminescent fungi within 9 genera, all of which are basidiomycetes. Examples of luminescent fungi include: ***Armillarea mellea***, ***Panellus stipticus***, ***Mycena citricolor*** *(synonym Omphalia flavida)*, ***Omphalotus olearius***(synonym *Pleurotus olearius*, *Clitocybe illudens*), ***Gerronema viridilucens***, ***Neonothopanus nambi***. [165]•.

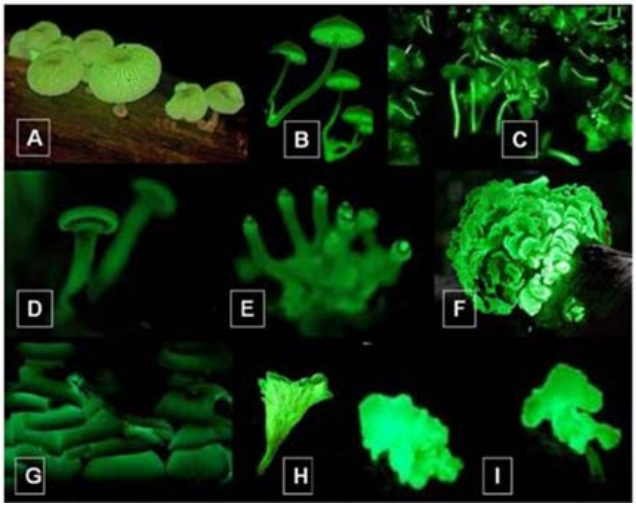

Image. [83]. Emission of visible light by mushrooms: A: *Mycena lampadis*; B: *Filoboletus sp.*; C: *Mycena sp.*; D, E, F: *Panellus stipticus*; G, H: *Omphalotus sp.*; I: *Neonothopanus nambi*. Photographs (A-H) official sites of National Geographic (http://news.nationalgeographic.com/news/) photo (I) Dao Thi Van (Bio-Lumi Co., Ltd., Ho Chi Minh City, Vietnam). [165]•.

[1.3.1] Fungal bioluminescence characteristics

Luminescence may occur in both **mycelia and fruiting bodies**, as for example in ***P. stipticus*** and ***O. olearius***, or only in mycelia and young rhizomorphs as in ***A. mellea***. Some luminescent fungi, for example ***A. mellea***, reportedly exhibit diurnal periodicity and seasonal variation of bioluminescence.

Bioluminescent mushrooms are generally **saprophytes** (less frequently pathogens) all of which are white-spored basidiomycetes. At different stages of their life cycle, they emit greenish light with maximum in range 520-530 nm. [165]•. **A luminous mushroom emits light only for a certain period (periods) of its life cycle; after and before that period, it practically does not glow**. [165]•. The

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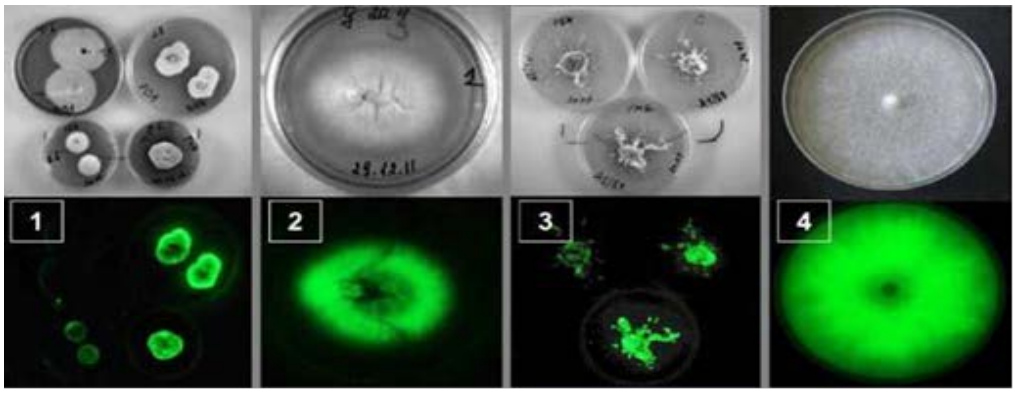
•[165]
• [165]
•[165] V. S. Bondar, et al., Luminescence of Higher Mushrooms, Journal of Siberian Federal University. Biology 4 (2012 5) 331-351, 2012.
• [165]
• [165]
• [165]

luminescence of younger fruiting bodies and young actively growing mycelium is brighter than mature fruiting bodies and old mycelium, even though the intensity of their luminescence varies widely with species and environment. The light emitted by bioluminescent fungi is often quite faint and typically requires very dark conditions to see. While the light or luminescence is difficult to observe in some species of *Mycena*, others are relatively brighter, bright enough to read and can be visible from a distance of up to 40 m.

Although the structural-functional organization and mechanisms of light emission of the luminescence system of mushrooms are currently not quite clear, [165]• bioluminescent fungi may be releasing light (not heat) as an energy by-product of enzyme-mediated oxidation reactions. As an example, a relationship of bioluminescence to lignin degradation has been suggested where it may act to detoxify peroxides that are formed during lignolysis. These wood-destroying fungi are specified by substantially higher luminescence (visible bioluminescence). Intensity of luminescence of these species correlates with the activity of enzymes of the secondary metabolism participating in lignin destruction. [165]•. For instance, many of the bioluminescent fungi are involved in wood and leaf litter decay, as A***. mellea*** and ***P. stipticus*** which are white rot fungi. [126]•.



[165]•

Image. [84]. Morphological identification (top) and luminescence (bottom) of the mycelium of different mushroom species grown in Petri dishes on liquid and solid nutrient media. 1. *A. borealis*, 2. *P. stipticus*, 3. *A. mellea*, 4. *N. nambi*. Bioluminescence intensity of mycelium samples recorded with Universal Hood II (USA) system.

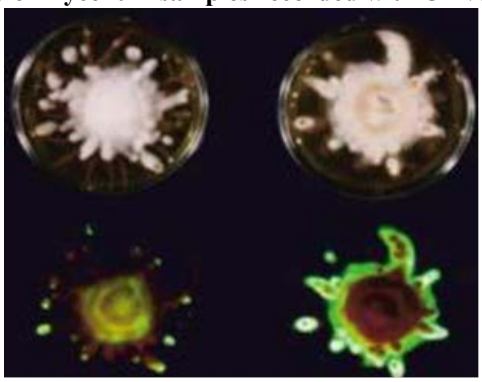

[126]•

Image. [85]. ***Armillarea mellea*** **ATCC 1113 grown on YM agar at 22°C in darkness for 3-4 weeks, taken in light (top) and darkness (bottom), with a Nikon F3 camera and a Micro Nikkor 60 mm lens. For the 'bioluminescence' photograph (bottom), exposure was 16 hours in total darkness. (Photographs taken by David Riley, the Macaulay Institute).**

• [165]
• [165]
• [126] H. J. Weitz, Naturally bioluminescent fungi, Mycologist, Volume 18. Cambridge University Press, UK, 2004.
• Op.cit.
• Op.cit.

[1.3.2] History of bioluminescence activity of fungi

J.F. Heller and von Derschau et al, associated luminescence of rotting wood with presence of mushrooms and fungal mycelium. [165]•. Luminous mushrooms have been found in North and South America, Europe, Asia, Australia, and Africa, specifically in the **subtropical and tropical zones**, where natural conditions are most favorable for their habitation.

Desjardin et al. (2008) attributed about 70 species of basidiomycetes to luminous mushrooms, further studies of fungal bioluminescence put in the list 80 species of luminous mushrooms. [165]•. Recently seven more new species of luminous mushrooms were found, attributed to *Mycena* family.

[1.3.3] Fungal bioluminescence system

Mushroom luminescence is also determined by functioning of the luciferase-luciferin system; however, attempts to isolate luciferin from luminous mushrooms were made by several groups, Kuwabara and Wassink (1966) extracted luciferin from 15 kilograms of mycelium of ***Omphalia flavida***, and obtained it in a crystalline form. The extracted luciferin exhibits chemiluminescence in the presence of H_2O_2, and emits light with a maximum emission of 524 nm in the presence of a luciferase preparation produced by Airth's process. [165].

Similarly Endo et al. (1970) extracted several similar compounds to luciferin from fruiting bodies of ***Lampteromyces japonicus*** mushroom, and a new substance, "**ergosta-4,6,8(14),22-en-3-one**", that exhibited fluorescence with maximum emission of 530 nm, which practically coincides with the maximum values of bioluminescence reported in previous studies in various mushrooms. [165]•.

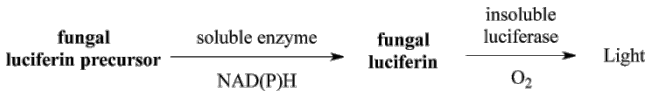
However, fungal luminescence has been conditioned to the presence of oxygen *in vitro, with a* light emission system that has been characterized as **an NAD (P) H-dependent luciferin-luciferase system** reported specifically for ***A.mellea*** and ***M. citricolor*** *fungal strains.* [126]•.

In 1959, Airth and McElroy demonstrated the luminescence of cell-free extracts from luminous fungi. [166]•. With the emission of light achieved by *adding* ***nicotinamide adenine dinucleotide phosphate (NADPH)*** *to a mixture of cold and hot aqueous extracts*, [167•; 168•] *prepared from the mycelium of* ***Collybia velutipes*** *and* ***Armillaria mellea*** *fungi*. [169]•.

According to these studies, the bioluminescence of fungi is thought to be a two-step process. In the first step, a luciferin precursor is reduced by an NAD (P) H-dependent enzyme to a true luciferin.

In the second step, luciferin is oxidized by air under luciferase catalysis to produce visible light. [170•; 165•].

Equation: [2]: ***the bioluminescence of fungi Two-step reaction. Proposed by Airth and Foerster.*** [171]•.



• **Op.cit.**
• **Ibid.**
• **[165] V. S. Bondar, et al., Luminescence of Higher Mushrooms, Journal of Siberian Federal University, Biology 4 (2012 5) 331-351, 2012.**
•**[126] H. J. Weitz, Naturally bioluminescent fungi, Mycologist, Volume 18, Cambridge University Press, UK, 2004.**
•**[166] R. L. Airth, W. D. McElroy, J. Bacteriol. 77, 249– 250, 1959.**
•**[167] R. Dubois, C. R. Soc. Biol, 37, 559 – 562, 1885.**
• **[168] K. V. Purtov, et al., The Chemical Basis of Fungal Bioluminescence, Chem. Int, Ed. 2015, 54, 8124 –8128, 2015.**
•**[169] E. N. Harvey, in A History of Luminescence From the Earliest Times Until 1900, American Philosophical Society, Philadelphia, USA, 1957.**
•**[170] R. L. Airthin, Characteristics of cell-free fungal bioluminescence:" Johns-Hopkins Press, Baltimore, pp. 262 – 273, 1961.**
•**[165]**
•**[171] R. L. Airth, G. E. Foerster, Arch.Biochem.Biophys, 97, 567 – 573, 1962.**

According to Airth and Foerster, mushroom luciferase can be a ***multi enzyme complex consisting of NAD (P) H-cytochrome of P-450-reductase and cytochrome P-450***. In their opinion, the **reductase can be a complex-structured enzyme, a part of which is NAD (P) H-oxy luciferin-oxydoreductase**.

They explained failures to obtain steady luminescence *in vitro* by dissociation of luciferase into individual components and loss of a certain substrate. [165]•.

The first attempt to produce fungal luciferin precursors in pure states resulted in Panal, PS-A and PS-B, which were extracted from luminous mushroom ***Panellus stipticus***. [165]•.

Recently, extracts from the mycelium of light-emitting mushroom ***Neonothopanus nambi*** have been found to contain a thermally stable low molecular weight component that activates the luminescence of the mushroom. It maintained the stimulating effect after boiling at 100ºC for several minutes and after concentrating at temperatures 45ºC and 60ºC. [165]•. These extracts stimulated luminescence of the mycelium, and the luminescence increased by several orders by adding hydrogen peroxide.

The spectral analysis of extracts showed two main peaks in the short-wave range (210 and 255 nm) and a slight bulge in the 400 nm range. It was found that the activator-containing extracts exhibit fluorescence in the visible range (wavelength range 480– 630 nm) with a maximum at 520 – 537 nm, which coincides with the data of luminescence spectrum of ***N. nambi*** mycelium *in vivo*. [165]•.

Numerous experimental facts have indicated that higher mushrooms, which do not show a visible luminescence, however, still exhibit weak chemiluminescent emission. For instance, Shimomura (1989) recorded the presence of chemiluminescent substances in non-luminous strain of luminous mushroom ***Panellus stipticus***. In addition to Luminescence of the hyphae in 13 mushroom species, Mihail and Bruhn (2007) also found bioluminescence activity in phyla ***Basidiomycota***, ***Ascomycota*** and ***Zygomycota***. This is supported by studies on the fruiting bodies of mushroom species from boreal and tropical zones that showed that most of them have chemiluminescence of various intensity. [165]•.

Another hypothesis suggest that, mushroom luminescence is **associated with oxidation of organic substrates and takes place without participation of a specialized enzyme**. Researchers adhering to the second concept express doubts about the presence of a specialized enzyme (luciferase) in fungi.

As the efforts of many researchers to demonstrate luciferin-luciferase reaction in luminous mushrooms have been failing for a long time. [165]•.

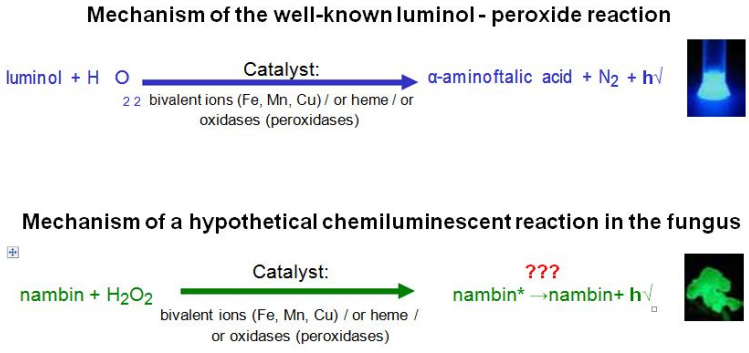
[1.3.4] synthesizing bioluminescence in fungi

Since bioluminescence activity in fungi is argued as mentioned above, a lot of attempt have arisen to synthesize the chemiluminescent luciferin-luciferase reaction in laboratory; some of these studies have reached positive results as in the study conducted by Airth and McElroy with «cold» and «hot» extracts from luminous mushrooms. **It is important to note that light emission was recorded by the authors only when NAD (P) H was added to the reaction medium**; this, suggests reversibility of any oxidation processes, affecting the native components. Later, additional information about luciferin-luciferase reaction was derived by Airth and Foerster. They used luciferin preparations from ***A. mellea*** mushroom and luciferase from the luminous mushroom ***C.velutipes***, proposing a scheme to describe luciferin-luciferase reaction providing for luminescence of mushrooms. [165]•.

•[165] V. S. Bondar, et al., Luminescence of Higher Mushrooms, Journal of Siberian Federal University, Biology 4 (2012 5) 331-351, 2012.
• Ibid
• Ibid
• Ibid
• Ibid
• Ibid
• Ibid

Another method suggested the involvement of ***active forms of oxygen*** in the light emitting reaction of higher mushrooms, which indicate that the mechanism of mushroom luminescence involves oxidase enzymes for the oxidation of organic substrates. Such as in the luminous mushroom ***Neonothopanus nambi***. Experiments with globular mycelium grown deep in vials showed that luminescence was stimulated by the additions of ***hydrogen peroxide*** only in the case of ***N. nambi***, which is an indicative of the involvement of peroxidase or peroxidases in mushroom luminescence mechanism.

Although rarely reported in fungal bioluminescence, in ***N. nambi****, em*ission intensity of mycelial globules can be so high, that it is visible in darkness by naked eyes, and the high luminescence level can continue for several hours. After the luminescence decreases to the initial steady state level, a new addition of H_2O_2 produces a rapid spike of emission with the light signal amplitude comparable to the initial one. [165]•. It should be emphasized that luminescence of ***N. nambi*** can be stimulated repeatedly without new additions of extract. This may be additional evidence that favors the presence in the extract of the luminescence emitter that is not spent in the course of luminescence reaction, thus providing the possibility of repeated stimulation of luminescence.



[165]•

Figure. [41]. Chemiluminescent luminol-hydrogen peroxide reaction (top) and assumed chemiluminescent reaction for the luminescence of *N. nambi* mushroom (bottom).

From the scheme presented in Figure. [41]. it is evident that to activate mushroom emission, a third component – reaction catalyst – is required in addition to the emitter and active oxygen forms (e.g., hydrogen peroxide). In the luminol reaction, the role of catalyst is performed by ions of transition metals (e.g., Mn, Fe, Cu) or enzymes with oxidase function (specifically peroxidases). It should be noted that ***N. nambi*** mycelium was found to be stimulated by manganese ions. This suggests involvement in mushroom light emission reaction of peroxidase enzymes, e.g., Mn-peroxidase (or Mn-peroxidases) of lignin-destroying enzyme complex. [165]•.

Factors to stimulate fungal luminescence are often processes attended by the formation of active oxygen forms. *These processes formation can be conducted by mechanical, physical or chemical impacts on the mushroom cell wall.* This may be also associated with metabolic changes in mushroom, which disturb enzyme system operation to form active oxygen forms. The chemiluminescent reaction as the basis of mushroom luminescence, in turn, may be supported by those enzyme systems catalyzing oxidation of organic substrates (including emitter) with participation of active oxygen forms. [165]•.

• **Ibid**
• **Ibid**
••**[165] V. S. Bondar, et al., Luminescence of Higher Mushrooms, Journal of Siberian Federal University, Biology 4 (2012 5) 331-351, 2012.**
• **Ibid**

[1.3.5] Bioluminescent fungi's ecological value

Many researches have been developing luminescent mushrooms into applications in testing for pollutants **[e.g. ions of mercury (Hg)]** in water supply when concentrations are too low to detect by conventional means. Luminous mushrooms were used to detect toxicity of salts of heavy metals. These compounds have been shown to inhibit the luminescence of mycelium of ***Armillaria mellea*** and ***Mycena citricolor***. The light generated by this reaction has been utilized by scientists as a bio indicator or biosensor to the level of pollutants present in the tested medium.

[2] Bioluminescence application in architecture and interior design

In this section, applications of bioluminescent microorganisms as ecological lighting sources will be exhibited, these applications include urban, architecture, interior and furniture design. In biology, the amount of light emitted by an organism is measured in photons, making the quantity of emitted photons by any light source directly proportional to the amount of generated energy. In architectural and lighting international measurement systems, the base unit to measure light output is the lumen (lm), referring to the "luminous flux" of total visible light emitted by a source at any angle, which must be considered when designing such lighting devices. Fixtures, device system, microorganism availability, and safety are strongly important considerations in this case. [172]•.

Most significant presented applications are the artistic, including art installation, land art, piers and marina illumination, reef delimitation and ephemeral art installations. [172]•.

[2.1] Architecture design

[2.2] Interior design

Project category	Urban –Architecture and Interior Design –three phase project
Project name	Genetic Barcelona
Designer	Professor DDr. Alberto T. Estévez, Universitat Internacional de Catalunya, Barcelona
Year	First phase: 2003-2006; second phase: 2007-2010; third phase: ongoing
Place	Barcelona –Spain
Microorganism	Luminose protein GFP (green fluorescent protein)
Description	

Genetic Barcelona Project **is a research about the genetic creation of bioluminescent plants for urban and domestic use.**

- **First phase: focus on possibilities of** *GFP (Green Fluorescent Protein*) **in natural lighting in urban design.**

• **[172] A. C. Gonzalez Veron, et al., Bioluminescent algae and possible implications in architectural design, PLEA2013-29th Conference, Sustainable Architecture for a Renewable Future, Munich, Germany, 2013.**

• **Ibid**

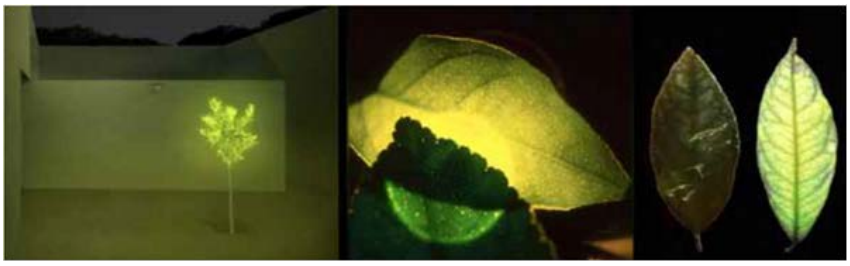


Image. [86]. Genetic Barcelona Project, 1st phase, 2003-2006. Genetic creation of bioluminescent plants for urban and domestic use. Left: Manifesto-image, the light of the bioluminescent trees (© A. T. Estévez). Center: Comparison between a real lemon tree leaf with GFP (Green Fluorescent Protein) and another without GFP, from the same lemon tree type (Photo: A. T. Estévez, with conventional reflex camera). Right: Comparison between a real lemon tree leaf with GFP (Green Fluorescent Protein) and another without GFP, from the same lemon tree type (Photo: J. Clotet and A. T. Estévez, with special UV photo camera). [45]•.

- **Second phase: the** Biolamp, bioluminescent batteries, with infinite number of applications and variant level of installation**, in walls, panels, ceilings, doors, skirting boards, decoration, wearable technology, and exhibitions. In this phase of the project, *Bio lamps* were utilized to illuminate a whole apartment without cables or installations.**

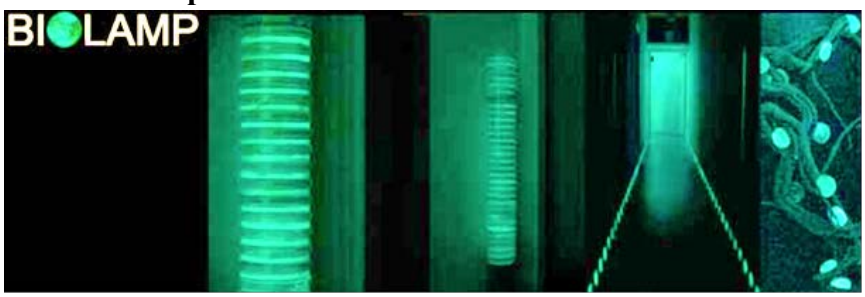


Image. [87]. Biolamp, Genetic Barcelona Project, 2nd phase, 2007-2010 (© A. T. Estévez). Left: Bioluminescent batteries applied at the "Biolamp 2". Center: Bioluminescent batteries applied at the "Biolamp skirting board", used for the first time to illuminate a whole apartment without cables, without installations, and without electricity. Right: Bioluminescent batteries applied at the "Biolamp Roots" (Photos: A. T. Estévez, with a conventional camera). [45]•.

Project category	**Furniture design**
Project name	Philips Bio-Light
Designer	Philips co.
Year	2011
Place	Germany
Microorganism	Bioluminescent Bacteria

Description

As one of proposed systems in its Microbial Home (MH) concept, Philips created *a lighting system driven by the wastes typically generated in the average home. To feed the bacteria housed in the bio-light's glass compartments, methane, which could be generated by the MH kitchen's bio-digester unit from composted bathroom solids and kitchen vegetable waste, is piped in through thin silicon tubes connected to a reservoir at the bases.*

•[45] A. T. Estévez, The Future of Architecture: Bio digital Architecture and Genetics, ESARQ the Architecture School of the Universitat Internacional de Catalunya, Barcelona, Architecture Research, 4(1B), pp.13-20, 2014.

• Ibid

Light produced by bacteria, or luminescence, is heat-free Chemiluminescence, usually employed in lighting unites inside glass tubes that generates the bioluminescence reaction by the familiar shaking, these sticks or tubes contains a mixture of phenyl oxalate, fluorescent dye and hydrogen peroxide, but those are closed one-use systems with a limited light-production period.

The bio-light's living bacteria, on the other hand, which utilize the enzyme luciferase, and its substrate, luciferin, to generate light, can be driven indefinitely, as long as key nutrients are supplied. While the light given off is not bright enough to fully replace artificial light just yet, it could be enhanced by the design of the system. [173]•.

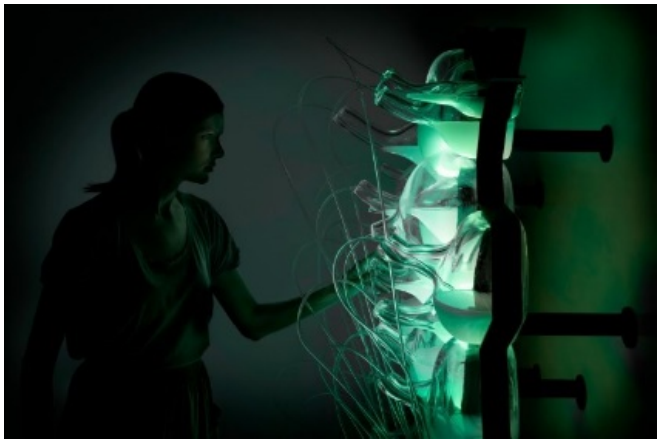


Image. [88]. The Philips bio-light is 'powered' by glowing bioluminescent bacteria.

Image. [89, 90]. Philips bio light cells inspired by the living cells shape, each cell is a container for bioluminescent bacteria

[3] Conclusions

1. Natural bioluminescence occur in different species of microorganisms including (Bacteria, Fungi and algae). This bioluminescence activity occurs in different ranges of light spectrum and for specific periods in the microorganism‘s life. The intensity of the emitted bioluminescent light, the specific period of bioluminescence activity, and the bioluminescent light spectrum; varies from one species to another, according to the organism’s DNA composition (Lux genes (Lux AB-CDE), the existence of certain proteins (YFB, YEP, GEP), the nutrition level of microorganism’s media, and microorganism’s growth curve.
2. Natural bioluminescence produce light without heat, which add an ecological value for its utilization in applications in architecture design, as it will not result in heat louds in architectural and interior applications.

• **[173] https://www.sott.net/article/238256-Philips-Bio-Light-Concept-Lights-The-Home-Using-Bacteria**

3. Bioluminescence reaction in most species depend on the luciferin/luciferase reaction.
4. Bioluminescence activity in bacteria exhibits the maximum emission of light in comparison with other luminose species. Varies in range (400-650) nm that makes bioluminescent bacteria the most applicable ecological lighting source.
5. Bioluminescent bacteria is used also as toxicity biosensors for different toxic compounds and elements; which have a double benefit of using these bioluminescent strains as lighting sources and biosensors at the same time and that depends on the lighting –biosensor device design.
6. Bioluminescence activity is found in some species of fungi (mushrooms); although it is not proven that these luminose strains contain considerable luciferase enzyme levels, which is crucial for luciferin/luciferase reaction of bioluminescence activity.
7. Bioluminescence activity could be stimulated in non-luminescent species through genetic manipulation by insertion of the lux genes into the receptor cell wither prokaryotes (*E.coli*) or eukaryotes (yeast).
8. Embedding living bioluminescent species (bacteria, algae) in ecological lighting systems highlighted main considerations and obstacles that should be taken into account when designing these devices:
    - ***The estimated required lighting unites conversions between photons to lumen (lm) or luminous flux to calculate the needed lighting intensity and achieving it, through employing specific luminose strains (according to their emitted light spectrum), device materials selection and amplifying the amount of emitted light by reflectors.***
    - The type of installation for the device (horizontal /vertical –fixed /suspended – wall, ceiling, floor or on surface of other space elements); as circulation in interior or exterior spaces should be considered to avoid accidents.
    - The design of the device of ducts and recharging of the mediated compartments, which contain the organism and the relation of the device duct with the main water suppliers and general duct.
    - The level of enclosure and plugging.

CHAPTER 3
Non-Pathogenic Microorganisms as Biocatalysts in Electricity Production

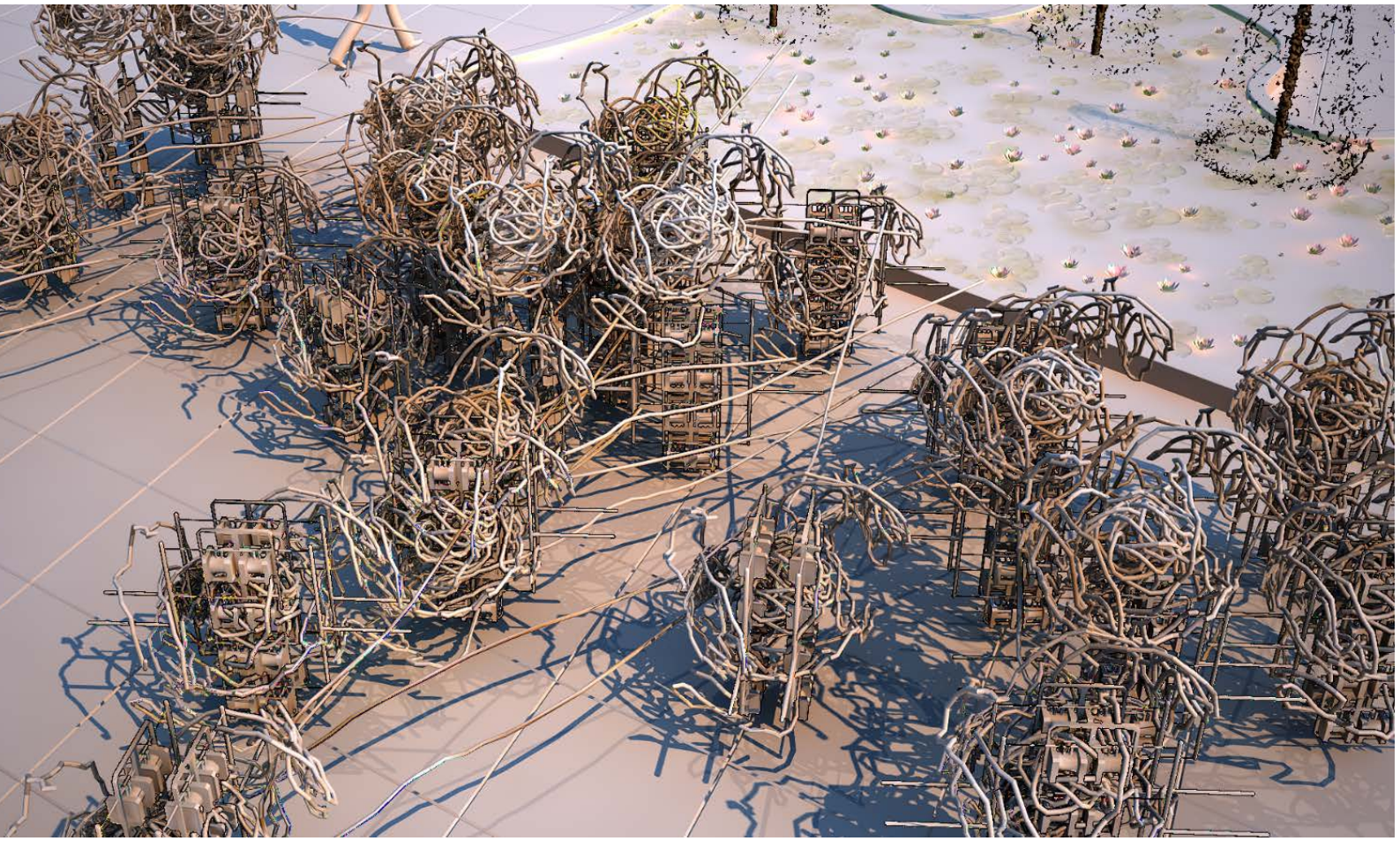


Image. [91]. Bioelectricity production by self-sufficient system employing clusters of Microbial Fuel Cells. For semi-exterior spaces. By the author.

Introduction

As mentioned previously, microorganisms play a crucial role as biocatalysts in energy production by exploiting its natural exoelectrogeneses characteristics in electricity production in microbial fuel cells. Thus, the concrete knowledge about this bio catalytic activity of microorganisms in different type of microbial fuel cells; elements, aspects and specification is an articulated phase of this research.

It is worth mentioning that reviews on microbial fuel cell will be exhibited briefly, not on purpose of electrical production optimization through cell design and architecture, but rather to categorize these types, highlight each cell's element in electricity production and manipulate MFC unites as a clusters in interior design elements; which is the main core of thesis hypothesis.

Microbial fuel cells (MFCs) have emerged in recent years as contributors to a low-carbon society, as they reduce the dominance of fossil fuels. MFCs can generate electricity through the catalytic activity of exo-electrogenic microbial strains involved in the oxidation of organic substrates acting as alternative renewable fuels. Although the energy produced by MFCs is relatively low compared to other fuel cell technologies, they can produce chemical energy effectively and directly and convert several non-purified organic substrates and varied classes of wastes into electrical energy. [174]•.

MFCs possess a high-energy transformation efficiency since it converts the chemical energy stored in substrates into electricity directly, which lowers operational costs. As biomass-based systems, MFCs (like other Bio electrochemical Systems) are considered carbon neutral: as the biotransformation of organic matter into chemicals through microbial metabolism prevents the primary production of CO2 emissions. [174]•.

[1] MFC emergence

[1.1] Fuel cells

Fuel cells (FC) are Generators of energy from varies organic compounds utilizing electrochemical reactions in the presence of catalysts that are mainly high value metal catalysts in this case. FC is of advantages over other kinds of energy generators as they do not produce emissions of environmental polluting gases as SOx, NOx, CO_2 and CO, they also exhibit higher efficiency, and they do not need mobile parts, they are also free of sonic pollution. However, the main disadvantages of these systems are high cost and high mass generation. [175]•.

Some certain types of fuel cells employ biocatalysts; they are called ***bio fuel cells (BFCs)***. These sort of fuel cells could be based on utilizing **microbes as in microbial fuel cells MFCs**, which use whole living cells to extract power from different substrates and oxidants, or an enzyme, as in enzymatic bio fuel cells, which instead use redox enzymes directly. [176]•.

In Bio electrochemical systems (BES), microorganisms are interacting with electrode using electrons, which are either removed or supplied through a closed circuit. The most utilized type of these systems is **Microbial Fuel Cells (MFCs)**, which has the capability to employ microbial communities as the catalyst and capture the electricity from a broad range of organic matters including wastes.

• **[174] R. A. Nastro, et al., Performance evaluation of Microbial Fuel Cells fed by solid organic waste: parametric comparison between three generations, The 8th International Conference on Applied Energy – ICAE2016, Energy Procedia 105. 1102 – 1108, 2017.**

• **Ibid**

•**[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

• **[176] M. Falk, et al., Direct electron transfer based enzymatic fuel cells, Electrochimica Acta 82, 191– 202-191-192, 2012.**

[1.1.1] Bio Fuel Cells

These devices as mentioned above are able to transform chemical to electrical energy via electrochemical reactions involving biochemical pathways. BFCs are attempting since they operate under mild reaction conditions, namely ambient operational temperature and pressure. They employing neutral electrolytes and using inexpensive catalysts and anodic fuel that can range from simple organic molecules like glucose or acetate to complex organic waste, like wastewaters and urine [177]•. These systems can be divided into two main types as exhibited.

[1.1.1.1] Enzymatic fuel cells (EFCs)

In an enzymatic fuel cell, either one or both electrodes, i.e. the bio anode and/or the biocathode, ***utilize enzymes to bio electrocatalytically oxidize the fuel and to reduce the oxidant***. A wide range of fuels and oxidants can be used to extract electrical power; the particular fuel will be dependent on the enzyme used. The biofuel, e.g. carbohydrates, alcohols, or even amino acids, is oxidized by an oxidoreductase, transferring electrons from the fuel to the bio anode. Electrons are then released at the bio cathode, where the bio oxidant, e.g. molecular oxygen (O2), hydrogen peroxide (H2O2), or organic peroxides (ROOR), is reduced by another oxidoreductase. [176]•.

Generally, enzymes have better electrochemical catalytic performance but are unsustainable and less durable compared to microbes. Enzymes are attractive to use as FC electron shuttles due to their high substrate specificity. As they are very selective in terms of the fuel or oxidant that is oxidized or reduced, making half-cell separation and usage of membranes unnecessary. These advantages of enzymatic fuel cells make miniaturization possible and enables using enzymatic fuel cells in wearable and implantable devices.

Enzymes are also biocompatible, and have high activity under mild conditions, and high transformation efficiency. Enzyme production is relatively inexpensive, which enabling their use in non-generic applications. In addition to their ability to utilize biologically derived fuels, such as e.g. glucose, fructose, lactose, ascorbate, dopamine, and alcohols, along with abundant O_2 as the bio-oxidant, these reasons make the use of enzyme based ***BFCs very smart for an array of applications, especially as electric power sources in implantable devices in living organisms***.

Enzymes along with their reaction products can also be considered as relatively safe compared to non-biogenous catalysts; which is an important consideration in implantable situation. Enzymatic Fuel Cells can be divided into two main groups according to how the electric connection between a particular electrode/enzyme pair is realized:

(i) **Mediated Electron Transfer (MET):** in these devices, redox mediators are used either in solution or as immobilized redox polymers, to channel the electrons to (and from) the electrode surfaces.

(ii) **Direct electron transfer (DET):** these devices are bio based, where the enzyme is able to communicate directly with the electrode with no mediators. [176]•.

One main disadvantage of EFCs is that the enzymatic lifetime is short and it is even further shortened in the presence of pollutants. The development of direct electron transfer devices such as **Mediator less enzyme-based biocathodes** and **bio anodes** has addressed the issue of the use of mediators, and increased in-active lifetime of the immobilized enzymes that avoid enzyme

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•[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources 356, 2017.
• Op.cit.
• Ibid

denaturation and provide a biocompatible hydrophobic and pH-buffered environment.[177]•. These developments made EFCs more compact, miniaturized and flexible bio electrochemical devices.

[1.1.1.2] Microbial fuel cell (MFCs)

Microbial fuel cells (MFC) use an active microorganism as a biocatalyst in a usually anaerobic anode compartment for production of bioelectricity. [178]•. Although Potter observed electric current produced by bacteria in 1911. [179]•, limited feasible results were recorded in this field by the next 50 years. [180•; 181•; 182•]. ***The use of whole microbial cells in MFC for the bio electrochemical oxidation of fuels is advantageous since it eliminates the need for enzyme isolation and still allows multiple enzymatic reactions to take place in conditions close to their natural environment, with the organisms regenerating the required enzymes as part of their natural life. On the other hand, they have a slower response time owing to the more complex chemical pathways.*** MFC target applications could span across various scales, they can be typically utilized for large-scale applications for wastewater treatment or in small-scale for small and portable applications. [177]•.

[2] MFC Architecture

This is an important aspect of the MFC design as it determines how the system will perform in terms of power output, Coulombic efficiency, stability, and longevity. [183]•.

Usually almost all MFCs consist of two-chamber system [184]•; **anode (usually anaerobic anode chamber**) and **cathode (usually aerobic cathode chamber)**, physically separated by **a proton exchange membrane (PEM)**. The biochemical reaction depends on the active biocatalyst in the anode that oxidizes the organic substrates and produces electrons and protons. In the absence of a suitable electron acceptor in the **anolyte**, electrons pass to the anode interface and are transferred through an external wire to the cathode, where they join oxygen molecules (O_2) and protons that are conducted to the cathode through the PEM to form water molecules, thus completing a circuit. [185•;186•;175•].

• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources 356, 2017.**
• **[178] M. Rahimnejad, et al., Power generation from organic substrate in batch and continuous flow microbial fuel cell operations, Appl. Energy 88. 3999–4004, 2011.**
• **[179] M.C. Potter, Electrical effects accompanying the decomposition of organic compounds, Proc. Royal Soc. London, Ser. B, Containing Pap. Biol. Charact. 84, 260–276.**
•**[180] K. Lewis, Symposium on bio electrochemistry of microorganisms, IV. Biochemical fuel cells, Bacteriol. Rev, 30. 101–113, 1966.**
• **[181] R.M. Allen, H.P. Bennetto, Microbial fuel-cells, Appl. Biochem. Biotech, 39. 27–40, 1993 .**
• **[182] A. N. Z. Alshehria, et al., Application of a five level central composite design to optimize operating conditions for electricity generation in a microbial fuel cell, Egypt Science Direct, 2015.**
• **[177]**
• **[183] B. E. Logan Microbial Fuel Cells. John Wiley & Sons, Inc, 2008.**
• **[184] H. Liu, B. E. Logan, Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane., Environmental Science & Technology, 38.4040–4046, 2004.**
• **[185] C.Y. Lai, et al., Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188. 392–398, 2017.**
• **[186] J. M. Sonawane, et al., Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells, Biosensors and Bioelectronics, 90. 558–576, 2017.**
• **[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

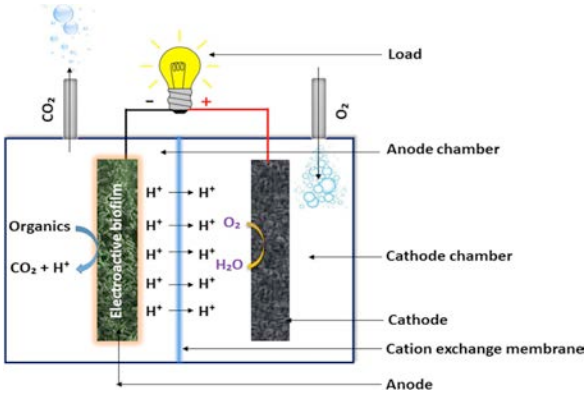

Figure. [42]. Working principle and basic construction of MFC. [186]•

MFC can use a wide variety of substrates, materials, and system's architecture configurations with different species of microbes to achieve bio-energy production. Although power levels in these systems are relatively low [183]•, it is particularly preferred for sustainable long-term power applications. [187•; 188•; 189•; 190•; 191•]. The main objective of MFCs is to achieve a suitable current and power for the application in small electrical gadgets. For instance, Rahimnejad, ***et al., turn on ten LED lamps and one digital clock with fabricated stacked MFC as power source and both devices were successfully operated for the duration of two days.*** [175]•.

Normally in two-chambered MFC system, oxygen diffusion in the anode chamber inhibits the production of electricity as it affects the anaerobic conditions of microbial growth and, thus, a pragmatic system is needed to keep the microbes separated from oxygen to maintain the anaerobic conditions in anodic chamber. This could be achieved by the use of PEM which is as mentioned before a membrane between the two separate chambers of anode and cathode that allow charge to be transferred between the electrodes. Since the difference in the potential coupled to electron flow produces electricity in MFC, maintaining a high rate of electron transfer is essential to optimize the MFC electricity production. This transfer of electrons could be more facilitated by the addition of mediators that are specific chemical compounds. Accordingly based on the mechanism of electron transfer, MFCs could be of two different categories: Mediated MFCs (with mediator), and Mediator less MFCs.

In mediated MFCs, artificial electron carriers (mediators), such as ***neutral red*** or ***anthraquinone2,6-disulfonate (AQDS)***, or ***humic acids*** can be used to carry electrons from inside the cell to an external electrode.

However, the need for high concentrations of electron carriers, that many of them are toxic chemicals, is thought to make electricity generation on a large-scale application impractical. The alternative is utilizing a biocatalyst that performs double benefit of being the biocatalyst and a facilitator of electron transfer, these biocatalysts could be found in some strains of bacteria, such as

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• Op.cit.
• [183]
• [187] Z. Du, et al., A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioener, Biotechnol. Adv, 25. 464–482, 2007.
• [188] M. Ghasemi, et al., Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance, Int. J. Hydrogen Energy, 38. 5480–5484, 2012.
• [189] G. Antonopoulou, et al., Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell, Biochem. Eng. J, 50. 10–15, 2010.
• [190] M. Rahimnejad, et al., Effect of mass transfer on performance of microbial fuel cell, Intech, 5. 233–250, 2011.
• [191] Y. Sharma, B. Li, The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs), Bioresource. Technol, 101. 1844–1850, 2010.
• [175].

Shewanella putrefaciens, that can produce their own mediators ***(soluble quinones)*** which eliminate the need to add external mediators.[192]•.

Moreover, recently, it was discovered that the respiratory enzymes of certain ***iron-reducing bacteria*** span their outer membrane allowing direct transfer of electrons to external metals such as Fe (III) or Mn (IV). The attachment of these iron-reducing bacteria to carbon electrodes results in electron transfer to the anode, with oxygen reduction at the cathode. Suggesting these enzymes are responsible for the electron transfer process. [192]•.

Performance of MFCs is mainly influenced by several factors such as: (1) The Supply and consumption of oxygen in cathode chamber, (2) The Oxidation rate of substrates in anode chamber, (3) Electron shuttle from anode compartment to anode surface, (4) Permeability of proton exchange membrane. [192•; 193•; 194•; 178•].

Accordingly, a two chambered-MFC has encountered several challenges in scale-up and practical application, such as: the turbulence in each compartment, the membrane resistance in the proton transportation process. In addition, there are two main problems in power generation, such as the power generation prevention in certain substrate concentrations, and the MFCs current decrease coupled with the high internal resistance that consumes considerable amount of the output power production in MFCs causing power loses. It should be added here that the proton exchange membrane (PEM), has been found the main source of high internal resistance (R_{in}) in two chambers of MFCs. [195•; 191•].

Other alternatives for optimizing performance of MFCs include utilizing bio cathodes to overcome the requirement for catalysis by oxygen oxidation on the cathode. [195]•. In addition, MFC reactor must have both a large surface area for biofilm formation, a high void volume, and biomass separation from any dissolved oxygen. [192]•.

[3] MFC types

[3.1] Double chamber

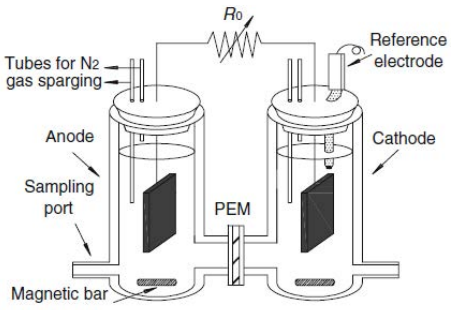

Figure. [43]. The two-chamber MFC constructed by connecting two plastic (Plexiglas) cylindrical chambers; net volume of 250 ml each) by a tube containing a proton exchange membrane (PEM). Both electrodes made of graphite plates and completely immersed in the anode and cathode solutions. A reference electrode placed into the cathode chamber to measure cathode potentials. The electrodes in the two chambers are connected with a copper wire for electron transfer.

[196]•

• [192] H. Liu, et al., Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell ,University Park, Pennsylvania, p2281.
• [192]
• [192]
• [193] G. Najafpour, et al., The enhancement of a microbial fuel cell for electrical output using mediators and oxidizing agents, Energy Sourc, 33. 2239–2248, 2011.
• [194] M. Rahimnejad, et al., Low voltage power generation in a biofuel cell using anaerobic cultures, World Appl. Sci. J, 6.1585–1588, 2009.
• [178] M. Rahimnejad, et al., Power generation from organic substrate in batch and continuous flow microbial fuel cell operations, Appl. Energy, 88. 3999–4004, 2011.
• [195] G.W. Chen, et al., Application of biocathode in microbial fuel cells: cell performance and microbial community, Appl. Microbiol. Biot, 79. 379–388, 2008.
• [191] Y. Sharma, B. Li, The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs), Bioresource. Technol, 101. 1844–1850, 2010.
•Op.cit
• [192]
• [196] G. Wang, et al., Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells, Biotechnol Lett, 30:1959–1966, 2008.

[3.1.1] Flat-plate MFC (double chamber): Min and Logan, (2004), [177]• developed a *flat-plate MFC with a cation exchange membrane CEM (Nafion) sandwiched in between the anode and cathode*. The plates cut to provide a serpentine flow path through the system, providing an approximation of a plug flow type of reactor. [183]•.

[3.1.2] Two Chamber bushing reactor (double chamber): You et al, (2006) developed a two-chamber "bushing" reactor using permanganate as the catholyte. The reactor consisted of a plastic bottle (3.5 cm in diameter, 10 cm high) containing four bars that held a *cation exchange membrane* CEM hot-pressed onto the bars, with a cathode placed in a concentric cylindrical shape inside the bottle. The anode was plain carbon paper. The reactor produced 3990 mW/m^2 of power.

Novel designs of MFCs have been developed to amplify the power generation, by reducing internal resistance by removing PEM, which resulted in the emergence of new type of microbial fuel cells. The single chamber microbial fuel cell.

[3.2] Single chamber MFC (SCMFC)

In the single chamber configuration, the MFCs has no separate chambers, they have one compartment with both electrodes at the two opposed ends of the compartment with no PEM or CEM, and their cathodes are exposed directly to the air to increase oxygen availability. [185]•. There are several advantages of using a single-chamber MFC versus a two-chamber system including; increased mass transfer to the cathode, decreased operating costs, an overall decrease in reactor volume; and a simplified design.

Power output is increased in a single-chamber MFC by the removing of the PEM. ***Although there is increased oxygen diffusion into the anode chamber in the absence of the PEM, the formation of an aerobic biofilm on the cathode inner surface (facing the anode) removes any oxygen that diffuses into the chamber, preventing the loss of anaerobic conditions in the anode chamber***, the lack of a PEM also substantially decreases the cost of the materials needed to make a MFC. [197]•

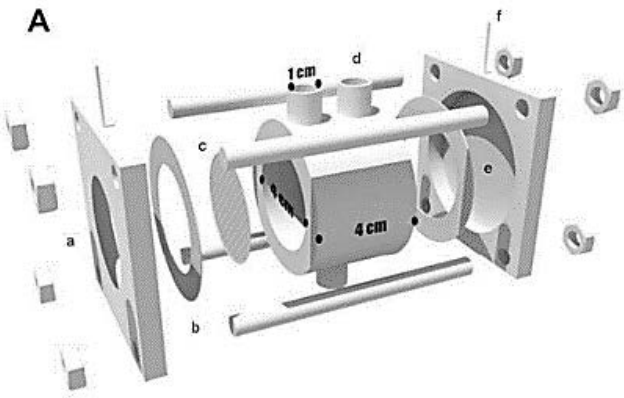

Figure. [44]. Schematic of the SCMFC system. From left to right, a. composed of a cathode, b. gasket, c. titanium net, ports, d. reactor cylindrical body, gasket, e. anode side, f. connection wires at both sides, and two upper cylindrical portals for medium inlet.

• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.**
• **[183] B. E. Logan Microbial Fuel Cells. John Wiley & Sons, Inc, 2008.**
•**[185] C.Y. Lai, et al., Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188. 392–398, 2017.**
• **[197] H. Liu, et al., Production of Electricity from Acetate or Butyrate Using a Single-Chamber Microbial Fuel Cell, Environ. Sci. Technol, 39, 658-662 p.658, Environmental Science and Technology, VOL. 39, NO. 2, 2005.**

[3.2.1] Air cathode single chamber MFC

[3.2.1.1] Cub reactor

Liu and Logan (2004) designed a Single chamber air-cathode cube system. The cube reactor consists of a single 4-cm block of Acrylic, which is a material that can be autoclaved, drilled through producing a 3-cm-diameter chamber. The two electrodes are placed on opposite ends, resulting in a surface area per volume of reactor of 25 m2/m3. The reactor has two openings on the top to allow draining and filling of the reactor, these portals are sealed with thick stoppers to prevent oxygen from entering the reactor while operating. The electrodes in the ends are cut to be slightly recessed, with a round rubber gasket placed over the electrode to prevent leakage; the flat plate ends are attached on top of the gasket to form a watertight seal. In this model, the cathode made of carbon cloth containing 0.5 mg/cm2 of Pt catalyst facing the waterside of the reactor. Two pieces of platinum (or stainless steel) wire are inserted through drilled holes that line up with the bottom of the recessed opening so that good contact is made when the electrode is inserted and pressed onto the wire. Four screw bars are then used to compress the end plates onto the ends, holding the reactor together. The circuit is completed with an external resistor, usually 500 or 1000 **ohm.** [183]•.

Another model developed by Cheng *et al.* **(2006) depending on** removable sections so that the anode can be placed at distances of 4 cm, 2 cm and 1 cm from the cathode. Piercing the anode with a few holes to allow fluid to move from one side to the other. Moving the anode closer to the cathode increases power output due to a reduction in internal resistance of the reactor. In addition, when operating the reactor in continuous flow mode with the flow directed through the anode towards the cathode this helped to reduce the effect of oxygen diffusion from the cathode to the anode. [183]•.

[3.2.2] Single chamber MFC with multiple concentric anode

This model of SCMFC consists of a single cylindrical chamber containing multiple graphite rods acting as the anode. These rods are placed in a concentric arrangement around a single cathode. The graphite rods would be of rough textured material to enhance microbial attachment. The cathode material should be an air-porous material that consist of a proton exchange membrane with carbon/ platinum catalyst layer fused to a support tube. The cathode/PEM would be placed onto a tube containing pores. To connect the circuit a copper wire would be used. [192•; 198•].

[3.3] Stacked MFC

MFCs can be stacked together in series or in parallel to achieve higher voltage or current, ***however, stacking multiple MFCs together in series can result in problems, such as voltage reversal, contact voltage losses, and erratic operation.*** Producing larger stacked MFCs can alter electrode spacing, and thus affect power density through changes in the area specific internal resistance. ***In small air-cathode MFCs, electrode spacing and anode surface area have been shown to affect power output positively. Thus, Scaling up MFCs requires compact reactor designs and the use of multiple electrodes. Electrodes should be closely spaced to minimize ohmic losses***. However, this can still decrease MFC performance due to oxygen crossover from the anode to the cathode, which reduces

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• **[183] B. E. Logan Microbial Fuel Cells.John Wiley & Sons, Inc, 2008.**
• **[183]**
• **[192] H. Liu, et al., Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell ,University Park, Pennsylvania, p2281.**
• **[198] M. Di Lorenzo, et al., A single-chamber microbial fuel cell as a biosensor for wastewaters, water research, 43. 3145–3154-p3146, 2009.**

power generation due to aerobic respiration of microbe. [199]•. Ion exchange membranes (CEM, PEM) can help reduce oxygen crossover, but they substantially reduce power production.

An alternative to a using a membrane is to place a separator between the electrodes, allowing for more compact designs known as ***separator electrode assemblies*** **(SEAs)**. MFCs with SEAs can be used to generate higher power densities than systems with large electrode spacing. [183]•.

Stacking MFCs together in series liable to increase the voltage, despite from the ***losses mentioned previously that is caused by in series configuration of group of individual cells, thus, the final voltage may not be equal to the sum of the individual cell voltages.*** *One of the precursors of these voltage losses is* ***voltage reversal*** that is the main obstacle for successful increases in the voltage. Aelterman ***et*** *al.* (2006) examined power production in a 6-cell stack. Reactors were connected in series or in parallel, the coulombic efficiency was only 12% when cells were operated as a stack in series, but increased to 78% when reactors were operated in parallel. [183]•.

Oh and Logan, (2007) further investigated the reasons for voltage reversal in stacks using a two-cell stack. It was shown that substrate depletion could drive one cell into voltage reversal. These findings indicate that linking cells in series to form stacks may be a difficult way to increase voltage as variations of output in individual cells could drive the stack power output to rapidly falter. It might be possible to eliminate charge reversal in a cell in a stack using a diode. [183]•.

Apart from stacked MFC advantages, there remains no proven design yet that is economical for scale-up. However, that situation is likely to be changed soon for the following reasons: power densities are continually increasing with air cathodes, high-cost anode materials such as graphite plates are being replaced by low-cost materials such as graphite fiber brushes, metal catalysts such as Pt are being replaced with non-precious metal catalysts using iron or cobalt, and reactor designs utilizes closely spaced electrodes, to increases power output. In addition, the reactors are being designed to maximize electrode packing (surface area per volume) so that power per reactor volume is increasing. [183]•.

[3.4] Up flow MFC

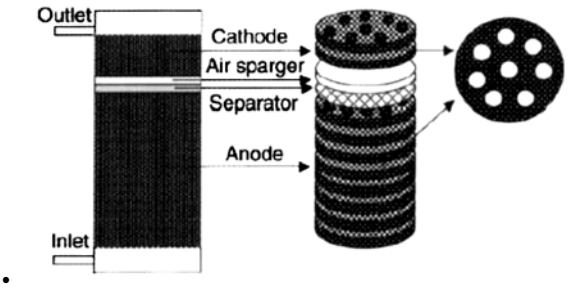

Figure. [45]. Continuous-flow tubular reactors developed by Moon *et al.* (2005) for electricity generation. The flow moves from the anode chamber into the cathode chamber where it is sparged with air. [175].

• **[199] Y. Ahn, B. E. Logan, A multi-electrode continuous flow microbial fuel cell with separator electrode assembly design, Appl Microbiol Biotechnol, 93:2241–2248, 2012.**
• **[183] B. E. Logan Microbial Fuel Cells. John Wiley & Sons, Inc, 2008.**
• **[183]**
• **[183]**
• **[183]**
•**[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

[3.4.1] Tubular up flow MFC

Several researchers have investigated the use of reactors in a tubular shape that contains a conductive packing and are specifically designed to run in a continuous flow mode. Liu ***et*** *al.* (2004) ***used a tubular MFC that contained eight graphite rods and a central cathode tube***. Some of these reactors use oxygen at the cathode, while others rely upon the use of chemical catholytes such as ***Ferricyanide*** for power production. Jang ***et*** *al.* (2004) used a novel tubular reactor approach based on the flow moving through an anode chamber and then directly into the cathode chamber in the same column. The cathode chamber is sparged with air to provide oxygen at the cathode. A perforated plate is used as a separator, and the cathode is of graphite felt, perforated and Pt-coated. In this model, Power generation was reported to be stable for over two years. One disadvantage of this reactor system is that if the organic matter is not removed sufficiently in the anode chamber it will flow into the cathode chamber and create an oxygen demand.

[4] MFC elements

[4.1] Anode in MFCs

Anode compartment is one of the main parts of MFCs. All the essential conditions to degrade the biomass are provided in the anode chamber. This compartment is filled with substrate, mediator (optional), microorganism, and the anode electrode as electron acceptor. The general reaction in the anode chamber is summarized in the following equation.

Equation: [3]:Biodegradable organic substrate $+ \frac{\text{Active microorganism}}{anearobic\ environment} \rightarrow CO^2 + H^+ + e^-$ [200]•.

As mentioned previously, one of the most effective factors which influences the performance of MFC is anodic microbial electron transfer, thereby amplifying microbial electron transfer rate, is achieved through diverse methods such as ***adding electron mediators, and optimizing cell design and electrode*** accordingly, ideal electrode materials should be of certain features including: [201•; 202•; 203•]. good electrical conductivity and low resistance, strong biocompatibility, chemical stability and anti-corrosion, large surface area, and appropriate mechanical strength.

The mechanism of the electron transfer process necessitates the donation of electron from the *anode respiring microbe to the anode surface via extracellular electron transfer (EET)* and, consequently, resulting in the passage of a current in the external circuit. This current generation mechanism is similar to direct electron transfer from cell to anode surface through diffusion of soluble electron shuttles and electron transfer through solid component of microbes. Three scales can affect this interaction: 1. ***The single-cell level***, 2.***The biofilm-level***, 3. ***The integrated system-level***. [203]•

Microbial-level attachment can be improved by changing the surface chemistry through changing surface charge with positive charges, utilizing hydro phillicity /hydrophobicity with hydrophilic surface during microbial attachment, adding oxygen or nitrogen functional groups that facilitate microbial surface interaction, or by using immobilized mediators.

• **[200] B. Virdis, et al., Microbial Fuel Cells, Elsevier Science publisher, Amsterdam, 2011.**
• **[201] B.E. Logan, et al., Microbial fuel cells: methodology and technology, Environ. Sci. Technol, 40 . 5181–5192, 2006.**
• **[202] B.H. Kim, et al., Challenges in microbial fuel cell development and operation, Appl. Microbiol. Biot, 76. 485–494, 2007.**
• **[203] U. Schroder, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency, Phys. Chem. Chem. Phys, 9. 2619–2629, 2007.**
• **[203]**

The attachment of microbes can also be affected by the ***surface morphology*** and the roughness that can be controlled at the nano micro scale. Different chemical treatments, surface coatings, electrochemical treatments, and thermal treatments have been reported to affect both chemical and morphological surface area and increasing power generation in MFC.

Moving to ***the biofilm-level***, surface morphology plays a key role in the biofilm growth and the current produced. ***Moving from a flat 2-D surface towards a 3-D electrode material in order to increase the available surface area and enhance the bio-interface between microbes and electrode have been examined and proved an improved performance in this regard.*** [186]•.

There are several classes and types of anode materials that can be categorized into four main groups as follows:

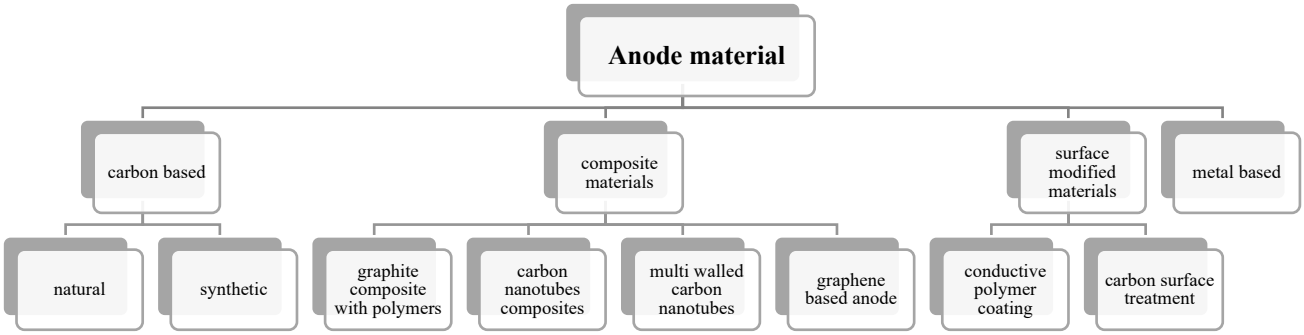

Diagram. [6]. Classification of anode materials used for MFCs. [186]•.

The most frequently used materials in anode are made of **carbon materials, as** they are of; low cost, biocompatibility, excellent electrical conductivity, chemical stability, and accessible surface area. There are several types of carbon-based materials. Here the author exhibits the most frequently used materials:

Carbon brush: is based on a titanium core in which carbon fibers are twisted. The surface area is quite high with an optimal area to volume ratio. The high electrical conductivity is guaranteed by the central titanium metal. Although it at the same time increases the material cost.

Carbon cloth: This material has high surface area and relatively high porosity demonstrating also high electrical conductivity, as well as flexibility and mechanical strength, in forming more complex 3D structures. The negative aspect is related to the cost that is generally quite high. [175]•.

Carbon mesh: Carbon mesh is commercially available, relatively low-cost and with a relatively low electrical conductivity, and low mechanical strength that could lead to low durability under high flow conditions. Carbon mesh can be also folded to make a 3-D electrode, but its porosity is low. [175]•.

Carbon paper: Carbon paper is a planar carbonaceous material, relatively porous but expensive and fragile with mainly lab scale demonstrations under batch conditions. [175]•.

•[186] J. M. Sonawane, et al., Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells, Biosensors and Bioelectronics, 90. 558–576, 2017.
• Ibid
• [175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.
• Ibid
• Ibid

Carbon veil: is a very cheap with relatively high electrical conductivity and high porosity, of extreme importance for allowing microbes to access and colonize all the available material sites. The single layer of carbon veil is quite fragile but since the material is versatile, it can be folded to form a robust and porous 3-D electrode. [175]•

Carbon felt: possess high porosity and high electrical conductivity. The large pores allow microbes to penetrate through the structure and colonize the biofilm internally. The cost is relatively low and the mechanical strength is high depending on the thickness of the material. [175]•

Carbonized cardboard: ***Carbonized cardboard is a 3-D material composed by a single wall corrugated cardboard from recycled paper. The material is very low-cost, has high electrical conductivity and high porosity.***

Reticulated vitreous carbon: very conductive and have great porosity. Unfortunately, the material is quite fragile and very expensive. It is used in MFCs for their stability in microbial cultures, high electric conductivity and vast surface area.

Graphite granules (GGs) or granular activated carbon (GAC): have a high degree of micro-porosity and catalytic activities. In addition, GGs are less expensive with higher conductivity and biocompatibility. In order to increase conductivity, GAC has to be packed and this might lead to possible clogging in a flow-through MFC configuration. The overall surface area is quite high but the surface area available for microbes' interaction is rather low because the majority of the surface area available is in the nanometric scale. Usually, GAC is combined with carbon rods as current collector. GAC has very high surface area that can help the adsorption of organics pollutants or heavy metals.

Composite anodes***: Graphite-polymer composites (carbon scaffold (CS) and carbon scaffold – graphite (CS–GR))***. [177]•. Heterogeneous fabrication methods and modification manners involving nanomaterials have been tried for enhancing the power density and enlarging the capability of electron accepting. ***Carbon nanotubes (CNTs)*** could amplify the electron transfer feasibility and electrode surface area with utilizing ***carbon nanotube/polyaniline nanostructure*** composite as anode materials.

Recent studies have tried to overcome two dimensional electrode materials limitations, such as low *surface area, high internal resistance, high activation and mass transfer over-potential, which hinders their ability to achieve high performance* with MFCs. Through advances in materials science and nanotechnology, the use of second-generation ***three-dimensional (3D) electrode*** material has been tested in MFCs. such as ***carbon nanotubes (CNTs), carbon nanofibers (CNF), gold/poly (e-caprolactone) microfiber (GPM),*** for reducing the internal resistance of MFCs. Thus, 3D surface anodes have proved to provide higher surface areas for efficient colonization of microbial communities, hence, increasing substrate access to the anode respiring microbes, and minimizing mass transfer limitation. 3D surface anodes also have high volume to surface ratio, and good biocompatibility. [186]•.

However, the transition from a flat surface to a more complex 3-D surface implies also the possibility of facing limitations due to diffusion transport phenomena of both products and reactants and relative pH gradients.

• **Ibid**
• **Ibid**
• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.**
• **[186] J. M. Sonawane, et al., Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells, Biosensors and Bioelectronics, 90. 558–576, 2017.**

Carbon nanotubes composite: this material has excellent intrinsic properties, which include high conductivity, corrosion resistance, high surface area and electrochemical stability.

CNT- textile material is also biocompatible and has high conductivity in nature. The 3D space structure provided by the CNT textile enables formation of 10 times more biofilm than with an unmodified textile. The 3D space facilitates an efficient substrate transport of biofilm and internal colonization of diverse group of microbial community. CNT-textile anode has also been found to produce 10 times less charge transfer resistance.

Polytetrafluoroethylene (PTFE) is utilized in MFCs as electrode. The graphite/PTFE composite could be excellent anode for bioelectricity production.

Among metals-based materials used as anode materials are stainless steel plate, stainless steel mesh, silver sheet, gold sheet and titanium plate. [177]•.

The anode surface can also be modified to become favorable habitats for biofilms, which are capable of enhancing electron transfer from microbes to anode surface. That enables the generation of more power with minimum loss. It has been demonstrated in a recent study that surface modification increases MFC system performance, and decreases the MFC initiation time [177]•. It should be considered also to design anode electrode in a way that enables avoiding clogging or "dead zone". This is mainly related with the design of the reactor and the overall system in order to guarantee long lasting operation. [175]•.

Natural anode materials: natural and recyclable materials have been proposed for synthesizing high performance anode materials; this trend provides a green approach for generating renewable and sustainable bioenergy all from nature. One example is the development of ***layered corrugated carbon (LCC)*** anode from cheap packaging material. [186]•. *LCC gives up to four-fold increase in current density when compared to conventional graphite felt*.

Natural anode materials are suitable low-cost alternatives for conventional metal based or graphite-based anode materials in MFCs because ***of their meso/microporous 3D structures, high electron transfer rate and the achievable high kinetics of electroactive microbial community.*** [186]•.

Along with recyclable natural materials, It has been reported that ***carbonized king mushroom***, ***wild mushroom and corn stem*** exhibited good electrode properties. As carbonized corn stem exhibits eight times better performance than plane graphite electrode. [186]•. Similarly, prepared ***bamboo charcoal*** has also been found to exhibit good electrode properties compared to a conventional graphite rod. Such as low internal resistance, better biocompatibility and rougher surface, which promoted biofilm adhesion.

• [177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.

• [177]

• [175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.

•[186] J. M. Sonawane, et al., Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells, Biosensors and Bioelectronics, 90. 558–576, 2017.

• [186]

• [186]

[4.2] Cathode in MFCs

After electrons are generated from the biochemical reaction at the anode, they travel to cathode chamber and transmit onto oxygen. This radical oxygen and produced positive ions in the anode participate in the following reaction to form water, which spreads through the ion permeable membrane on the cathode along with the assistance of catalysts as follows:

$$H_{2 \rightarrow 2\,H^+ + 2e^-}$$
$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Equation: [4]: oxidation-reduction reaction of Biocatalysis in MFC. [175]

The reaction yield of the cathode is affected by multiple factors including the concentration and species of the oxidant (electron acceptor), proton availability, catalyst performance, and electrode structure and its catalytic ability. Oxygen has usually been a final electron acceptor in the cathode due to its accessibility, intense oxidation potential, and not being a chemical waste product **(water is formed as the end product)**, and producing no poisonous end products.

Since catalyst usually is immobilized on the cathode, they play a crucial role in enhancing the reaction rate and decreasing the activation energy, thus, the presence of an adequate catalyst is of vital importance for MFC performance optimization [175]•.

One certain type of developed cathode is the Biocathodes, these are more beneficial in contrast to ***abiotic cathodes,*** as configuration and expenses could be decreased by utilizing them; hence, expensive catalysts (e.g. Pt) and mediators would not be needed. Another advantage of biocathodes is generating practical products or removing secondary products using microbial metabolism. Hence enhancing the MFC sustainability. Biocathodes can be classified into two major categories: Aerobic, and Anaerobic. [175]•.

Air-cathode MFCs are promising for practical applications due to their simple configuration, sustainable operation, and relatively high power densities. ***In aerobic biocathodes***, oxygen is reduced coupled with the oxidation of transition metal compounds (Fe(II), Mn(II)) that are able to be catalyzed by the biofilm which is on the surface of cathode. Moreover, MFCs utilizing aerobic biocathodes generate greater power density compared to MFCs using anaerobic biocathodes. Although, microbial activities within the cathode chamber could be prevented by the accumulation of microbial metabolites, experimental results show that the charge transfer (part of internal resistance) of cathode decreases using biocathode, which enhances the overall performance of the MFC.

One type of biocathodes is the ***fungal cathode***, ***these biocathodes are based on oxidative enzymes secreted by the fungus e.g. laccase. These enzymes are sometimes immobilized on the surface of the cathode to catalyze the reaction instead of noble metals catalysts that are expensive and can deteriorate rapidly under the moist and corrosive conditions in which most MFCs operate***. Another method is proposed by ***Wu et al. they demonstrated that growing a laccase secreting white-rot fungus in the cathode chamber increased the power density of a two-chamber MFC sevenfold, instead of applying a purified enzyme to the cathode. However, such a setup requires the continuous addition of nutrients and mediator compounds into the cathode chamber***. [185]•.

•[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.

•[175]

• [185] C.Y. Lai, et al., Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188. 392–398, 2017.

It is worth mentioning that the performance of cathode is considered the main limitation to make an MFC scalable, this implies that the cathode material is a crucial parameter in MFC performance.

Although it was reported that the surface area of cathode has insignificant effect on power output, cathode efficiency can be improved using high surface area materials or granular materials. The right cathode material can also affect maximizing power generation and columbic efficiency and minimizing expenses. Some of used materials in cathode are: carbon paper, carbon felt, carbon brush, carbon fiber, graphite of various type, Pt (Pt commonly used as cathode catalyst), polymer binders such as perfluorosulfonic acid (Nafion), Cu, Cu–Au, granular graphite, and reticulated vitreous carbon (RVC). [175]•

[4.3] Catalyst in MFC

In microbial fuel cells, ***two redox couples are required for coupling the reduction of an electron mediator to microbial oxidative metabolism and for coupling the oxidation of an electron mediator to the reduction of the electron acceptor on the cathode surface***. In aerobic cathodes, the electron acceptor is regenerated by atmospheric oxygen on cathode surface. [204]•. The power output of MFC would be enhanced by raising the air pressure of cathode by controlling oxygen diffusion of membrane to the anode. Thus, there are two general techniques of transmitting the electrons to the anode:

Mediated Electron Transfer, MET: the use of external redox mediators to link the electrode biofilm interaction(s).

***Direct Electron Transfer, DET*:** physical adherence between the microorganism and the anode electrode surface [205]•.

It is worth mentioning that the surface layer of the microorganism has to have a conducive surface (e.g. cytochromes or forming nanowire (pili)). Thus, microbial community that is able to release electrons from degradable substrates in addition to consume easily oxidizable organic substrates are very crucial feature that have to be considered.

The oxygen reduction reaction in neutral media can be facilitated by the utilization ***of enzymes, microbes or abiotic catalysts***. An ideal electron mediator for converting metabolic power into electricity should, form a reversible redox couple at the electrode, have a high negative E^0 to maximize electrical energy generation, be stable in both the oxidized and reduced form and should not decompose during long-term redox cycling.

The ***abiotic catalysts*** adopted in MFCs are mainly based on ***platinum-based materials, carbonaceous (metal-free) materials and platinum-group-metal free (PGM-free) based materials on a carbon support***. [177]•. The oxidation-reduction reaction involving abiotic catalysts can follow two different pathways (acidic and alkaline):

- The ***acidic*** pathway has the intermediate production of H_2O_2 (involving $2e^-$) with the final product being H_2O (involving 2 more electrons i.e. a total of $4e^-$ involved).

• **[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

• **[204] D. H. Park, et al., Electricity production in biofuel cell using modified graphite electrode with Neutral Red, Biotechnology Letters, 22: 1301–1304, 2000.**

• **[205] D. Z. Khater, et al., Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 15, 127–137, 2017.**

• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.**

- The ***alkaline*** pathway on the other hand, involves OH^- and it has the intermediate production of HO_2^- þ OH^- (involving $2e^-$) with the final product being OH^-. (Involving again 2 more electrons i.e. a total of $4e^-$ involved).

A $4e^-$ transfer mechanism is preferred since double the amount of electrons is transferred with the utilization of half of the reactant (oxygen) amount.

The slow oxygen reduction kinetic on plain carbon-based electrode could utilize ***Potassium Ferricyanide (K3 [Fe (CN) 6])*** as a catalyst. Although oxygen does not adequately oxidize K3 [Fe (CN) 6] and its regeneration could be a problem; needed to be refilled periodically, Ferricyanide has been advantageous for its low over potential on plain carbon electrodes. However, Ferricyanide have proved to be hazardous to environment, limiting its appeal to be used in MFCs. [175]•.

Other abiotic catalysts used in MFCs can be grouped in three main categories according to ***the presence or absence of platinum and the presence or absence of earth abundant metals.*** These categories are:

- **Platinum-based (PGM-based)** with a $4e^-$ transfer mechanism,
- **Carbonaceous-based (metal-free)** with a $2e^-$ transfer mechanism,
- **Platinum-group metal- free** (PGM-free) with a more complex electron transfer mechanism ($2e^-$ or $2x2e^-$ or $4e^-$).

A frequently used abiotic catalyst is Platinum owing to the cathodic reaction. However, due to its poisoning sensitivity toward some substances in the substrate solution, platinum must be used in trivial amounts as catalyst in MFCs.

Carbonaceous materials used as cathode catalysts are generally based on **graphene** (the most expensive among all the carbonaceous materials), activated carbon, carbon nanotubes, and carbon nanofibers. Among the above-mentioned carbonaceous materials, **activated carbon (AC)** is the most used catalyst for oxidation-reduction reaction in MFCs.

Other abiotic electron redox mediators also were tested in cathode compartment, such as ***potassium permanganate***; previous research have shown that low concentration of potassium permanganate as the oxidizing agent had a very good ability to increase the current, power, and voltage in MFC.

It is also possible as mentioned previously to use biocathodes in order to overcome the requirement for catalysis by oxygen oxidation on the cathode, ***in which cathodic reactions are catalyzed using microorganisms, this*** improves electricity production in a MFC. This biocathode replaces a wide spectrum of catalysts including: **nitrate, sulfate, tetrachloroethene, fumarate, perchlorate, and trichloroethene, O_2, Ferricyanide, H_2O_2, Acidic permanganate, Acidic hexavalent chromium, Co_2, H^+, $Fe^{(III)}$, $Cr^{(VI)}$, $U^{(VI)}$ or $Mn^{(IV)}$.**[175•; 196•].

Several methods are used to apply the catalyst into the cathode. These include spraying technique, drop casting, pressing and rolling. The first two techniques are based on a preparation of a slurry or liquid solution that is applied on the current collector using a spray gun, or directly through drops release respectively. ***Rolling and pressing techniques for preparing the cathode are the most common techniques of applying catalyst to the electrode surface. In both techniques, AC is***

• **[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

• **Ibid**

• **[196] G. Wang, et al., Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells, Biotechnol Lett, 30:1959–1966, 2008.**

previously mixed with a binder, generally polytetrafluoroethylene (PTFE), and then applied on the current collector using pressure.

Unfortunately, most soluble mediators cannot be easily bound to cells, and all must be continually added or recycled. This problem can be solved by immobilizing the electron mediator on the electrode. [207]•.

Catalysts can also be added to the anode chamber, as it has been reported that mediators such as **methylene blue (MB)**, **neutral red (NR), thionin, ferricyanide, humic acid or methyl viologenare** artificially added to anode chamber, improved the performance of MFC. [206]•. Park & Zeikus (1999) have previously shown that **neutral Red (NR)** ***functions as an electronophore for electron transfer across the cytoplasmic membrane***, enabling the microbe to use electrical reducing power for both growth and metabolite production. It is also reported that the electricity production was increased 10 fold higher in biofuel cell using NR as an electron mediator. [207]•.

[4.4] Membranes

Membranes are of significant importance in MFCs, they physically separate the cathodic and anodic chambers and biological reactions (cathode for a single-chamber MFC) However, besides the advantages of using membranes, utilizing them can bring some drawbacks. [175]•.

MFCs work on the principle of ***similar metals in dissimilar solutions, or dissimilar metals in identical solutions, since liquid electrolytes are used in the anode and cathode, provided that the cathode is not open to air. These liquid solutions contain ions, and so the ion-exchange-membrane is in principle not an essential requirement, provided that the anode and cathode are either dissimilar***, which is the equivalent to electrochemical separation, or they are physically a certain distance apart, so that short-circuit can be avoided.[177]•. If this is not the case, protons produced in the anode chamber should be transferred via a membrane, unfortunately this membrane prevents the *permeation of substrate and oxygen. Thus, removing the membrane will improve the penetration of substrate and oxygen*. Moreover, using a membrane, which increase pH in the cathode chamber and reducing it in anode chamber will consequently, decrease the performance and system stability. In addition to decrease in conductivity ,capacity of ion transfer and diffusion coefficient due to Biofilm enrichment during long-time operation which will hinder membrane fouling. These factors affect MFC performance negatively, reduce electricity generation and finally enlarge operation costs due to membrane replacement.

All these factors along with amplification of overall internal resistance and the overall cost of MFCs, outweighs membrane benefits and make it more attempting to remove it from MFCs design, to overcome these problems, various membranes were developed continuously in the past decade. The materials of diverse membranes can be classified into three categories in terms of their traits of filtration as follows: **salt bridge, size-selective separators, Ion exchange membranes (IEMs)**.

A typical ion exchange membrane is based on the sort of ionic groups fastened to the membrane matrix; these could be in two major categories:

• **[207] D. H. Park, J. G. Zeikus, Improved Fuel Cell and Electrode Designs for Producing Electricity from Microbial Degradation, East Lansing, 2002.**

• **[206] D. Permana, et al., Preliminary Investigation of Electricity Production Using Dual Chamber Microbial Fuel Cell (DCMFC) with Saccharomyces cerevisiae as Biocatalyst and Methylene Blue as an Electron Mediator, Procedia Chemistry, 17. 36 – 43, 2015.**

• **Op.cit.**

• **[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**

• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.**

- **Anion exchange membranes. AEM**: **this type of membranes *utilizes carbonate and phosphate as pH buffer to improve proton transfer***. This type is used when the substrate permeability is commonly higher than Cation exchange membrane (CEM). AEM includes ions with positive charges (e.g. –PR +3⁻ ,SR +2⁻ ,NH+3 ,COO) that join the membrane and transmit anions through it. [175]•.
- **Cation exchange membranes. CEM**: CEMs influence MFC performance significantly, they mainly transport produced protons to cathode chamber in MFCs. Moreover, CEMs must be able to prevent the transfer of other materials such as substrate or oxygen from anode and cathode compartment. CEM is a widespread ion-penetrable membrane that transfer positive charges through it. ***Groups with negative charges are inclusive cation exchange membranes (e.g. –PO 3, –COO, –C6H4O) attached to the backbone of membrane, which subsequently permit the cations to pass through them***, but in contrast, negative ions are refused. Hence, they are often referred to as ***proton exchange membranes (PEMs)*** which have been extensively utilized as membranes in MFCs. due to low internal resistance and high conductivity of cations. Different kinds of materials were used as CEM in MFCs such as, ultrex, Nafion, bipolar membranes, dialyzed membrane, glass wool, nano-porous filters and microfiltration membranes. [175]•.

For instance, **Nafion** is one of the most common CEMs in MFCs; due to the existence of the negatively charged sulfonate groups, which enables Nafion to provide high conductivity to different kinds of cations. Nafions used as CEMs enhances MFCs performance as they are of more lifetime, and have an appropriate level of hydration and thickness, however, Nafion is not suitable for neutral pH and in the presence of cation species such as Na+, K+, and NH4+. Other alternatives are **Zirfon and Hyflon** that are used as CEMs. Zirfon is a macro porous organ mineral material. In comparison with Nafion, Zirfon is of higher oxygen permeability, that is detrimental to anodic reactions, but its specific resistance is much lower in conductivity and chemical stability than Nafion, Hyflon is better than Nafion; yet, it demonstrated larger internal resistance in comparison with Nafion.

Recent efforts have focused on nanoparticle and nanofibers membrane as they are cost effective materials, these separators generally integrated in: **ultrafiltration membrane (UFM)**, **salt bridge**, **bipolar membrane (BPM)** which *consists of two mono polar membranes (CEM and AEM)*.

For bipolar membranes, metallic materials have been vastly exploited for their electrical conductivity and chemical stability. In addition, Graphite was used for bipolar membrane due to its easy fabrication, good conductivity and low density.

In fact, bipolar membranes play crucial role in the stack version of MFC, for five typical functions that they perform that supports at least two adjacent cells in stack design. These functions are: to detach single cells in the stack, to facilitate water management inside the cell, to transport current away from the cell, to distribute fuel and oxidants of fuel inside the cell and to facilitate heat management.[175]•.

However, recent studies have showed that the use of proton exchange membranes in most MFCs causes ***accumulation of cations on the anode side because these selective materials favor the passage of proton***. Non-proton cations that migrate toward the cathode tend to be stopped at the anolyte

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• **[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**
• **Ibid**
• **Ibid**

membrane interface. ***During prolonged operations, the build-up of cation concentration near the membrane surface in turn produces a large resistance to the passage of proton, and thus the flow of current***, leading to a rapid acidification of the anolyte. To overcome this, gel membranes were developed, e.g. PVA-H gel. These gel membranes serve in lowering the cost and mitigating acidification during prolonged operations. [208]•.

Recently, rapid fabrication techniques, ***such as 3D printing***, have been employed extensively for bespoke MFC architectures, designed for specific practical applications, which can have the dual purpose of being the structure as well as the ion-exchange bridge. ***Rapid prototyping using 3D printing can also have the advantage of monolithic, complete MFC reactors, which widens the range of applications and environments***. [177]•.

[4.5] MFC Media

Anolyte components not only act as feed for microbial culture but also determines the economic viability and performance output of MFCs. Thus, optimizing media components is a crucial step in determining MFCs overall performance, efficacy and economic feasibility of the process. [209•; 177•].

[4.6] MFC Organisms (biocatalysts)

The microbial electro-catalysts of bio-electrochemical systems have been identified in a large variety of natural ecosystems such as: soils, sediments, seawater, also in samples collected from a wide range of different microbial-rich environments such as sewage sludge, activated sludge, or industrial and domestic effluents. ***Microbes produce more electricity in the stationary growth phase than in the exponential growth phase because***, ***during rapid growth, cell synthesis competes with the electrode for transfer of electrons. The electrochemically active microbe cell*** *(bacteria, fungi) referred to* ***as electricigens, anodophillic and exoelectrogens*** on the anode *are attached and colonized on the surface of anode forming a biofilm which is called* ***EA biofilms-electrochemically-active biofilms****,* ***which act as a biocatalyst that is able to degrade the substrate and generate electricity***.[205]•.

Many bacterial strains have been identified to be potent to generate electricity in MFCs, most of which are ***metal-reducing bacteria***, include ***Geobacter sulfurreducens, Geobacter metallireducens, Shewanella putrefaciens, Clostridium butyricum, Rhodoferax ferrireducens, and Aeromonas hydrophila .***

Since, electron transfer in MFC occurs by either direct electron transfer or indirect **electron transfer**, as mentioned before, direct transfer occurs by **direct contact between the outer membrane of the electroactive microbe and the surface of the anode**. This is performed by exchange of electrons, between the cell and the electrode, through the cytochrome membrane proteins. Additional direct transfer mechanism has been described in 2005, which occurs ***via extracellular conductive connections called conductive pili or bacterial nanowires in case of bacterial biocatalyst.***

• **[208] H. Liu, et al., Scale-up of membrane-free single-chamber microbial fuel cells, 2008.**
• **[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications, A review, Journal of Power Sources, 356, 2017.**
• **[209] C. Yuvraj, V. Aranganathan, Enhancement of Voltage Generation Using Isolated Dissimilatory Iron-Reducing (DIR) Bacteria Klebsiella pneumoniae in Microbial Fuel Cell, Arab J Sci Eng, 42:65–73, 2017.**
• **Op.cit.**
• **[205] D. Z. Khater, et al., Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 15, 127–137, 2017.**

Alternatively, the other method is by **indirect electron transfer** may also be performed **via the oxidation of a by-product resulting from microbial metabolism**, an example is the **hydrogen** produced by **fermentative bacteria** and which is then oxidized at the surface of the anode. [198].•

Transplanting of EA biofilms is a technique of cultivation of successive generations of biofilms on a solid or porous conductive support (electrodes). Biofilms used may be mature communities collected from natural environments or from an already colonized electrode. This strategy allows less microbial diversity within the biofilm, by eliminating non-EA or non-attached to electrodes microbes (planktonic microbes).

The EA biofilms formation has been performed in many modes such as ***batch, fed-batch or continuous-flow modes***. ***Fed-batch mode is often conducted by adding the inoculum as part of the medium in batch mode or inside a recirculation loop for 24 h to a few days when the electrical output falls below a baseline, or by replacing the whole solution or a part of the solution***. The continuous feeding phase is then performed by providing the reactor with only the carbon-energy medium. The repeated addition of an inoculum is in some cases necessary in the first batches, and then only fresh medium is added in further replenishments. However, ***Continuous mode*** ensures a more stable electrolyte environment and allows controlled changes in its composition.

There are methods that optimize the performance of MFCs in terms of acclimation of the EA species, these are: ***the addition of vitamins, essential nutrients, substrates***, ***the absence of oxygen i.e. anaerobic conditions in the anode compartment or electrode, the specific elimination of other contaminant microbial groups*** by physical methods (ultrasound, temperature, etc.) or by chemical methods (antibiotics, fungicides, etc.), and ***the chemical modification of the inoculum*** (change in conductivity, pH, etc.).

It has also been recently shown that electricity generation in a MFC resulted in large part from the production of mediators, or electron shuttles, by a microbial community consisting of certain species of bacteria including: ***Alcaligenes faecalis, Enterococcus faecium, Pseudomonas aeruginosa*** [197]•, ***Pseudomonas alcaliphila, Shewanella oneidensis***, these strains can produce their own **redox mediators**. For instance in case of employing ***S. oneidensis*** as the biocatalyst, the production of a **quinone** mediator by the strain increases by a factor of 2 the power density of a MFC compared with a MFC without the mediator. [210]•.

[5] MFC electricity

MFC electrical aspects and efficiency is tested through main parameters; these parameters include: ***open circuit voltage, closed circuit voltage at different external louds, current density and power density at different external louds***, power curve and most important ***polarization curve***. In order to estimate these aspects, there are some important terms and relations explained in this section including:

• **[198] M. Di Lorenzo, et al., A single-chamber microbial fuel cell as a biosensor for wastewaters, water research, 43. 3145–3154-p3146, 2009.**

• **[197] H. Liu, et al., Production of Electricity from Acetate or Butyrate Using a Single-Chamber Microbial Fuel Cell, Environ. Sci. Technol, 39, 658-662, p.658, Environmental Science and Technology, VOL. 39, NO. 2, 2005.**

• **[210] D. H. Park, J. G. Zeikus, Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens, Appl Microbiol Biotechnol, 59:58–61, 2002.**

-Potential (Voltage) E: Each fuel cell provides a voltage dependent on operating conditions such as ***temperature, applied load, and fuel/oxidant flow rates***. *The standard measure of performance for fuel cell systems is the* ***polarization curve***, ***which represents the cell voltage behavior against operating current density.***

-Open-circuit voltage *(OCV)*: is the difference of electrical potential between two terminals of a device when disconnected from any circuit when there is no external load connected and no external electric current flows between the terminals. Open-circuit voltage may be thought of as the voltage that must be applied to a battery to stop the flow of current. It is sometimes given the symbol Voc. [211]• OCV is used to maximize produced power and obtain the maximum current density.

-Voltage losses: these losses are the electrical energy obtained from a fuel cell only when current is drawn, and the cell voltage drops due to several irreversible loss mechanisms. The loss is defined as the change in the cell potential (V_{irrev}) from the theoretical potential (V_{rev}) and is calculated by the following Equation: [5];

$$V(i) = V_{rev} - V_{irrev}$$

Equation: [5]: Total Voltage Loses Calculation

The actual open circuit voltage of a microbial fuel cell is lower than the theoretical model due to species crossover from one electrode through the electrolyte and internal currents. The three major classifications of losses that result in the drop from open circuit voltage are: (1) Activation polarization, (2) Ohmic polarization, (3) Concentration polarization. Therefore, the operating voltage of the cell can be represented as the departure from ideal voltage caused by these polarizations in following equation: Equation: [6];

$$V\ (i) = V_{rev} - V_{act-anode} - V_{act-cathode} - V_{ohmic} - V_{conc-anode} - V_{conc-cathode}$$

Equation: [6]: Operating Voltage of the Cell

Where $V_{act}, V_{ohmic}, V_{conc}$ represent activation, ohmic (resistive), and mass concentration polarization. Activation and concentration polarization occurs at both the anode and cathode, while the resistive polarization represents ohmic losses throughout the fuel cell.

It is worth mentioning that ***higher voltage production lead to a reducing the cell life*** thus, the voltage generation in microbial fuel cells decreases with time.

In order to get full study of MFC performance there are multiple parameters that can be calculated as follows:

-**Current in MFC**, I (milliampere), could be calculated according to $I = E/R_{ex}$, where E (millivolt) is the voltage and R_{ex} (ohm) is the external resistance.

-**Current density** ***is the electric current per unit area of cross section***. The current density vector is defined as a vector, which its magnitude is the electric current per cross-sectional area at a given point in space; *its direction is of the motion of the charges at this point.* The electric current density is measured in amperes per square meter. [212]•. It is worth mentioning that there is a direct relationship between potential and resistance, while in case of current density the relation is inversely. ***Higher resistance leads to lower the generated current.*** Moreover, ***high internal resistance consumes***

• **[211] http://www.learnabout-electronics.org/resistors_18.php-1/6/2018.**
• **[212] R.G. Lerner, G.L. Trigg, Encyclopedia of Physics (2nd Edition), VHC publishers, 1991.**

amount of power output inside MFCs and low electrochemical activity causing decrease of power generation.

-Power in MFC could be calculated according to P = V x I, **where** P is the power in watt, V is the potential in volts, and I is the current in amperes. The power density is thus calculated from the division of MFC total power output by the surface area of the electrode as $P_d = P / A$, **where** P_d is the power density in Watt per m^2, and A is the surface area of the anode in m^2. [206]•.

-Polarization curve is ***the change of MFC electrode potential from its equilibrium state due to a flow of current***. It represents a great tool for the examination and classification of microbial fuel cells. ***The polarization curve displays the voltage output*** of ***the microbial fuel cell for a given current density loading***. Internal resistance is calculated from the polarization curve, ***whereas the slope of voltage versus current plot is considered***. [205]•.

Polarization curves are usually obtained with a potentiostat/galvanostat, ***which draws a fixed current from the microbial fuel cell and measures the output voltage***, by gradual increasing the load on the potentiostat, the voltage response of the microbial fuel cell can be determined. An alternative primitive method of determining the **current/voltage relationship** from the microbial fuel cell is to take ***several different types of small resistors (to act as a load) and measure the voltage output on a multimeter***. [213]•.

There are three distinct regions of a fuel cell polarization curve:

- ***At low power densities***, the cell potential drops as a result of the activation polarization.
- ***At moderate current densities***, the cell potential decreases linearly with current due to ohmic losses.
- ***At high current densities***, the cell potential drop departs from the linear relationship with current density as a result of a more pronounced concentration polarization.

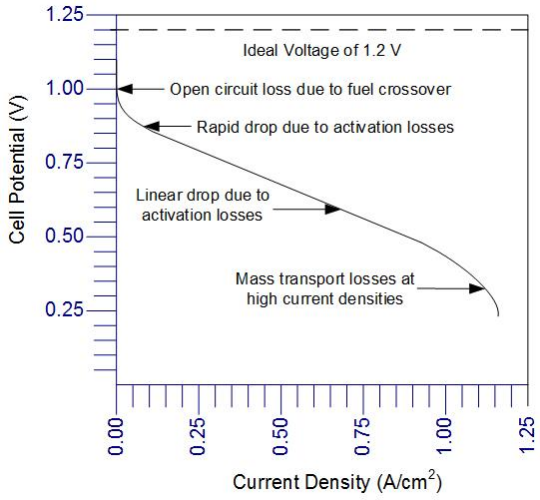

Figure. [46]. Example of a PEMFC polarization curve. [213].

The relationship between the fuel cell potential and current density (fuel cell polarization curve) is given by the following Equation: [7];

• **[206] D. Permana, et al., Preliminary Investigation of Electricity Production Using Dual Chamber Microbial Fuel Cell (DCMFC) with Saccharomyces cerevisiae as Biocatalyst and Methylene Blue as an Electron Mediator, Procedia Chemistry, 17, 2015.**

• **[205] D. Z. Khater, et al., Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 15, 127–137, 2017.**

• **[213] http://www.fuelcellstore.com/blog-section/polarization-curves-1/6/2018.**

$$E = E_r - \frac{RT}{\alpha F}\, ln\left[\frac{i+i_{loss}}{i_0}\right] - \frac{RT}{nF} ln\, \left[\frac{i_l}{i_{L-i}}\right] - iR_i$$[213]•.

Equation: [7]: MFC polarization curve calculation

When the current (load) on a fuel cell is changed, the fuel cell heat and water balance change, and it may take time to reach a new equilibrium point. During testing, a designated time should be used to allow the fuel cell to reach the new equilibrium. The establishment of an equilibrium period varies depending upon whether the fuel cell load has been increased or decreased. ***A typical method for obtaining measurements is beginning at open-circuit voltage and then collecting five to six points between 600 mV/cell and 850 mV/cell and waiting 15 minutes between each point***. The data from the last five minutes should be averaged and then plotted as average current versus average voltage. [213]•

-Coulombic Efficiency (CE), also called ***faradaic efficiency*** or ***current efficiency***, ***describes the charge efficiency by which electrons are transferred in batteries***. ***CE is the ratio of the total charge extracted from the battery to the total charge put into the battery over a full cycle.*** [214]•.

-Degradation rate of organic substrate or Chemical Oxygen Demand (COD) test *is used to determine either the availability of converting fuel in the MFC, into electricity, or growth of biomass*, through competitive reactions with other electron acceptors (e.g., oxygen, nitrate, and sulfate). An increase in COD led to the current production increase, with the decrease in resistance.

[6] MFC Applications

Generation of bioelectricity: MFC is almost the only technology that can ***generate energy out of waste, without the input of external/additional energy,*** and this enables MFCs from remote area access via the robotics or remote power generation (extreme environments colonization e.g. Mars cities).

MFC technology as sensor for pollutant analysis and process monitoring: MFC can directly capture the microbial response and metabolism, ***and produce this as an analogue electrical signal***, which can be used in any environment, compatible with the microbes of choice. [177]•. Thus, ***MFCs are suitable for powering electrochemical sensors and small telemetry systems to transmit obtained signals to remote receivers*** e.g. MFCs as biological oxygen demand (BOD) sensor.

Wastewater treatment: MFC technology was considered to be used for wastewater treatment early in 1991. MFC captures energy in the form of electricity or hydrogen gas .In addition to its high operational sustainability and low material costs. ***The antagonism of the established biofilm on the anode electrode against any non-electroactive pathogenic organism results in the elimination of pathogens simply by exposure to the MFC environment,*** *which can improve sanitation;* this is of particular interest for countries and regions of the Developing World. [177]•. Therefore, from the environmental and economic point of view, MFCs could be exploited as a bi-functional system to facilitate simultaneous wastewater treatment and electricity generation.

•[213] http://www.fuelcellstore.com/blog-section/polarization-curves-1/6/2018.
• Ibid.
[214]http://batteryuniversity.com/learn/article/bu_808c_coulombic_and_energy_efficiency_with_the_battery-1/6/2018.
•[177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.
• Ibid

Bio hydrogen production: MFCs can be readily adjusted to the harvest of bio hydrogen, instead of producing electricity. Hydrogen can be accumulated for later application. [175]•.

[7] MFC implementation in practical applications

Since the emergence of MFC technology, there were countable manifesting projects that employed MFC in domestic application moving from just a laboratorial device. These early proof-of-concept projects showed that it is possible to have artificial agents powered by microbial metabolism inside MFCs, but did not demonstrate the essential element of self-sustainability (or energy autonomy), since human intervention was still required to either replenish/replace chemicals and feed the MFCs.

The first demonstration of a self-sustainable MFC stack, exhibited sufficient energy to power its own feeding and hydration pumps and sustain long-term operation; other projects functioned in the charging of a basic mobile phone and more recently LEDs for interior lighting.

[8] MFC implementation in architectural and interior design

1. Project name **Living Architecture (LIAR)**
Project date **April 2016**
Project field **Power generation (electricity), environmental purification (wastewater treatment), Architectural and interior design.**
Project team **UNEW, UWE, CSIC, UNITN, (EXP, LSG).**

Description

LIAR is a modular bioreactor-wall, based on the operational principles of MFC technology and synthetic 'consortia' of microbes. It is conceived as a next-generation selectively programmable bioreactor that can be an integral component of human dwelling. [215]•. The project is capable of extracting valuable resources from sunlight, wastewater and air and utilize them to generate oxygen, proteins and biomass by manipulating consortia performance. Its operational principles are grounded in distributed sensing, **decentralized autonomous information processing**, high-degree of fault-tolerance and distributed actuation and reconfiguration.

The project's applications within (existing) urban systems can include: customizable micro-agriculture for installation in domestic, public (schools, hospitals) and office environments, the improvement of building performance through resilience and resource recycling, a mediator between the built environment and local ecosystems. The project establishes Protocols for 'synthetic ecosystem' design and engineering, Foundational concepts for computationally processing, recycling, remediating and synthesizing valuable compounds from wastewater.

LIAR Prototype take the form of a modular wall-like partition, installed into a building interior. It is exposed to sunlight and receives resources from flue exhaust and grey water which it treats by removing pollutants (CO2, N2O, organic matter) from domestic waste products and turn into sustainable resources (fresh water, polyphosphate, O2) and small amounts of consumable products

•[175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 2015.

• [215] A. R, Ferracina, et al., Living Architecture (LIAR): Metabolically engineered building units, In: Heisel, F; Hebel, D, ed. Cultivated Building Materials: Industrialized Natural Resources for Architecture and Construction, Berlin, Germany: Birkhauser, 2017.

(fertilizers, biodegradable detergents). The LIAR 'system' is housed within 'block reactors' each operating according to the complex interweaving of 'green' and 'grey' technologies. Its structural and operational flexibility allows it to be modified for a variety of contexts, both indoor and out, including use as a building façade.

A freestanding partition composed of bioreactor 'building blocks' was prototyped. The 'building blocks' are conceived as standardized building segments and can be incorporated into common building construction methods. The outcome are two bioreactor 'building blocks'; one, a programmed and configured Microbial Fuel Cell (MFC) to produce electricity, the other to purify air and water.

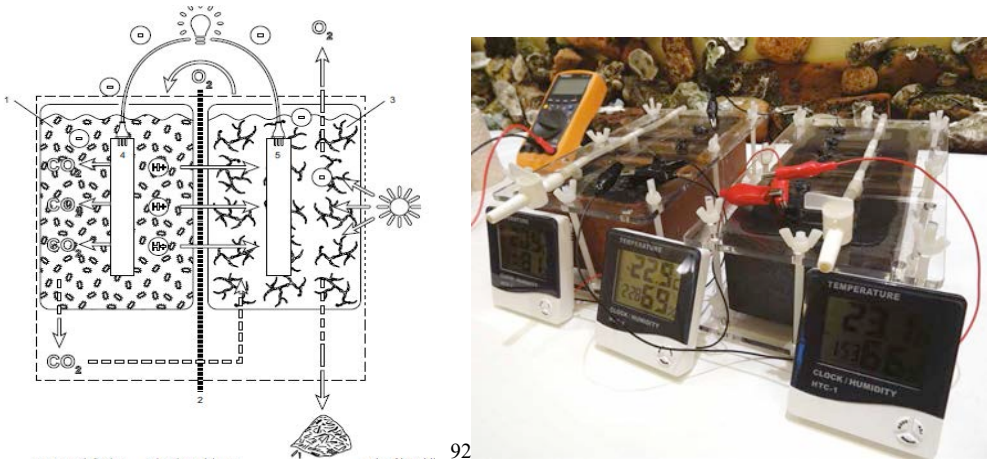

Figure. [47]. Left, Scheme of the intended final configuration of the microbial fuel cell (MFC) with a photocathode used as a modular building block wherein bacteria generate bioelectricity through the oxidation of organic wastes that can be coupled with renewable biomass. Image. [92]. The microbial fuel cell (MFC) in operation, as exhibited at the Biennale, 15th International Architecture Exhibition, in Venice in 2016.

An array of bioreactors act in parallel to a computer that is capable of both sensing local conditions within a building and controlling the bioreactor system to optimize the building's environmental impact. .[216]•

*A photo-bioreactor is a device that can be programmed to utilize a variety of inputs such as: grey water, microbial consortia (algae and bacteria), nitrous oxide and carbon dioxide, visible light, temperature, different types of nutrient (nitrogen and phosphate as macronutrients, trace metals and vitamins as micronutrients). To generate outputs such as; "polished" water, fertilizer, extractable products (recoverable phosphate), oxygen, next generation biodegradable detergents, electricity, recoverable biomass, bio-fluorescence and to a certain extent, heat. [217]•.

2.Project name	**BIOCLAD: Adaptive Biodigital Cladding System**
Project field	**Energy production, food production, biosensor, environmental purification Architectural design, bio façade design.**
Project date	**2014**
Project team	**F. Borello, C. Griffa, R. Giordano. Biologic Consultant: M. Tredici.**

• **[216] http://livingarchitecture-h2020.eu/project-description**
•**[217] http://emciblab.com/-www.liquifer.at-www.explora-biotech.com.**

Description

BIOCLAD is an adaptive biodigital cladding system for the cultivation of microalgae able to transform the solar energy into chemical energy, through the photosynthetic process, fixing CO2 and producing O2. Microalgae function as a sort of micro bio refinery, from their biomass can be extracted *proteins for the food industry, omega 3 and amino acids* for the nutraceutical industry, cosmetic and pharmaceutical molecules, in addition to bio plastics and biofuels such as ethanol and biodiesel.

The system is based on the allocation of a sensor system that is able to map in real time the environmental conditions and use data as input for mechanical and biological transformations, allowing propagation of the autonomous intelligence of the single component to a system of components as distributed intelligence. This ability to self-management and self-definition provided by the system of sensors and solenoid valves synchronizes with the natural biological capacity of microorganisms within the components to adapt to environmental conditions by carrying out metabolic processes of growth and oxygen production. [218]•.

Image. [93]. BIOCLAD: Adaptive Biodigital Cladding System façade design.

The project is also based on the integration of self-specificity of the microbiological processes of algal cells, and the benefits that can be obtained at the architectural and urban scale through the development of coating opaque envelopes. The process of defining the project was done through the following steps:

-The geometric definition of the component using parametric modeling techniques, controlling complex NURBS surfaces, patterns and gradients via attractor points.

-The definition of the hydraulic system necessary for the management and distribution of algal solution within the entire system. The project proposal was manufactured as a prototype in 1: 5 scale of a series of four components: using digital manufacturing technologies in numeric control, laser cutting and thermo-vacuum forming. The prototype was also equipped with an artificial intelligence by use of an Arduino platform and the application of a system of sensors (temperature and light intensity) able to simulate the actual properties of the adaptive real technological system.

•[218] https://federicoborello.com/2014/10/09/bioclad-adaptive-biodigital-cladding-system

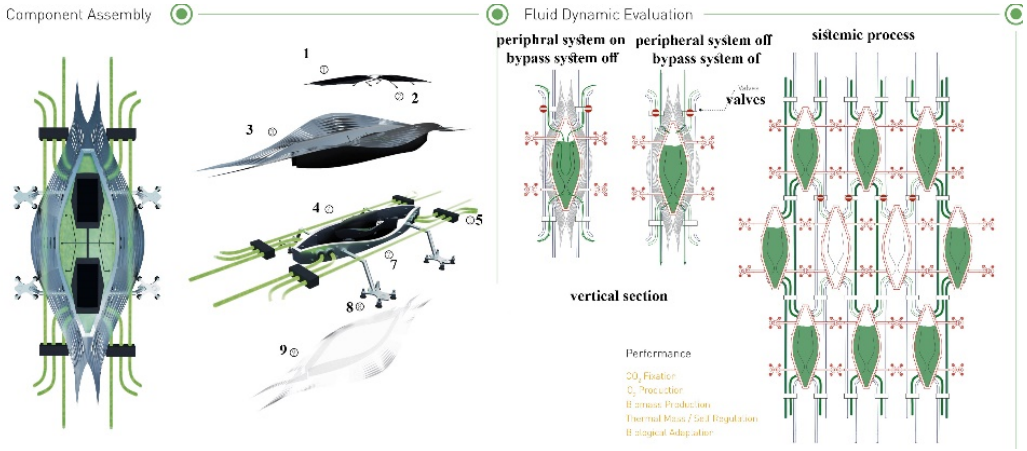

Figure. [48]. Left, Single component explosion [1.Amorphic photovoltaic cells, 2.sensors system co_2, temperature, solar radiation, 3.covering shell, 4 .secondary piping system (bypass), 5.primary piping system (peripheral), 6.flow controller, 7.structural core, 8.fixing structural elements, 9.covering shell, 10.valves]. Figure. [49]. Right, fluid dynamic evaluation. [219]•.

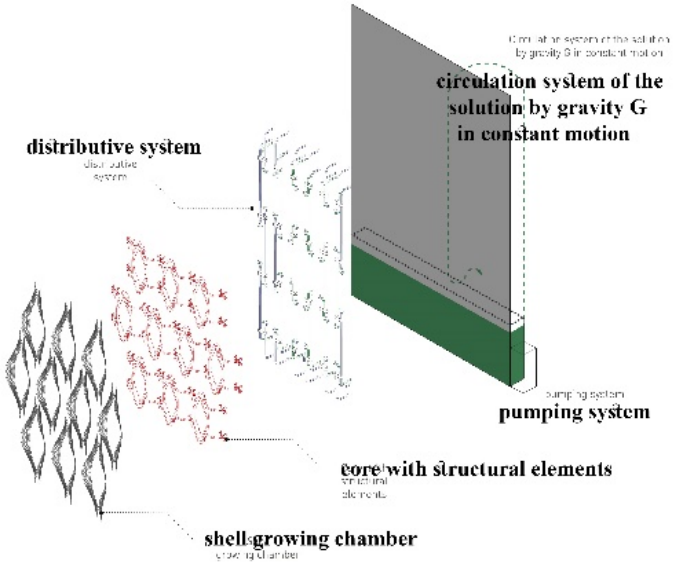

Figure. [50]. Circulation system of the Bio-clad façade.

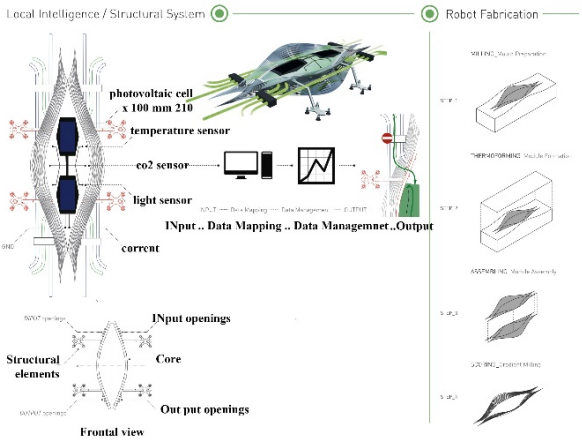

Figure. [51]. left, local intelligence system for environmental data mapping through sensors and output transformation / adaptation, right fabrication process.

• **[219] https://federicoborello.files.wordpress.com/2014/10/presentation_12.png.**

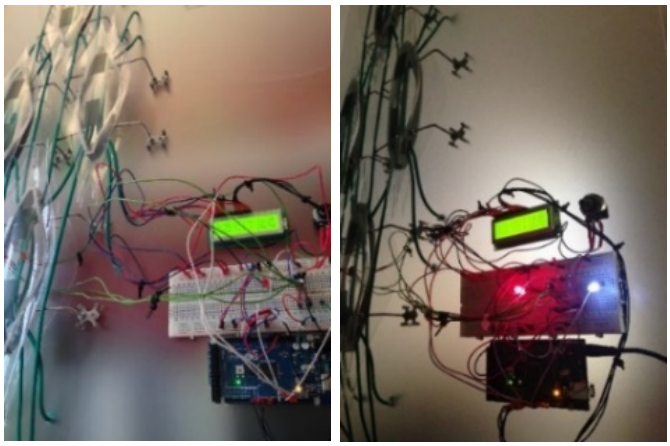

Image. [94]. Final 1:1 prototype of the Bio-Clad system [220]•.

3.Project name **Bacteria-Activated Wallpaper**
Project field **Electricity generation**
Project team Imperial College London (ICL), University of Cambridge. 2017

Description

The project takes the form of wallpaper-like covering that acts as both a solar bio-battery and solar panel, and it is environmentally friendly. An inkjet printer was used to print photosynthetic microorganisms called *cyanobacteria* onto conductive carbon nanotubes, all of which were then printed onto paper. The printed cyanobacteria continue to perform photosynthesis, creating small amounts of electricity, which could power a small digital clock or LED lamp. [221]•.

Image. [95]. The bacteria activated wall paper detail

This project furthers current research into microbial *bio photoltaics (BPV)*, considered to be a new alternative renewable energy technology. While current BPVs are still expensive to make, have low-power output, and relatively short lifespans, the organisms photovoltaic cells are capable of producing electricity in the dark from energy molecules produced in the daylight. Paper-based BPVs could be used to construct power supplies that are both disposable and biodegradable. Their low power output means they are more suited to devices and applications such as environmental sensing and biosensors.

• **[220] http://www.architesi.polito.it/de_aglio_tesi.asp?id_tesi=24528.**
• **[221] http://www.architectmagazine.com/practice/bacteria-activated-wallpaper-that-generates-electricity.**

[9] Conclusions

1. Microbial fuel cell (MFCs) is type of Fuel Cells that uses a wide variety of substrates, materials, and system architectures with bacteria or fungus as an active biocatalyst in an anaerobic anode compartment for production of bioelectricity. ***The use of whole microbial cells in MFC for the bio electrochemical oxidation of fuels is advantageous as it eliminates the need for enzyme isolation and still allows multiple enzymatic reactions to take place in conditions close to their natural*** **environment**. ***With the organisms regenerating the required enzymes as part of their natural life.***
2. Artificial (mediators) are used to carry electrons from inside the cell to an external electrode, however, the need for high concentrations of electron carriers, which are toxic chemicals, is thought to make electricity generation on a large scale impractical. Some bacterial strains, such as ***Shewanella putrefaciens*** can produce their own mediators that can eliminate the need to add mediators.
3. *There are several advantages of using a single-chamber MFC versus a two-chambered system including; increased mass transfer to the cathode, decreased operating costs, an overall decrease in reactor volume and a simplified design.*
4. Power output is increased in a single-chamber MFC by removing the PEM, the formation of an aerobic biofilm on the cathode inner surface removes any oxygen that diffuses into the chamber, preventing the loss of anaerobic conditions in the anode chamber.
5. *Electrodes are essential in enhancing the MFC efficacy. Hence, ideal electrode materials should be of certain features such as good electrical conductivity and low resistance; strong biocompatibility; chemical stability and anti-corrosion; large surface area; and appropriate mechanical strength and toughness.*
6. *Microbial cellular level attachment to anode electrode surface can be improved by changing the surface chemistry such as; surface charge with positive charges, utilizing hydro phillicity /hydrophobicity surfaces during organism attachment, adding oxygen or nitrogen functional groups that facilitate organism surface interaction, developing immobilized mediators, controlling surface morphology and the roughness at the Nano micro- scale. In addition, employing different chemical treatments, surface coatings, electrochemical treatments, and thermal treatments.*
7. On the biofilm-level formation on the electrode, surface morphology plays a key role in the biofilm growth and the current produced. Moving from a flat 2D surface towards a 3D electrode material in order to increase the available surface area and enhance the bio-interface between organism and electrode.
8. At the system-level, the anode electrodes have to be designed in order to avoid clogging or dead zone. This is mainly related with the design of the reactor and the overall system in order to guarantee long time operation.
9. The advantages of using carbon-based anode materials include low cost, biocompatibility, excellent electrical conductivity and chemical stability, accessible surface area.
10. An important advantage of biocathodes is generating practical products or removing by-products using microbial metabolism. In addition, **obviating challenges such as the requirement for electron mediators that have poisonous effect and** enhancing the MFC sustainability.

11. The oxygen reduction reaction (ORR) in neutral media can be facilitated by the ***utilization of enzymes, microbes or abiotic catalysts***. Abiotic catalysts are mainly based on ***platinum-based materials***, ***carbonaceous materials and platinum-group-metal free***-based ***materials***. However, noble metal and enzyme catalysts are expensive and can deteriorate rapidly under the moist and often corrosive conditions of MFCs operation. ***Instead of applying a purified enzyme to the cathode, growing a laccase secreting fungus in the cathode chamber increases the power generation of MFCs.***
12. The surface layer of the microorganism has to have a conducive surface (e.g. cytochromes or forming nanowire (pili) .
13. Rapid fabrication techniques, such as 3D printing, have been employed extensively for bespoke MFC architectures, which can have the dual purpose of being the structure as well as the ion-exchange bridge.
14. Biofilm enrichment during the long-time operation of MFC contracts with the PEM as it decreases its conductivity, and capacity of ion transfer; these factors affect MFC performance negatively, reduce electricity generation and finally enlarge operation costs due to PEM replacement.

CHAPTER 4

MFC Experimentation
Bioelectricity generation from Microorganisms

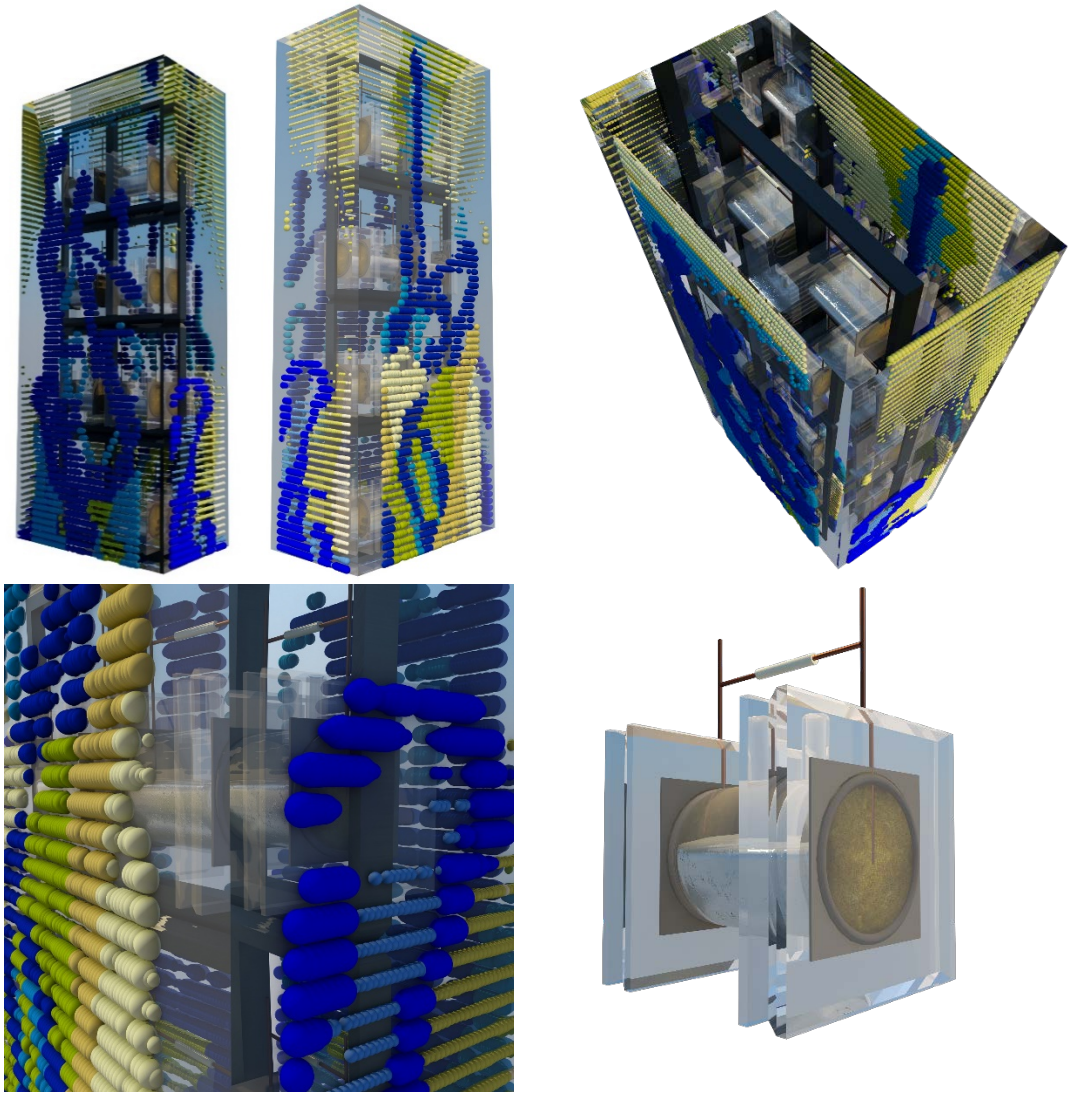

Image. [96]. Self-sufficient unit (cluster) for bioelectricity production using a system of MFCs employing *Aspergillus sydowii* NYKA 510 as the bio catalyst, and employing biodigital design procedures in the unite form generation simulating the formal pattern of the fungal strain culture. Up-left, two different perspective views of the self-sufficient unit; exhibiting the different patterns around 360 degrees. Up-right, unit's top view perspective showing the two stacks of MFCs. Bottom-left, detail of silicon rubber spheres fixed on the perforated transparent glass container that shield the inner system of MFCs and gives the unit is formal pattern. Bottom-right, the MFC (bio battery) model showing the accumulation of the fungal spores on the anode and the composition of the biofilm (yellowish white thin layer on top of the medium inside the MFC). By the author.

Introduction

This chapter is based on the experimental work conducted by the author in order to examine the biocatalysis ability of fungal strains and employ them in MFCs for domestic use. The reached results were published in Journal of Microbiology and Biotechnology, accepted in 27 October 2019, online in 1 November 2019.

As concluded from the previous chapter, microorganisms play crucial role as biocatalysts in electricity production by employing their natural exoelectro-genesis in microbial fuel cells. Recently, MFCs have been contributing to a society with low-carbon, with ability to reduce the dominance of fossil fuels. MFCs can generate chemical energy from different types of wastes. They are capable of converting various organic wastes into electrical energy. The main purpose of MFCs is achieving appropriate power and current to be applied in small electrical gadgets.

For instance, Rahimnejad et al. (2015) [175]• fabricated a stacked MFC as a power source to turn on a digital clock and ten LED lamps. Both devices operated successfully for two days duration.

Moreover, as highlighted from the previous chapter, utilizing microbial cells, as a whole in MFC is advantageous, as there is no need for isolation of enzymes. This allows multiple enzymatic reactions to occur in almost their natural environmental conditions. The organisms can naturally produce the desired enzymes. [177]•.

As exhibited in the previous chapter, a certain type of MFCs is the air cathode, membrane less, single chamber MFC (ACMSCMFC) that has no separate anode or cathode chambers. Here, cathodes are directly exposed to air thus increasing availability of oxygen [185•; 184•]. It has no proton exchange membrane (PEM), to increase power output by minimizing the intrinsic resistance (R_{in}) as mentioned previously. [195•; 191•]. This type of MFCs does not need mediators or electron carriers. There are other several advantages when using the single chambered MFCs such as the decreased costs of operation, increased transfer of mass to the cathode, an overall decrease in volume of reactor and a simplified design. [194•; 195•].

Laccases are blue multicopper oxidases. They can be employed in various applications comprising biofuel cells and biosensors. [222•; 223•]. They are found in fungi, bacteria, plants as well as insects. Fungal laccases are of special interest among these sources. This is due to their ability to break down lignocellulosic biomass. [223]•.

• [175] M. Rahimnejad, et al., Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.

• [177] C. Santoro, et al., Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.

•[185] C.Y. Lai, et al., Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188, 2017.

• [184] H. Liu, B. E. Logan, Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane, Environmental Science & Technology, 38, 2004.

• [195] G.W. Chen, et al., Application of biocathode in microbial fuel cells: cell performance and microbial community, Appl. Microbiol. Biot, 79, 2008.

• [191] Y. Sharma, B. Li, The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs), Bioresource. Technol, 101, 2010.

• [194] M. Rahimnejad, et al., Low voltage power generation in a biofuel cell using anaerobic cultures, World Appl. Sci. J., 6, 2009.

• Index- [195]

•[222] S. H. Kacem, et al., New efficient laccase immobilization strategy using ionic liquids for bio-catalysis and microbial fuel cells applications, Journal of Chemical Technology & Biotechnology, 93, 2017.

• [223] U.N. Dwivedi, et al., Review: structure-function relationship among bacterial, fungal and plant laccases, Journal of Molecular Catalysis B: Enzymatic, 68, 117–128, 2011.

• [223].

This chapter focuses on testing the potential of generating sustainable electricity from fungal strains employing microbial fuel cells; and will exhibit three sets of experimentation as follows:

- Biocatalyst Microorganism survey & selection.
- Biocatalyst growth optimization.
- Bioelectricity generation with microbial fuel cell.

Microbial fuel cell system configuration and indulging in interior design elements will be discussed extensively according to interior design criteria in the following chapters, focusing on interior design manipulation and adjustment of these self-sufficient systems.

Experiments were conducted at Faculty of Science, Cairo University, Department of microbiology facilities.

[1] Material and methods

[1.1] Fungal strains

For the isolation of fungal strains, a soil sample was collected from an agricultural soil inside Cairo University, Giza, Egypt. Isolation of fungal species was accomplished according to Johnson et al. (1960) [224]• by the method of soil dilution plate. The isolated fungal cultures were identified up to the species level according to the morphological characters and microscopic examination. This was achieved through the help of Moubasher (1993) [225]• and Watanabe (2002) [226]•. *Pleurotus ostreatus* was kindly provided from Ain Shams University, while *Saccharomyces cerevisiae* was purchased from the local market. Fungal strains utilized in this work were maintained on potato dextrose agar (PDA) slants and kept at 4ºC.

[1.2] Laccase production survey

The basal medium [227]• was used for screening fungal isolates for laccase production. It contained (g/l): Peptone, 3.0; Glucose, 10.0; KH_2PO_4, 0.6; $ZnSO_4$, 0.001; K_2HPO_4, 0.4; $FeSO_4$,0.0005; $MnSO_4$, 0.05; $MgSO_4$,0.5; Agar,20.0. Triplicate flasks containing 100 ml of basal sterilized medium were inoculated with fungal discs (1 cm in diameter, each) cut from the periphery of cultures (7 days old) grown on PDA plates. These flasks were then incubated for 7 days at 30º C. Culture filtrate was obtained by filtering the liquid culture through Whatman filter paper no. 1. The filtrate was then used for measuring extracellular laccase production.

• **[224] L. F. Johnson, et al., Methods for Studying Soil Mycoflora: Plant Diseases Relationships, Burgess Publishing Co, Minneapolis, pp: 179, 1960.**

• **[225] A. H. Moubasher, Soil Fungi in Qatar and other Arab Countries. The Scientific and Applied Research Centre., First ed. University of Qatar, Doha, Qatar, pp. 566, 1993.**

• **[226] T. Watanabe, Soil and Seed Fungi. Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species, Second ed. CRC Press, Boca Raton London New York Washington D.C, 2002.**

• **[227] V. K. S. Olga, et al., Purification and characterization of the constitutive form of laccase from basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis, Biotechnology and Applied Biochemistry, 28. 47- 54, 1998.**

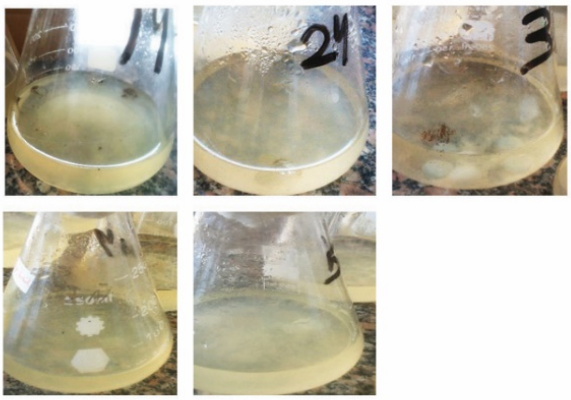

Image. [97]. Erlenmeyer Flasks inoculated with 1 cm diameter discs (1. *A.niger, 2.A.sydowii, 3. Pleurotus ostreatus* 4.penicillum .*5.S.Cerevisiae).* By author.

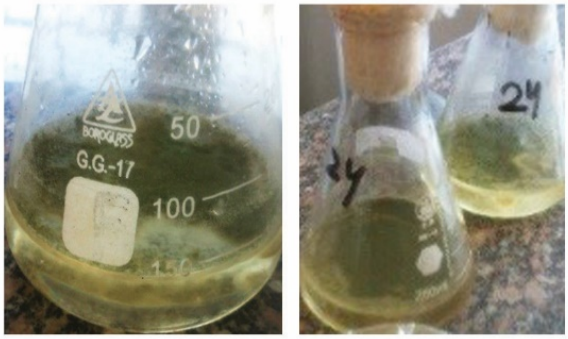

Image. [98]. Pure resulting culture of *Asprigillus sydowii.* By author.

[1.3] Enzyme assay

Guaiacol was utilized as substrate to assay laccase activity. The reaction mixture was composed of 1 ml guaiacol (2 mmol/l), 3.9 ml acetate buffer (10 mmol/l, pH 5.0) and 0.1 ml enzyme solution at the appropriate dilution. The mixture was incubated for 30 min at 35°C. The absorbance was read at 470 nm. Acetate buffer was used to replace guaiacol in the blank [228]• .

Laccase activity unit is expressed in μ mol of substrate oxidized per milliliter per minute, in the following equation: U ml-1 min-1 = ΔAbs (10^6) (ε RT)-1. Where ΔAbs is the difference between final and initial absorbance, ε is the extinction coefficient of laccase product (ε max = 6,740 Mol-1 cm-1), R is the volume in milliliters of supernatant, and T is the reaction time in minutes. [229•; 230•].

• [228] P. Das, et al., Improved bioavailability and biodegradation of a model poly aromatic hydrocarbon by a biosurfactant producing bacterium of marine origin, Chemosphere, 72, 1229–1234, 2008.

• [229] M. I. A. Ali, et al., Biosynthesis of laccase by Aspergillus flavus NG85 isolated from Saint Catherine protectorate, Egypt. J. Bot., Vol. 55, 2015.

•[230] N. Bhuneshwari, et al., Lignocellulolytic Fungal Isolation and Screening for Their Laccase Producing Ability, Indian J.Sci.Res, 13 (2): 188-191, 2017.

Image. [99]. Filtration of different tested fungal strains by Whatman paper (left) or centrifugation for unicellular *Saccharomyces cerevisiae* (right). Image. [100]. From left to right; solution (A), solution (B), the acetate buffer solution (A+B), adjusting pH of the acetate puffer solution to pH4.7 by pH meter. By author.

[1.4] Molecular identification of *Aspergillus sydowii*

Identification of *Aspergillus sydowii* was further confirmed using nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing. The genomic DNA was obtained through the protocol of GeneJet Plant genomic DNA purification Kit (Thermo) # K0791 [231]•. Internal transcribed spacer (ITS) region of 5.8S rRNA was amplified by the primers ITS1 with sequence 5′TCC GTA GGT GAA CCT TGC GG 3′, and ITS4 with sequence 5′TCC TCC GCT TAT TGA TAT GC 3′. PCR sequencing of the amplified product was carried out at GATC Company (Germany). A strain identifier and an accession number were obtained from GenBank of NCBI database.

[1.5] Optimizing growth conditions of *A.sydowii* NYKA 510

Physical and chemical growth conditions of *A.sydowii* NYKA 510 were optimized to reach maximum production of laccase. Czapek-Dox broth medium was prepared which contained (g/l): sucrose, 20; $NaNO_3$, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5 and $FeSO_4.7H_2O$, 0.01. The physical factors included, incubation periods at 2, 4, 6, 8, 10, 12 or 14 days; incubation temperature at 25, 30, 35, 40, or 45 ^oC and pHs at 2, 3, 4, 5, 6, 7 or 8. The chemical factors comprised carbon source: glucose, banana peel, orange peel, potato peel, or cartoon paper; banana peel concentrations at 5, 10, 15, 20, 25, 30 or 35 g/l; the banana, orange and potato peels were prepared by peeling, drying in 60 C, and grounded to powder; the **cartoon paper was** prepared by cutting to 0.5 cm x0.5 cm small squared pieces. Nitrogen source: 2 g/l NaNO3. Sodium nitrate was substituted with other nitrogen sources such as ammonium sulfate (2.1 g/l), peptone (2.64 g/l), and glycine (1.8 g/l). These sources of nitrogen were utilized at equimolecular nitrogen weight; peptone concentrations at, 1, 1.5, 2, 2.5, 3, 3.5 or 4 g/l and metal ions (10 µM) as $MnSO_4.H_2O$, NaCl, $CuSO_4.5H_2O$ or $CaCl_2.2H_2O$. In each tested condition, the

• **[231] http://www.thermoscientificbio.com.**

optimized Czapek-Dox's medium was prepared in triplicate flasks. Fungal discs were used to inoculate the medium and then incubation was under the previous successive treatments.

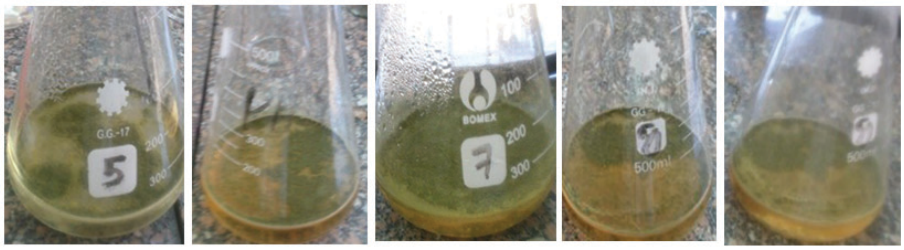

Image. [101]. Different pH *A.sydowii* cultures (from left to right 5, 6, 7, 8, 9). By author.

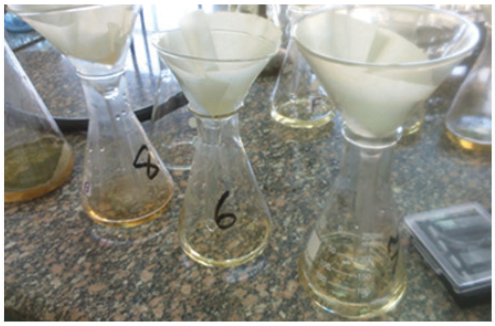

Image. [102]. Filtration process of *A.sydowii* culture by Whatman filtration paper. By author.

Image. [103]. Different types of organic waste (from left to right, banana peel, orange paper, Potato peel, cartoon paper 10 g/100 ml each) as a carbon source in growth media of *A.sydowii.* By author.

Image. [104]. Pure cultures of *A.sydowii* with different Carbon source from waste (from left to right, banana peel, orange peel, cartoon paper 10 g/100 ml each). By author.

Image. [105]. Filteration process of banana peel carbon source culture of *A.sydowii (left),* Filteration process of potato peel carbon source culture of *A.sydowii (right).* By author.

[1.6] ACMSC-MFC performance

A. *sydowii* NYKA 510 was employed as the biocatalyst in air cathode, membrane-less, single chamber microbial fuel cell (ACMSC-MFC). The cell had a volume of 117.25 ml (5 cm diameter, 6 cm length). It was manufactured with the use of the construction material transparent Perspex (Omega, Egypt). The methods of Cheng et al. (2006) [232]• and Khater et al., (2017) [205]• were followed for preparation of carbon cloth anode, carbon paper cathode, diffusion layers treatment with poly-tetrafluoroethylene (PTFE), catalyst ink layer (mainly platinum) for reducing loss of water and diffusion of oxygen to the MFC inducing an increase in power output as well as Coulombic Efficiency. Cathode and anode electrodes were then stationed on opposite sides. Connection of Cathode and anode electrodes was *via* an external circuit through different external resistances.

The optimized fungal growth medium was utilized inside the ACMSC-MFC for growth of *Aspergillus sydowii* NYKA 510 to produce laccase. Optimized medium constituents were: banana peel (15.1 g/L) as carbon source; peptone (2.6 g/l) as nitrogen source; K_2HPO_4, (1 g/l); $MgSO_4.7H_2O$ (0.5 g/l); KCl, (0.5 g/l); $FeSO_4.7H_2O$ (0.01 g/l) and additional metal ions of $CuSO_4.5H_2O$ (10µM) at pH 5.2. To inoculate the cell from the upper cylindrical portal of 1 cm diameter, some steps were followed. First, 10 discs (1cm) obtained from a solid pure culture of *A. sydowii* NYKA 510 fungus were used for the inoculation of 100 ml optimized fungal medium in a 250 ml Erlenmeyer flask. The flask was then incubated for 1 day (24 hours) at 31°C. After that, the flask was shacked gently to disperse spores into the liquid medium. Finally, the inoculated medium was transferred to the ACMSC-MFC using a sterile syringe.

[1.7] MFC operation

The MFC operated for 6 days for each cycle of three open circuit cycles. The ink Pt-loaded side of the cathode was facing the solution and the other side of the cathode was facing air. Anaerobic conditions were set for the anode. A multimeter and data acquisition system (SOOER–SD9205A) was used to record the cell potential between cathode and anode every 24 hours.

Open circuit voltage (OCV) was utilized for maximizing the generated power and to acquire maximum current density. In each cycle of the three open circuit-voltage cycles (6 days each), the addition of fresh medium with inoculations of *Aspergillus sydowii* NYKA 510 dispersed spores was repeated. That was to regenerate the function of MFC, as well as recovering the activity of the anode in the MFC in each cycle by continuing to supplement the anodic electrode with electrochemically active fungus. After stabilization of electricity production, operation of the MFC took place for several closed cycles until the formation of cathode-biofilm and recording the generated electricity of the MFC. After steady state of power and electricity generation, ACMSCMFC was discharged and operated under varied fixed external resistances: 1000 Ω, 2000 Ω or 3000 Ω, to measure the effect of external loads on current, cell potential and power density; also polarization curves were obtained by varying external resistance (R_{ext}).

After replicating the procedure of adding fresh medium with fungal spores in 3 consecutive series, there was establishment of a steady reproducible MFC operation. *A.sydowii* NYKA 510 formed anodic

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• **[232] S. Cheng, et al., Increased performance of single chamber microbial fuel cells using an improved cathode structure, Electrochemistry Communications. 8. 489–494, 2006.**

•**[205] D. Z. Khater, et al., Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 2017.**

biofilm producing electrons, protons and CO_2. It was in close attachment with the anode surface. The anodic bio-film transports electrons to the outside of the cell while protons were transported to O_2 (final hydrogen acceptor).

The current (I), the power (p) and the current density (mAm^{-2}) are calculated from the function of surface area of anode. [233]•. The method of the power density curve. [183]• was followed to procure maximum power density and the internal resistance of the MFC.

[2] Design application

[2.1] Design concept

A design proposal was based on applying biodigital design procedures in form generation of a self-sufficient lighting unit, by employing biocatalyst microorganism of choice (fungus: *Aspergillus sydowii* NYKA 510). The design process employed a bottom up strategy through three main phases:

[2.1.1] Phase 1: Bio-catalysis experimentation in ACMSC-MFC

This phase focused on evaluating the possibility of embedding the cells system as a self-sufficient unit for electricity generation in interior design. A designed model of the MFC was developed by the author using Rhinoceros 3D modeling software. The 2000 Ω external resistance was chosen to give maximum wattage, which achieved 0.4 W in average per cell for 100-150 h (4-6 days). For estimation of total wattage resulting from 4 cells, a set of 4 MFC cells were connected in series configuration. A Low wattage LED lamp of 0.8 Watt (Festoon Base - T3 Bulb (Length**:** 1.625", Lumens: 30, Weight (LBS): 0.06)) [234]• was used where a low amount of light is required. Besides its energy efficiency, it lasts up to 25,000 h, it contains no mercury elements and it does not release any hazardous gasses. LED Lamp with fixtures and cables was fixed inside a metal case (8 cm x 8 cm x 2 cm) with a transparent glass face to allow lighting.

[2.1.2] Phase 2: ACMSCMFC physical, chemical and electrical properties

The criteria that will affect the indulging of the cell unit in interior design were taken into consideration, including cell dimensions, shape and material, as well as operation conditions. A cell of 10 cm x 10 cm x 10 cm was used as the MFC case. The cells' cluster outer container was made of Perspex glass in order to engage fungal culture form in the final design appearance, to achieve natural form, and to ease monitoring of bio catalysis process inside MFC for periodical maintenance.

Temperature, lighting, orientation, installation and maintenance (refueling) conditions were adjusted. The cell operated under room temperature from 25-30^{o} C, and in aeriated conditions according to the air cathode requirements. The MFC was set to be horizontally stable. It fixed by screws on a stainless-steel frame (holder) **to maintain stability for electrochemical reaction to occur and to prohibit leakage of the inner solution of the MFC.** As MFC physical stability affects the electrochemical reaction and voltage stability. For safety, the set of cells is placed inside the glass cuboid container that gives the design its final form and keep cells from usage accidents and for

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• **[233] G. S. Jadhav, M. M. Ghangrekar, Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration, Bioresource Technology, 100, 717-723, 2009.**

• **[183] B. E. Logan, Microbial Fuel Cells, John Wiley & Sons, Inc, 2008.**

• **[234] https://www.energyavenue.com/LED-Light-Bulbs/Festoon/08-Watt (2019).**

maintenance including refueling (recharging with substrate), the glass container is openable from the front to enable recharging cells with substrate or further maintenance.

[2.1.3] Phase 3: Design generation based on biodigital procedures

For imaging the growth pattern (colonies) of *Aspergillus sydowii* NYKA 510, scanning electron microscopy was conducted for a 5-day old culture and the biofilm formed inside the MFC.

[2.1.3.1] Scanning Electron Microscopy (sample preparation)

Biofilm samples were washed three times with water and ethanol 30%. The samples were then dried for 24 h at 25ºC. The 5-day old culture samples were dried. Both samples were each mounted on the specimen stubs by graphite paste. Gold was used to coat the specimens for 20 minutes then the specimens were pictured by (JEOL JSM 5200) scan electron microscope at Experimentation Station, Faculty of Agriculture, Cairo University, Egypt.

[2.1.3.2] Form generation and manipulation

The design of the self-sufficient lighting unit is based on growth patterns of *Aspergillus sydowii* NYKA 510*,* extracted from the algorithmic analysis of the scanning electron microscope (SEM) imagery of the fresh culture. The algorithmic analysis offers a mathematical base of imagery data analysis. The analysis translates the image into 3D points that are the base for various 3D form generation and manipulation. Rhinoceros 3D + Grasshopper (Image sampler) was utilized for this purpose. This form generation approach allows infinite iterations of resultant patterns and form, which could be applied to the self-sufficient device design.

The design application methods were developed by the author in this work, these methods are: ***mass customization, patterning and patterned customized mass.***

[2.1.3.2.1] Mixed approach design manipulation (mass customization / pattern)

This design approach was developed specifically for living organism's behavior in order to achieve a patterned customized mass based on living patterns pixilation in 3D. It is a cellular automaton approach, which includes the three levels of mimicking, simulation and programming.

In the present work, the model employed was a mimicking approach of the growth pattern of *Aspergillus sydowii* NYKA 510 through algorithmic analysis of a scanned electron microscopy image of the organism. The SEM image was analyzed to 3D points in space by using image sampler tool in Grasshopper + Rhinoceros 3D. This tool converted each spore of the colony into a group of points, these points where then converted to spheres.

[3] Statistical analysis

In each experiment, data given are mean of triplicate assays. SPSS 20.0 software was employed for the evaluation of standard error (SE) and for regression analysis.

[4] Results and Discussion

This work aims to optimize media and growth conditions of most potent nonpathogenic-laccase producing fungal strains, employing it as a biocatalyst in Microbial fuel cell in a self-sufficient system for electricity production embedded in interior design elements. Thus; the study focused on achieving the most affordable, easy, reproducible and safest methods in biocatalyst growth media & conditions,

balancing these parameters with the required amount of laccase production, which is the pivotal parameter in oxidation reduction reaction controlling MFC efficiency and electricity production.

[4.1] Isolation and identification of fungal isolates

This study was conducted on laccase producing fungal strains, as laccases (EC 1.10.3.2, benzenediol: oxygen oxidoreductase) are multi copper polyphenol oxidases, which mediate the oxidation of a wide range of phenolic compounds and aromatic amines. [235]•. It contains copper atoms in the catalytic center called multi copper oxidases. [236•; 237•].

The catalysis of laccase occurs with reduction of one molecule of oxygen to water accompanied with one electron oxidation of a wide range of compounds. This oxidation results in generation of oxygen-centered free radical that can be converted to quinone in a second enzyme catalyzed reaction.

Laccase catalysis occurs in three steps: (1) type I Cu reduction by substrate; (2) electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster; (3) reduction of oxygen to water at the trinuclear cluster. [236]•.

Laccases have broad substrate specificity, which makes them excellent candidates for biotechnological applications and provide green route in various biochemical processing. One significant role of laccases in development of biosensors and biofuel cells.

Laccase degrade recalcitrant and xenobiotic compounds, it is a major contamination source in soil. Laccase also degrade mutagenic, carcinogenic compounds that responsible for risk to human health. [230]•. Use of laccase saves electrical energy, improves strength properties, and is environmentally compatible due to reduced effluent toxicity. [238]•.

The main problem of laccase applications for the commercial purposes is the high costs of their production. [239]•. Also the type of cultivation. Laccases can be produced by batch, fed-batch or continuous cultivation. [239]•. To make laccases available for industrial applications, methods to reduce costs include fermentation media optimization, novel fermentation methods, and genetic modification for large-scale production via eukaryotic recombinant strains. [236]•.

Several factors including type of cultivation (submerged or solid state), carbon limitation, nitrogen source, and concentration of microelements can influence laccase production, in this study, submerged cultivation in fed batch mode is the type of cultivation selected.

The process of submerged cultivation involves the growth of microorganisms in a liquid medium, rich in nutrients under aerobic conditions. It can be carried out using cheap material source considered as “waste”. This material contains considerable amount of soluble carbohydrates, nitrogen, minerals and vitamins, and inducers for enzyme production. The main disadvantage of this technique is the

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• **[235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate. Acta Biologica Szegediensis. 61, 25-33, 2017.**

• **[236] K. Brijwani, et al., Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, vol, 10, 2010.**

• **[237] H. V. Prajapati, F. P. Minocheherhomji, Laccase-a wonder molecule: a review of its properties and applications, International Journal of Pure & Applied Bioscience, 6, 766-773, 2018.**

•**[236]**

• **[230] N. Bhuneshwari, et al., Lignocellulolytic Fungal Isolation and Screening for Their Laccase Producing Ability, Indian J.Sci.Res, 13 (2): 188-191, 2017.**

• **[238] G. M. Scott, et al., Environmental aspects of biosulfite pulping. Proceedings of 1995 international environmental conference Atlanta: TAPPI, 1155-1161, 1995.**

• **[239] M. Hazuchová, et al., The optimization of propagation medium for the increase of laccase production by the white-rot fungus Pleurotus ostreatus, Nova Biotechnologica et Chimica, 16, 113-123, 2017.**

• **[239]**

• **[236]**

excessive growth of mycelium, which affects the production yield due to mass transfer and metabolic rate limitation. [237]•. For this reason, the author chose to limit the cultivation period in balance with highest laccase production rate achieved.

In the other hand, solid state fermentation (SSF) which is another method of fermentation occurs in absence or near absence of free liquid, employing an inert substrate (synthetic materials) or a natural substrate (organic materials) as a solid support. SSF is shown to be particularly suitable for the production of enzymes by filamentous fungi because they mimic the conditions under which the fungi grow naturally. The use of natural solid substrates, especially lignocellulosic agricultural residues as growth substrates has been studied for various enzymes including laccases. The presence of lignin and cellulose/hemicellulose act as natural inducers and most of these residues are rich in sugar promoting better fungal growth and thus making the process more economical.

Filamentous fungi grow following a branched pattern. The tubular hypha that emerges from the spore elongates at the tip and at the same time, new branches are formed along the hypha. The branching continues and forms a porous three-dimensional network of hyphae, which is known as mycelium. This unique morphological characteristic of filamentous fungi is suitable for SSF conditions, as it allows them to colonize and penetrate the solid substrate in the search for nutrients. [240]•. Moreover, the high level of enzyme production obtained by SSF is due to the lower the cost of the substrates, coupled with the reduced risk of bacterial contamination and low energy requirements as well as downstream processing of the enzyme. [241]•.

Although the tempting advantages of the solid-state fermentation method, the major disadvantage with SSF is ***lack of any established bioreactor designs***. There are several attempts to design SSF bioreactors trying to overcome limitations of temperature, pH, moisture, agitation, oxygen and mass transfer in solid media. [235]•.

SSF mainly is based on two great categories of used materials, the inert (synthetic materials) and non-inert (organic materials). The former only acts as an attachment place for the fungus, whereas the latter also functions as a source of nutrients, due to which it is called support-substrate. [242]•. In both cases, the success of the process is directly related to the physical characteristics of the support (particle size, shape, porosity, consistency), which favor both gas and nutrient diffusion and the attachment of the microorganisms. [242]•.

In a study conducted by V. Vivekanand., et al (2011). [243]•, banana peel was found to be an ideal support in the SFF method and resulted into higher levels of laccase (6281.4± 63.60 Ul−1) by *Aspergillus fumigatus* VkJ2.4.5. under 80% of moisture level, 6 days of incubation period, at 50°C and at varying pH range from 5–9 for up to 2 h. [243]•.

•[237] H. V. Prajapati, F. P. Minocheherhomji, Laccase - a wonder molecule: a review of its properties and applications, International Journal of Pure & Applied Bioscience, 6. 766-773, 2018.

• [240] J. F. Osma, et al., Morphology and laccase production of white-rot fungi grown on wheat bran flakes under semi-solid-state fermentation conditions, FEMS Microbiol Lett, 318(1):27-34, 2011.

• [241] T. Robinson, et al., P. Remediation of dyes in textile effluent, a critical review on current treatment technologies with a proposed alternative, *Bioresource Technology,* 77: 247-255, 2001.

• [235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis, 61. 25-33, 2017.

• [242] J. L. Toca-Herrera1, et al., Potential of solid-state fermentation for laccase production, Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Méndez-Vilas (Ed.), 2014.

• [242]

•[243] V. Vivekanand, et al., Banana Peel: A potential substrate for laccase production by *Aspergillus fumigatus* VkJ2.4.5 in solid-state fermentation, Applied Biochemistry and Biotechnology, 165. 204–220, 2011.

• [243]

It would be concluded that SSF lacks the robust control mechanisms. Especially control of the environment within the bioreactors, and scaling-up of SSF processes. Process complexity and the lack of reliable and affordable instrumentation make designing control strategies difficult. As the SSF bioreactors should be designed with economic, anticorrosive, and non-toxic materials. Other important aspect to be considered during the design of a bioreactor is the effective regulation of aeration, mixing, and heat removal. [242]•. For these reasons, the author did not employ solid-state fermentation in the current study and chose submerged fermentation.

Laccases are widely distributed in plants, fungi, bacteria and insects. Among all these sources, laccases from fungi are of special interest, because of their aptitude to degrade lignocellulosic biomass by elaborating extracellular laccase enzyme. [244]•. Fungal laccases exhibit higher redox potential when compared to plants or bacterial laccases. [236•; 244•]. ***Owing to the higher redox potential (+800mV) of fungal laccases compared to plants or bacterial laccases they are implicated in several biotechnological applications***. For instance, redox potentials of laccases from common laccase producing fungi are reported as 450mV (*Myceliophthora thermophila*), and 750mV (*Pycnoporus cinnabarinus)*. [236]•.

It was reported by many previous studies that white rot fungi present on decay wood and soil are the best laccase producer fungi [237]•, which proposes a sustainable use of these wastes (decaying wood) as a source of laccase production to generate renewable clean electricity in MFCs. Laccase production was also reported for some soil ascomycete species from the genera *Aspergillus*, and *Penicillium.* In the present study, three fungal strains were isolated from soil. They were purified and further identified *via* morphological and microscopic examinations. They were *Aspergillus niger*, *Aspergillus sydowii* and *Penicillium chrysogenum.*

[4.2] Quantitative screening of fungal isolates for laccase production

The selection of tested strains, in the present work, was according to availability, safety, and sustainability. The three fungal strains isolated from soil along with *Pleurotus ostreatus* and *Saccharomyces cerevisiae* were tested for their ability to produce laccase. Figure. [52]. reveals quantitative screening (U/ml) for laccase production by fungal isolates. It appears that *Aspergillus sydowii* was highly distinctive in laccase production (2.569 U/ml). It was followed by *A. niger* (0.323 U/ml). *Pleurotus ostreatus* came third (0.217U/ml). These results are congruent with previous studies reporting *Aspergillus sydowii* to produce oxidoreductases. [245]•. In addition, one sequence annotated as laccase was obtained in *A. sydowii* MS-19. Another peroxidase sequences were also found and annotated. They shared similar structures with manganese peroxidase and lignin peroxidase.

•[242] J. L. Toca-Herrera1, et al., Potential of solid-state fermentation for laccase production, Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Méndez-Vilas (Ed.), 2014.

• [244] S. S. Bhattacharya, et al., Optimization of laccase production using response surface methodology coupled with differential evolution, New Biotechnology, 28. 31–39, 2011.

• [236] K. Brijwani, et al., Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, 10, 2010.

• [244]

• [236] K. Brijwani, et al., Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, 10, 2010.

• [237] H. V. Prajapati, F.P. Minocheherhomji, Laccase - a wonder molecule: a review of its properties and applications, International Journal of Pure & Applied Bioscience, 6. 766-773, 2018.

•[245] B. Cong, et al., Isolation, characterization and transcriptome analysis of a novel Antarctic *Aspergillus sydowii* strain MS-19 as a potential lignocellulosic enzyme source, BMC Microbiology, 17. 129, 2017.

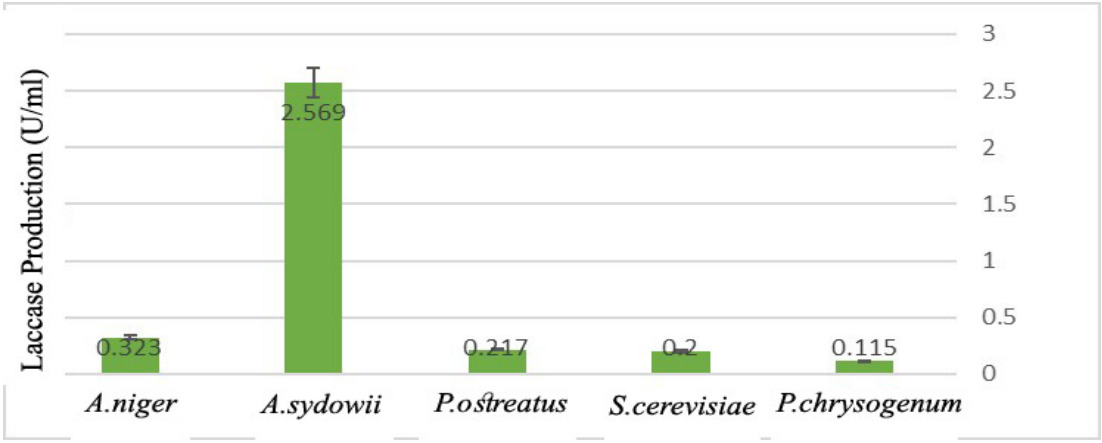

Figure. [52]. Laccase production by different fungal isolates. Bars show means. Error bars show mean ± SE. SE: standard error. By author.

From the obtained results, *A. sydowii* displayed highest production of laccase. Accordingly, it was chosen for further investigations.

[4.3] Molecular identification of *Aspergillus sydowii*

Further confirmation of *Aspergillus sydowii* identification was performed by nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing. A combination of the traditional Sanger technology and the new 454 technology was utilized in order to sequence the PCR product. The procured nucleotide sequence was deposited at the NCBI GenBank and a strain identifier and an accession number were provided. The fungal isolate was thus identified as *Aspergillus sydowii* NYKA 510 with accession number MK060010.

[4.4] Optimization of growth conditions of *Aspergillus sydowii* NYKA 510

The physical and chemical cultural factors of *A.sydowii* NYKA 510 were optimized to obtain highest amount of laccase as shown in Figure. [53]. The result revealed that the laccase production was growth associated, and the enzyme secretion was dependent on the fungal biomass and the specific growth rate of *A. sydowii*.

Laccase production by *A. sydowii* NYKA 510 was monitored along the incubation period of fourteen days as shown in Figure. [53-a]. Laccase production increased gradually up until **7** days where maximum amount of laccase **(2.25**U/ml) was noticed. After that, laccase production decreased with the increase in incubation period.

Figure. [53-b]. Demonstrates a significant increase in laccase production corresponding to the increase in pH until reaching maximum production (**2.40 U/ml**) at pH **5.2**. Laccase production exhibited a decrease after this pH. This is supported by various reports, which demonstrate that fungal laccases are active at lower pH values (3-5). [246•; 235•].

Pointing et al. (2000). [247]•, mentioned that the optimum range of temperature for the production of laccase is approximately from 25 to 30°C.The effect of different incubation temperatures is

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•[246] S.S. More, et al., Isolation, purification, and characterization of fungal laccase from Pleurotus sp., Enzyme Research, 2011.

• [235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis, 61. 25-33, 2017.

• [247] S.B. Pointing, et al., Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid culture, Mycologia, 92 (1). pp. 139-144, 2000.

illustrated in Figure. [53-c]. The statistical optimum temperature was **31**ºC for maximum laccase activity of **2.45 U/ml**. A decrease in enzyme production can be observed when changing this temperature. The results are concomitant with those obtained by Ghosh and Ghosh (2017). [235]•, for the effect of initial culture pH and temperature on growth of *A. flavus* PUF 5 where maximum laccase production took place at pH 5 and 30ºC after 14 days of incubation.

Carbon and nitrogen sources and concentrations as well as concentration of microelements can influence laccase production. [236•; 239•]. In the current work, alternative carbon sources from wastes were used to raise laccase production and to add ecological value to the domestic used MFC system. These alternative carbon sources from waste are of everyday use edible products in various regions domestically and worldwide. They are also cost effective as they are moderately cheap products affordable by different economic categories, they are also easy to prepare as they do not require extensive or complex procedures, their preparation could by applied personally at home and easily and they are toxins free.

Figure. [53-d]. Reveals the effect of various carbon sources at concentration 1 % on production of laccase by ***A.sydowii*** **NYKA 510** after **7** days of growth at **31**ºC and pH **5.2.** Obviously, banana peel induced highest laccase production (**3.12 U/ml**). Glucose exhibited 2.8 U/ml laccase production, while orange peel followed with 2.68 U/ml production. Potato peel achieved a moderate production value of 1.55 U/ml. Bulk amounts of banana peel are available. Disposal of such bulk amounts is of significant importance for industries concerned with fruit processing. In addition, banana fruit is among the highly used crops in the world accounting for 40% of the total world trade of fruits and their products. Banana peel also, appears as a good substrate for fungal consumption to adhere and penetrate due to its porous structure. The peel is highly rich in carbohydrates, ascorbic acid, and potassium. [243]•.

Statistically, increasing the concentration of banana peel up to 15.1 g/l led to a high increase in the amount of laccase enzyme to 3.22U/ml as shown in Figure. [53-e]. Enzyme production decreased above the optimum concentration of banana peel. Brijwani et al. (2010). [236]•, stated that higher concentrations of carbon can cause inhibition of the production of laccase by different fungal strains.

The effect of different nitrogen sources on *A. sydowii* NYKA 510 laccase production was tested. In Figure. [53-f]. It is clear that peptone was the most potent laccase inducer (3.67U/ml). followed with Sodium nitrate 3.00 U/ml induction of laccase. These results are congruent with the results obtained by Ghosh and Ghosh (2017). [235]•, and the results obtained by Hazuchová et al., (2017). [239]•, that turned out that from organic nitrogen sources, yeast extract, peptone, tryptone, albumin and casein, the most suitable substrate was yeast extract followed by peptone for growth. [239]•.

• [235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis, 61. 25-33, 2017.

•[236] K. Brijwani, et al., Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, 10, 2010.

•[239] M. Hazuchová, et al., The optimization of propagation medium for the increase of laccase production by the white-rot fungus Pleurotus ostreatus, Nova Biotechnologica et Chimica, 16. 113-123, 2017.

•[243] V. Vivekanand, et al., Banana Peel: A potential substrate for laccase production by *Aspergillus fumigatus* VkJ2.4.5 in solid-state fermentation, Applied Biochemistry and Biotechnology, 165. 204–220, 2011.

• [236]

• [235]

• [239]

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Laccase was reported to be produced earlier from different fungal strains when the fungal strains are cultivated in nitrogen rich media rather than nitrogen-limited media. [236]•. This is supported by a study conducted by Khalil et al, (2016). [235•; 248•].

Figure. [53-g]. Shows the effect of different peptone concentrations on laccase production by *A. sydowii* NYKA 510. The gradual increase in peptone concentration led to a concomitant increase for Laccase enzyme with maximum activity of 3.70 U/ml at 2.60 g/l peptone. A decrease in enzyme activity was noticed above this concentration**.** Sources of nitrogen as well as their concentrations are as significant nutritional factors as the sources of carbon, since they regulate production of laccase. [249]•.

The phenomenon of increased laccase production with addition of different metal ions has already been established in different reports. [235]•.

Figure. [53-h]. Depicts the effect of different metal ions on laccase production. NaCl caused a statistically non-significant change. An enhancement in laccase production was noticed for $MnSO_4.H_2O$ (123%). However, the highest significant promote effect was due to $CuSO_4.5H_2O$ addition with percentage increase competed with the control (no added metal ion to the medium) of 145 %. These results are supported by preceding reports, which showed that 2 mM $CuSO_4.5H_2o$ addition during fungal exponential growth phase caused a marked increase in production of laccase. [250]•.

• [236] K. Brijwani, et al., Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, 10, 2010.

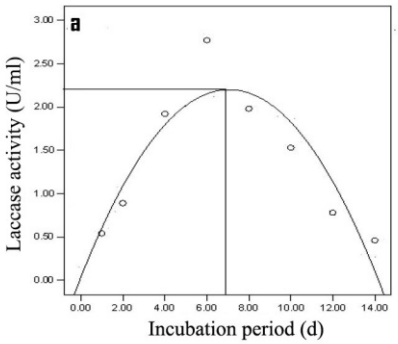
• [235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis. 61, 25-33, 2017.

•[248] N.M. Khalil, et al., Characterization of Aspergillus flavus NG 85 laccase and its dye decolorization efficiency, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7. 817-826, 2016.

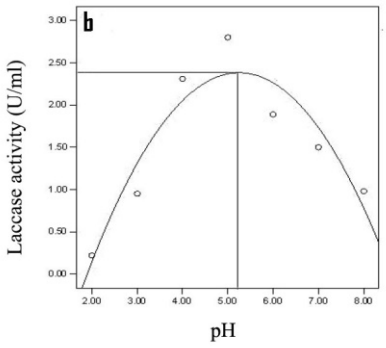
• [249] R.C. Minussi, et al., Potential applications of laccase in the food industry, Trends in Food Science & Technology, 13, 205–216, 2002.

• [235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis, 61, 25-33, 2017.

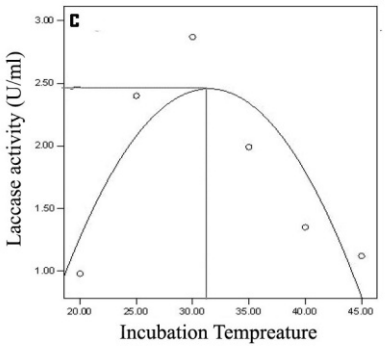
• [250] R. Couto, S.S. Maria, Application of solid-state fermentation to ligninolytic enzyme production, Biochemical Engineering Journal, 36. 211-219. 2005.



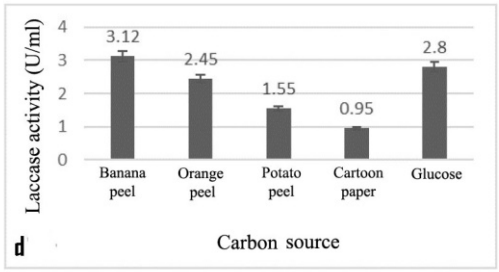
$Y=ax^2+bx+c= (-0.04)\ x2+ (0.61)\ x+.03 \quad R^2= 0.828$



$Y= ax^2+bx+c=(-0.211)\ x^2+ (2.22)\ x\ -3.45 \quad R^2= 0.868$



$Y= ax^2+bx+c= (-.009)x^2+0.570\ x-6.50 \quad R^2= 0.721$



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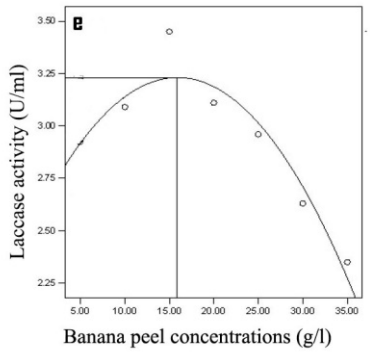
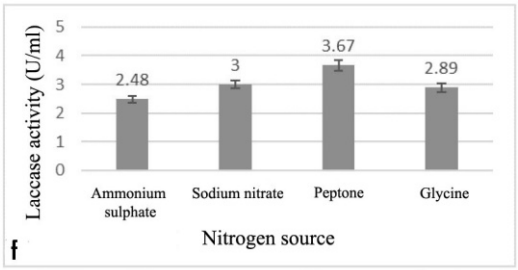
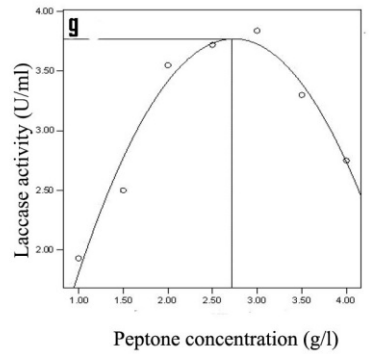


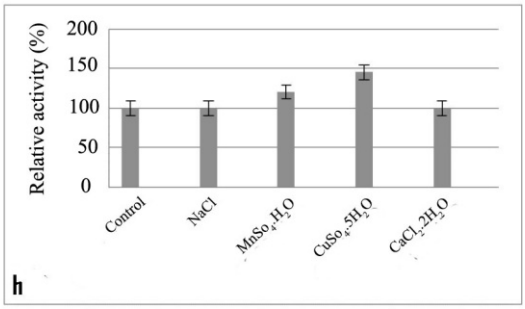
$y=ax^2+bx+c=-0.002x^2+0.083x+2.56 \quad R^2=0.891$



Bars show Means. Error Bars show Mean ± SE.



$y=ax^2+bx+c= (-0.63)\ X^2+3.49X-1.108 \quad R^2=0.957$



Bars show Means. Error Bars show Mean ± SE.

Figure. [53]. Optimization of cultural conditions of *A. sydowii* NYKA 510 for maximum laccase production. Linear regression model fit of different (a) incubation periods, (b) pH, (c) incubation temperatures, (e) banana peel concentrations or (g) peptone concentrations, as a function of laccase production. Effect of different (d) carbon sources, (f) nitrogen sources or (h) metal ions on laccase production. By author.

[4.5] ACMSCMFC operation and performance

Microbial fuel cells (MFC) can produce electricity directly from renewable sources by utilizing microorganisms. [251•; 252•]. Submerged cultivation is a process where microorganisms grow in liquid medium containing nutrients through aerobic conditions. Wastes can be used as cheap material source in submerged cultivation. This material contains considerable amount of carbon, nitrogen, mineral ions, and other compounds to induce production of enzymes. The author adopted this type of cultivation applied in the fed batch mode in the MFC. This technique causes excessive growth of mycelium, which operates a required function in MFC of forming biofilm between anode and cathode. This is essential for current boosting inside the MFC. [239•; 237]•. Different parameters including both operational and designing factors affect the MFC performance. Nature of the substrate, substrate concentration, temperature, microorganisms' species, alkalinity of anode and cathode chambers, external resistance, and residence time, are among the operational parameters. Anode and cathode material, type of membrane, and MFC architecture are among the designing parameters. [183]•. Two parameters can limit generation of current in the MFC; ***rate of oxidization of the substrate by microorganism and the electrons transfer rate to the surface of the electrode.*** [183]•. ***Rate of oxidation of the substrate depends on substrate concentration, which is usually considered as a reaction of the first order. However, parameters such as transfer of mass and thickness of the biofilm layer can cause suppression in generation of power***. [253]•. Developing an air-cathode single chamber MFC without a membrane has been to increase transfer of mass to the cathode, decrease costs of operation, and decrease volume and design of the reactor. Removing the proton exchange membrane (PEM) increase power output, as the aerobic biofilm formed on inner surface of the cathode removes oxygen molecules, which diffuse to the chamber. This substantially decreases the cost of the materials, hence enhancing the MFC sustainability. [254]•.

In this study, oxygen was chosen as an oxidant in the cathode due to the fact that it is available and its environmentally friendly reduction product *i.e.* water. Application of platinum as a main catalyst was for improving rate of reduction of oxygen in the cathode chamber.

Image. [106], (a-c). shows the ACMSC-MFC operation and performance with *Asperigillus sydowii* NYKA 510 fed batch mode. The open circuit voltage was recorded by SOOER-SD9205A multimeter. The *Aspergillus sydowii* NYKA 510 biofilm formed between anode and cathode in the membrane less single chamber microbial fuel cell is also shown.

• **[251] D. Pant, et al., A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production, Bioresource Technology, 101, 1533-1543, 2010.**

• **[252] P. Tanikkula, N. Pisutpaisala, Performance of a membrane-less air-cathode single chamber microbial fuel cell in electricity generation from distillery wastewater, Energy Procedia, 79, 646 – 650; Science Direct 2015 International Conference on Alternative Energy in Developing Countries and Emerging Economies, 2015.**

• **[239]**

• **[237] H.V. Prajapati, F. P. Minocheherhomji, Laccase - a wonder molecule: a review of its properties and applications, International Journal of Pure & Applied Bioscience, 6. 766-773, 2018.**

• **[183] B. E. Logan, Microbial Fuel Cells, John Wiley & Sons, Inc, 2008.**

• **[183]**

• **[253] S. E. Oh, B.E. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, Water Research, 39, 4673-4682, 2005.**

• **[254] A. Tardast, et al., Fabrication and operation of a novel membrane-less microbial fuel cell as a bioelectricity generator, Int. J. Environ. Eng, 3, 2012.**

Image. [106]. Microbial fuel cell in a) fed-batch mode, b) opencircuit voltage configuration and c) *Aspergillus sydowii* NYKA 510 biofilm formation. By author.

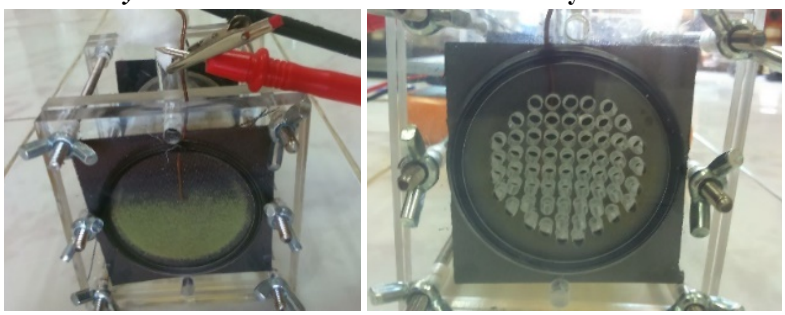


Image. [107]. left shows the aeriated cathode with accumulated spores of *Aspergillus sydowii* resulting from the oxidation reduction reaction of the bio catalysis. Image. [108]. shows the anode also with accumulated spores of *Aspergillus sydowii*. By author

The ACMSC-MFC performance over three open circuit cycles was recorded at zero current and unlimited resistance. Figure. [54-a]. Reveals that in the open circuit voltage (OCV), a rapid increase was noticed to values from 0.47 V to 0.54 V at the initial inoculation (first 24 h). The OCV reached a steady value of approximately 0.58 V after 50 h and continued to rise through the following 100 h, then reached to the maximum voltage of (0.765 V) MFC after 150 h (6 days). This production time was in accordance with laccase production achieved by *Aspergillus* sydowii NYKA 510 in this study.

The cell was then recharged with fresh media with dispersed spores of *A.sydowii* NYKA 510, and recorded the value of 0.65 V in the following 50 hours (200 h) (8th day). After that, OCV continued to rise again to 0.75 V up until 300 h (12^{th} day), as the fresh spores oxidize the carbon source in the fresh medium. Operating the MFC, in this work, in open circuit configuration for three successive cycles, each cycle lasting for 150 h (6 days), was targeted to produce the maximum power output that could be applied in an electrical device that depend on stable current.

To measure the effect of external resistances on current, cell potential and power density, the ACMSC-MFC was discharged then it was operated under the effect of fixed loads, 1000, 2000, or 3000 Ω. This was done for the purpose to explore the relationship between resistances, current density, potential, and power density. Figure. [54], (b–d). illustrates the performance of the ACMSC-MFC inoculated with *Aspergillus sydowii* NYKA 510 applied under external resistances (1000, 2000 or 3000 Ω). Figure. [54-b]. represents the effect of varied resistances on the produced voltage versus time, while, Figure. [54-c, d]. depict the production of current and power density at various resistances (1000, 2000 or 3000 Ω) versus time.

As observed in Figure. [54], (b-d). the potential, current density as well as power density show the same performance. There was a slow increase in output voltage during first 100 h, and then there was a significant increase in the following 50 h. Maximum Voltage (0.82 V) occurred at external load 3000 Ω which matched a current density of **160** mA m^{-2} and a power density of 80 mWm^{-2} after 150 h

(approximately 6 days). The voltage value of 0.76 V was achieved at external load 2000 Ω that matched 380 mAm^{-2} current density and 160 mWm^{-2} power density, which is the highest efficiency recorded in this study. The MFC maintained the same performance for the following 150 h (6 days) with minor fluctuations in the first 24 h after recharging each cycle. This maintenance of constant electrical performance is due to the formed biofilm inside the MFC. This is supported by Khater et al. (2017). [205]•, results achieving 0.79 V OCV and a stable current density of 354 mAm^{-2}, using acetate feed ACSCM-MFC. The results were also congruent with Lai et al. (2017). [185]•, where a maximum open-circuit voltage of 821 mV and a maximum current density of 330 mAm-^{2} were at external load of 1000 Ω. In the current study, the author used a MFC that depended on laccase utilization of oxygen as the final electron acceptor for catalyzing the reaction type of one-electron oxidation of phenolic compounds acting as a cathode catalyst.

The polarization curve as shown in Figure. [54-e], was acquired by varying the external resistance at the MFC maximum output voltage. To change the electrode potential of the MFC from its state of equilibrium due to a flow of current, this is polarization. It indicates dependence of power on the current. There was an increase in the power density to its maximum value against the current density then there was a sharp decrease. From Ohm's law, at the maximum power density, the external load is equal to the MFC internal load.

Power curves were plotted as factor of the current density versus electrode potential of the MFC. The internal resistance was determined from the polarization curve, considering the slope of the plot of voltage against current. The mean internal resistance was 32 Ω of standard. As the internal resistance decreases, the power density increases, where the increase in internal resistance causes consumption in power output in the MFCs and low electrochemical activity, which would decrease power generation. Power curve demonstrated the amount of power produced by the system. This is the main goal of MFCs when the MFC produces a steady electrical current by repeatable power cycles.

• [205] D. Z. Khater, et al., Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 2017.

• [185] C.Y. Lai, et al., Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188, 2017.

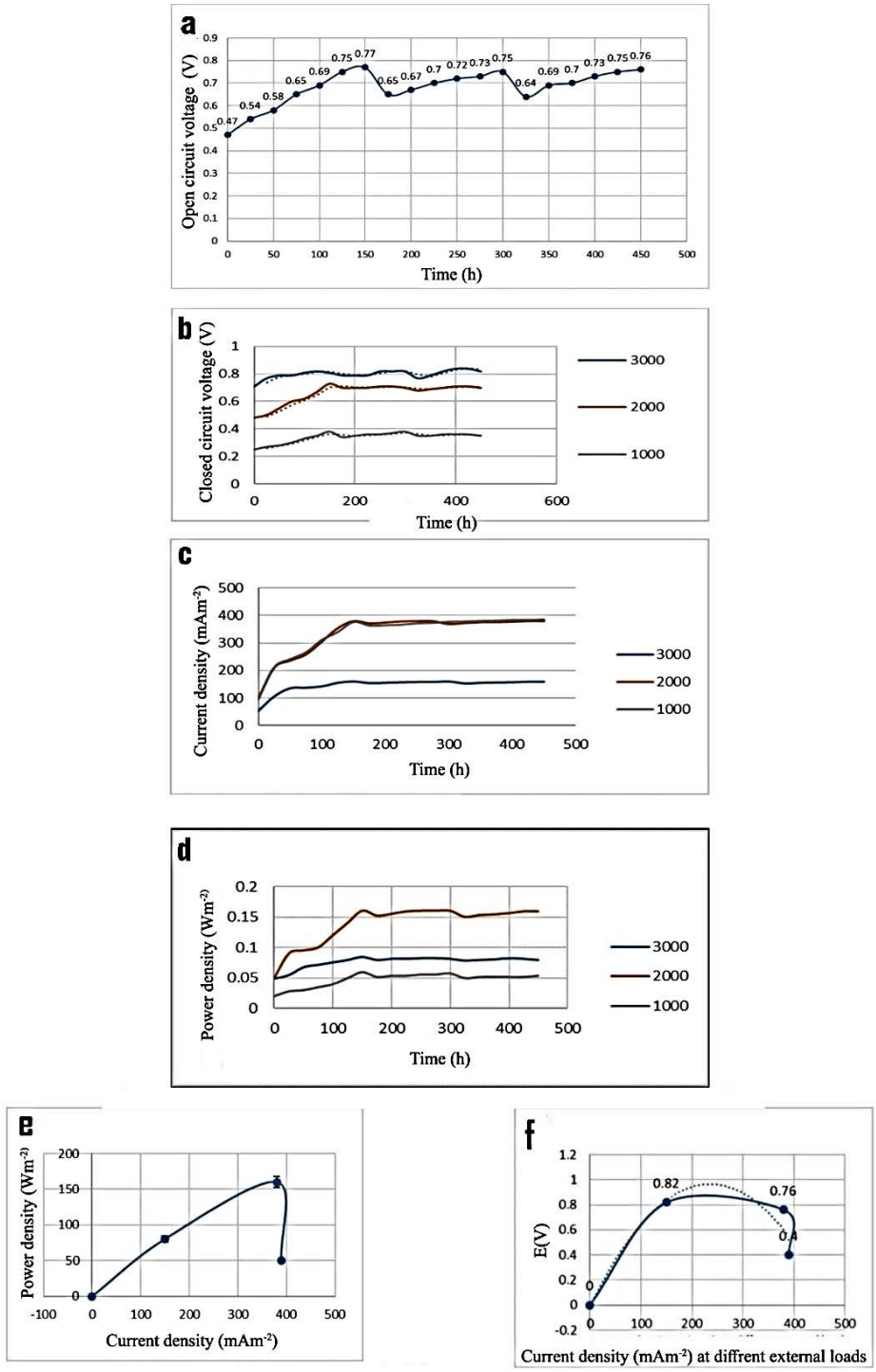


Figure. [54]. Performance of ACMSC-MFC operating with *A. sydowii* NYKA 510 as a biocatalyst. (a) Performance in open circuit for 3 cycles. Effect of varied resistances, 1000, 2000, or 3000 Ω, on voltage production (b), current density production (c) or power density production (d). Polarization curve as a factor of current density against potential (e). Power curve (f). by author.

In the current work, there was a directly proportional relationship between potential and resistance, while in case of current density: it was an inversely proportional relation. As supposed by Ohm's low, higher resistance leads to lower the generated current. The maximum voltage peak value at 3000 Ω was slightly higher than that in case of 2000Ω and the voltage peak at 2000 was higher than that in

case of 1000 Ω. The maximum current density value at1000 Ω is slightly higher than at 2000 Ω and 2000 Ω is higher than in case of 3000 Ω. Comparing potential and current density values between the three tested external loads, the 2000 Ω achieved the best performance. The same is in the case of power density values, where maximum power density was achieved by 2000 Ω, which proves the best MFC performance under 2000 Ω.

The total wattage of the microbial fuel cell operated under 2000 Ω was calculated from Ohm's law to be 0.4 W, and maintained the same value for the following 150 h (6 days).The results reached in the current study were optimized for electrical applications for domestic use in terms of closed circuit voltage, current density, and power density at 2000Ω. It employed the simplest MFC architectural, without the use of mediators or inducers that may be toxic or pollutant, reducing the total cost of MFC and committing to safety criteria for domestic use (home, office).

A set of four series-connected microbial fuel cells producing 1.5 W is sufficient for lighting a 0.8-watt LED light bulb for 150 h (6 days). This implies the need of recharging the cell with substrate every 4-6 days.

[4.6] Design proposal

A designed air cathode membrane less single chamber microbial fuel cell (ACMSC-MFC) modeled by the author using Rhinoceroses 3D modeling software is shown in Figure. [55-a]. The MFC is loaded with optimized fungal growth medium. It is in closed circuit configuration with external load.

Figure. [55-b]. shows a lighting unit design composed of 2 sets of connected in series 4 MFCs. this unit contains two 0.8 watt LED lamps. In Figure. [55-c]. there are two sets of connected in series MFCs, each set composed of 4 cells. This is to light two 0.8 W LED light bulbs. Fixtures and cables are adjusted inside a metal case of (8 cm x 8 cm x 2 cm) for each bulb. The figure also shows 5 cm maneuver margins for maintenance and recharging with substrate every 100-150 h (4-6 days). The design is to be employed in a self-sufficient lighting unit for domestic use. This design process followed a bottom-up methodology in coupling function and form concerning design criteria, including: physical, chemical and electrical properties. These criteria are cell dimensions, shape and material, as well as operation conditions.

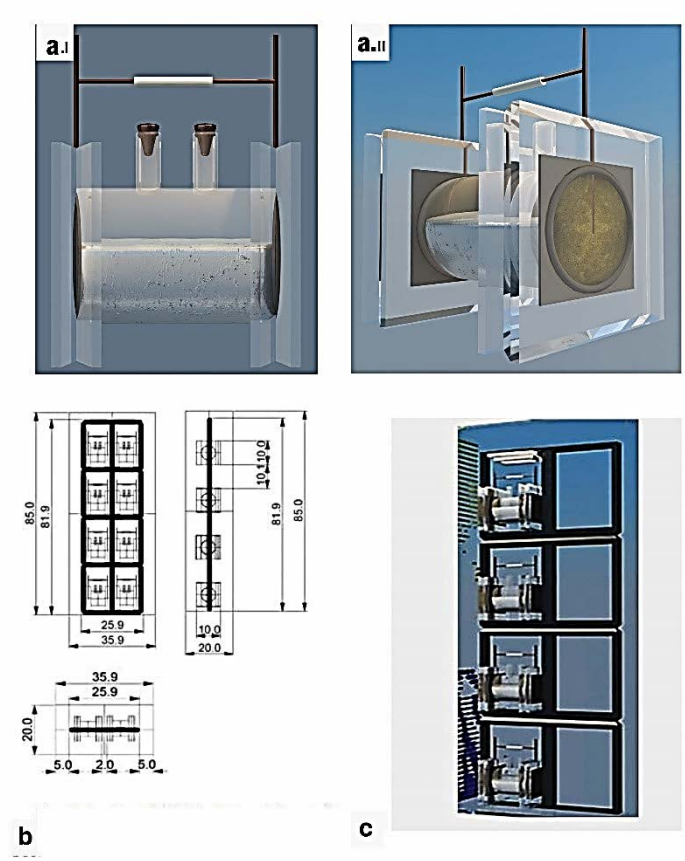


Figure. [55]. Lighting unit design utilizing ACMSC-MFC with *A. sydowii* NYKA 510. Two different views of an ACMSC-MFC modeled by authors (a, II and I). Front elevation of cells fixed on stainless steel frame, side view and plan view, respectively (b). Cells fixed on stainless steel frame (c). By author.

The MFC was defined on a stable horizontal configuration to maintain stability for electrochemical reaction to occur and to prohibit leakage of the inner solution of the MFC. Stability affects the electrochemical reaction and voltage stability, and so dynamical forces affect the MFC operation**.** For safety, the set of cells is placed inside the glass cuboid container that gives the design its final form and keeps cells from usage accidents.

[4.6.1] Scanning electron microscopy

The scanning electron microscopy (SEM) analysis of the culture of *A. sydowii* NYKA 510 is shown in Image. [109], (a-c). The fungus appears as mycelium with conidiophores bearing vesicles. Phialides radiate from these vesicles, which carry conidia. The scanning microscopy of the *A. sydowii* NYKA 510 biofilm formed at the MFC. Image. [109-d]. Shows a tangled web of mycelium and conidiophores.

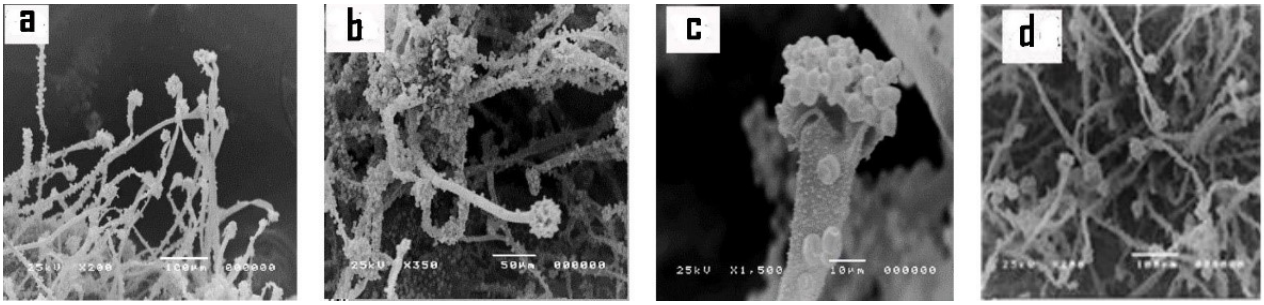


Image. [109]. Typical appearance of *Aspergillus sydowii* NYKA 510 growth culture at different magnifications (a, x200; b, x350; c, x1500) and biofilm formed at MFC (d). By author.

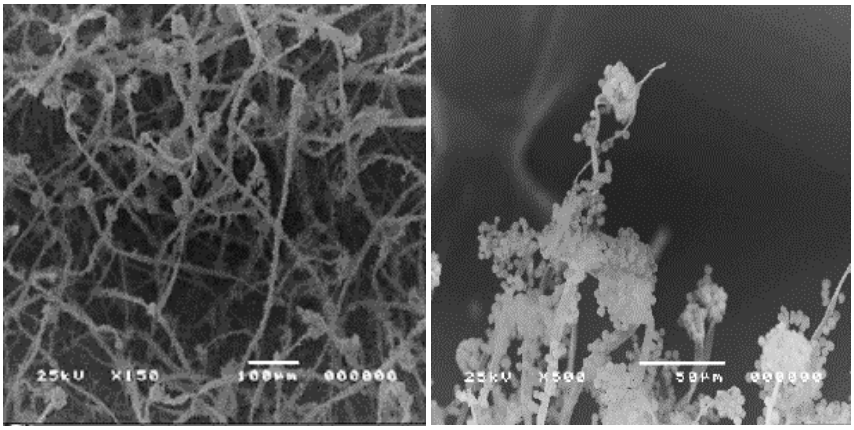


Image. [110, 111]. Left, tangled web of the *Aspergillus sydowii* mycelium, conidiophores (old culture) Right, tangled web of the fresh cultured *Aspergillus sydowii* mycelium and conidiophores. By author.

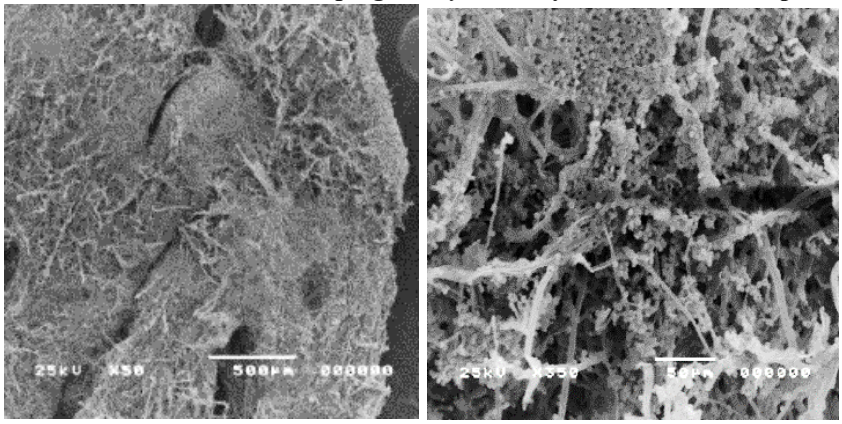


Image. [112, 113]. Bio film (formed from electro chemical reaction in MFC) showing old formed tangled webs of mycelium.

[4.6.2] Form generation and manipulation

The SEM image in Image. [109-a]. was utilized in this section. Figure. [56], (a-c). show the 3D points that were employed in the algorithmic form generation equations in the present design concept (designed by the author). The design form generation should reference to the microorganism's growth behavior patterns, which provide infinite form solutions with infinite potentials of manipulation and customization. The algorithmic analysis of SEM imagery offers a mathematical base of imagery data analysis and translates it into 3D points that are the base for various 3D form generation and manipulation as well. This form generation approach allows infinite iterations of resultant patterns and form applied to the self-sufficient device design.

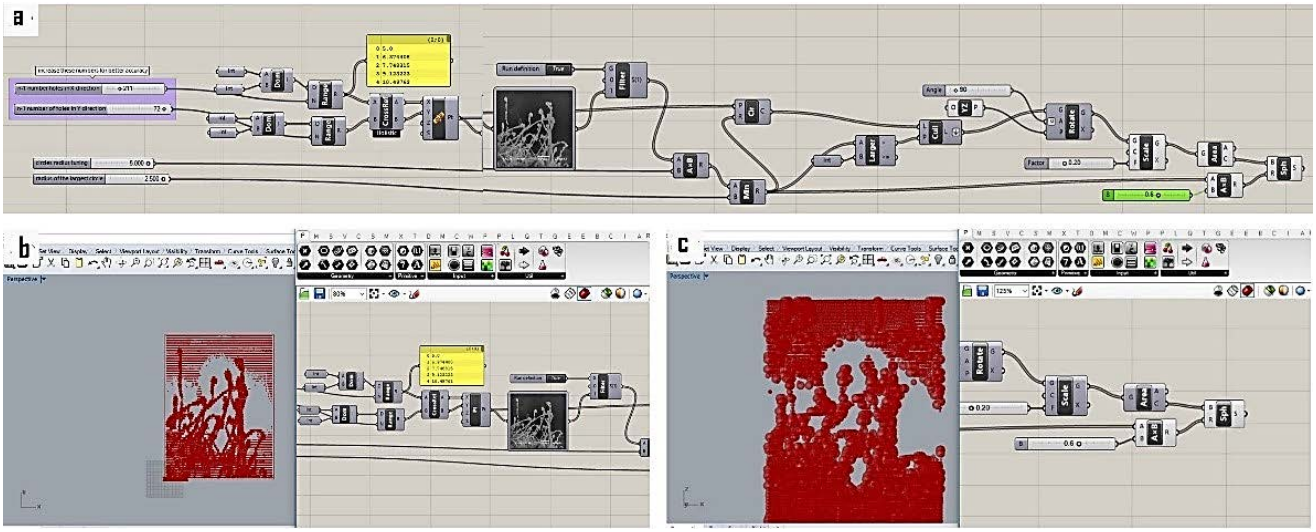
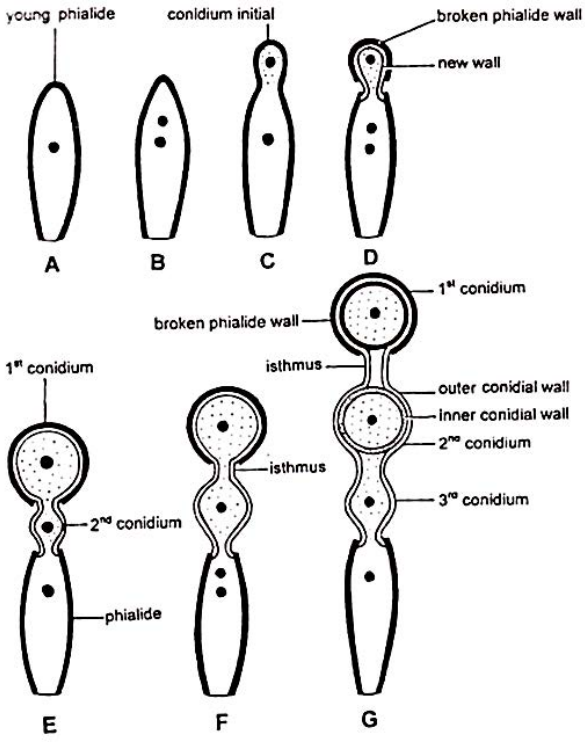


Figure. [56]. Algorithmic analysis of aggregation pattern and form generation equation (a). First (b) and second (c) steps in form generation development. By author.

The resulting patterns were studied to be employed in the unit's design. The patterns are porous allowing moderate transparency of the unit in order to indulge MFC living components as a part of the design aesthetics, and to maintain transparency and ventilation (aeriated cathode) conditions of MFC operation.



[255]•

Figure. [57]. Shows *Aspergillus* formation of conidia, by form analysis shows the 3D points developing to 3D spheres called conidium, which is the abstract algorithmic bases of the design form generation that was employed in this design concept.

• **[255] Aspergillus: Habitat, Reproduction and Importance, Ascomycotina- http://www.biologydiscussion.com/fungi/aspergillus-habitat-reproduction-and-importance-ascomycotina/24000**

The three main design application methods developed by the author were:

1) Mass customization (spatial configuration): by further algorithmic processing for resulting forms through scaling, randomization, animation and collision. The self-sufficient device is an interior standalone piece of furniture whose primarily function is generating electricity for lighting. This configuration is easier for maintenance and manipulation. The resulting forms in this method are unexpected and have infinite iterations; this provides 3D installation in the interior space or exterior space (pavilions, benches, etc.).

2) Patterning: In this method, the resulting form is being capsulated as a tile for repetition. The 2D configuration could be employed as panels for walls cladding or as standalone partitions. This configuration implies further processing for defining panels relation, electrical connections, estimated resultant power according to panels number and required total power, as well as maintenance. In addition to maneuver considerations (safety), this method can still provide 3D form design manipulation through controlling tessellations orientation and relation to each other including intersection, juxtaposition and separation. It would be applied for walls and partitions, as interior design element.

3**) Patterned customized mass**: a mixed approach of the aforementioned methods, based on manipulating patterns through overlapping and transition. This method was applied in the following design model of the proposed self-sufficient unit for lighting.

Image. [114-a]. Represents the mixed approach of patterned customized mass method: showing different forms along longitudinal axis. Silicon rubber spheres are distributed on the four surfaces, fixed on the perforated circles on the glass with the same distribution negative and positive joint, fixed by the grove on the diameter of the silicon rubber spheres.

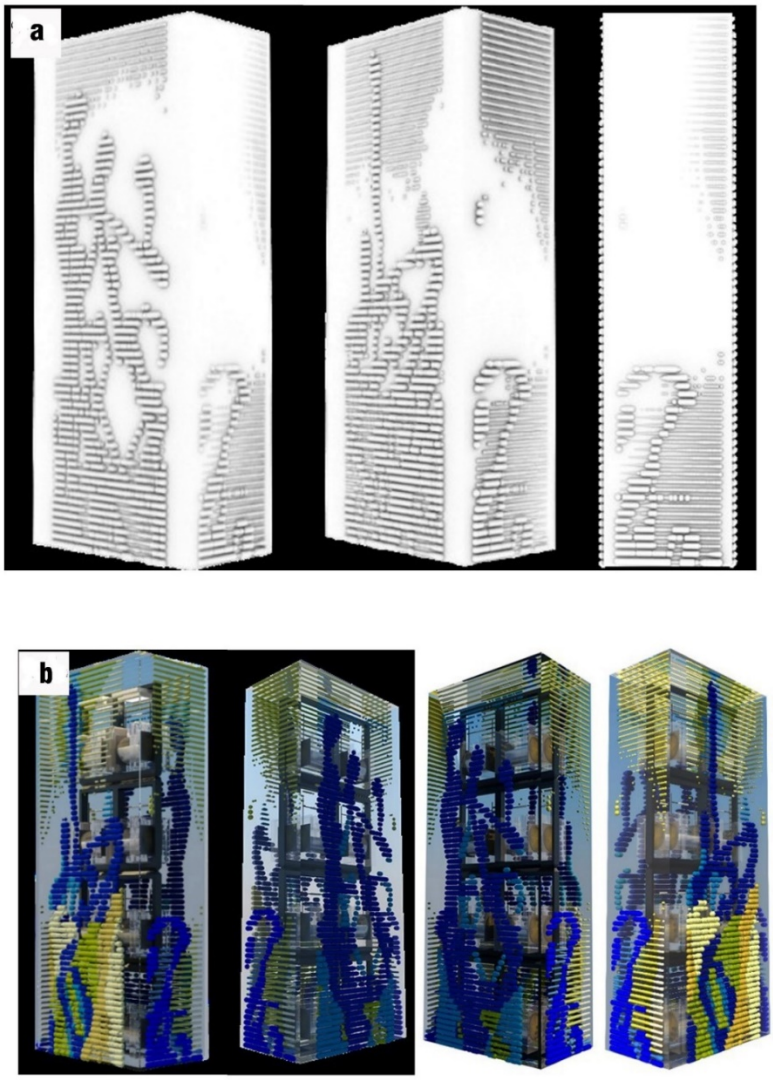


Image. [114]. Mixed approach of patterned customized mass method for a lighting unit design utilizing ACMSC-MFC system. (a) Patterned customized mass method: showing different forms along longitudinal axis. (b) The final design of a self-sufficient lighting unit powered by ACMSC-MFC from different views, designed by author using Rhinoceros 3D+grasshopper.

The color scheme was defined according to *Aspergillus sydowii* NYKA 510 culture colors (light olive green to light greenish blue) combined with (blue shades) to confuse the final design appearance between pattern and real organism (fungus). Image. [114-b]. shows the lighted unit by the power of MFCs from different views. It also shows pattern overlapping and transition by employing the glass container transparency to achieve various deferent views around 360 degree of rotation around the z-axis. The glass container also engages the MFCs with fungal cultures in the final design form to couple the appearance of the growth pattern applied in design and the real growth of *Aspergillus sydowii* NYKA 510 inside the MFCs. This application method is a cellular automaton approach including three levels of mimicking, simulation and programming. It can be applied in furniture and small sized installations.

Image. [115]. Details of the self-sufficient cluster, showing from left to right, planner perspective of the lighting unite showing overlapping and conjunction between different patterns applied on four faces of the glass container, two different sides detailes shows pattern's particles (silicon rubber) relation with transparent glass container and inner MFCs with fungus. By author.

[5] Conclusions

The current work focused on surveying laccase production by some fungi. The growth conditions of the most potent fungus, *Aspergillus sydowii* NYKA 510, were optimized for highest production of laccase. This fungus was employed as the biocatalyst in air cathode, membrane less, single chamber microbial fuel cell for electricity production. The novelty of this work is that the results reached were implemented in an interior design project based on applying biodigital design procedures in form generation of a self-sufficient lighting unit.

The aim was to achieve stable electrical current for lighting unites and power saving gadgets, the main concern was directed to:

1. Ecological aspect by achieving clean electricity that implies the ban of toxic chemicals (inducers, mediators) in the cultivation media of the biocatalyst.
2. Fast growth of the biocatalyst and laccase enzyme activity that leads to fast production of electric current, this affects latency period of electrical productivity of the cell.
3. Long life span of electrical productivity by the MFC.
4. Cost effectiveness by using the cheap materials in fabrication of the microbial fuel cell, by replacing expensive platinum-based electrodes with carbon cloth and carbon paper with platinum base catalyst to reduce cost.
5. Bioreactor simplified design: the design of the single chamber microbial fuel cell guarantees the ease of use in domestic scales, the ease of maintenance, refueling and fixation.

- Operation and embedding in interior design elements: the proposed self-sufficient device for electricity generation, could be applied in two main methods

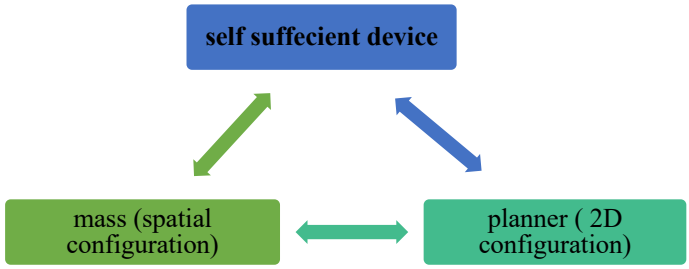


Diagram. [7]. Developed methods for applying behavioral pattern of *Aspergillus sydowii* NYKA 510 fungal cells in the formal design of the proposed self-sufficient cluster of bioelectricity, through pattern direct mimicking. By author

-For mass (spatial configuration), the self-sufficient device is an interior standalone piece of furniture generating electricity for lighting, this main function could be combined with other to form a multipurpose device. This configuration is easier for maintenance and manipulation.

-For the planner (2D configuration), the device is a tessellated cluster that could be employed as panels for walls cladding or as standalone partition, this configuration implies further processing for defining panels relation, electrical connections, estimated resultant power according to panels count and required total power, as well as maintenance and maneuver considerations (safety).

CHAPTER 5

Methodology of Embedding Microorganisms in Interior Design and Architecture to Achieve Design Ecology

Image. [116]. Detail of self-sufficient clusters of the bio-electricity generating system based on MFCs. Formal design extracted from a cellular automaton- agent based (biased random walk) model developed by the author to simulate fungal cells nutrient search and chemotaxis behavior.

Introduction

This chapter aims to design a methodology for embedding and employing microorganisms in interior and architectural design, functionally and aesthetically. This is tackled through defining biological processes, methods, tools, and introduce and emerge them into the design process in order to achieve space ecology and augment physical, functional and formal morphogenesis of living architecture**. In this chapter three levels of integration will be identified:**

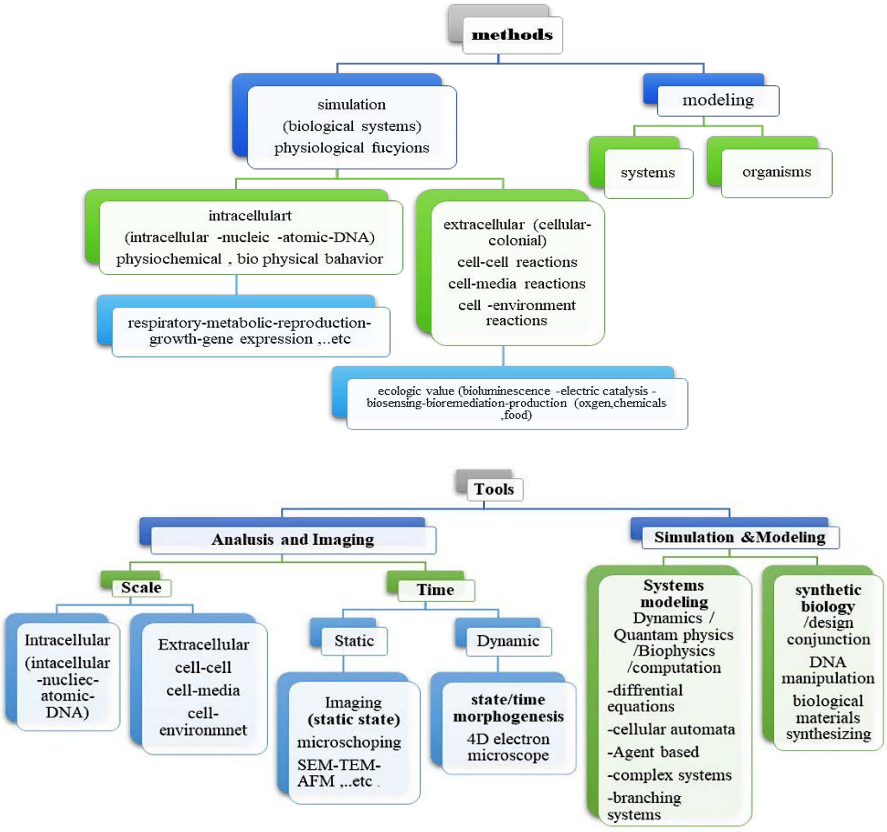


Diagram. [8]. Proposed methods and tools of embedding microorganisms in interior design and architecture. By author.

Recent revolutionary tools of analysis, simulation and modeling have made it possible to design engineered systems that behaves as natural ones. In these cases, biology integrates natural science and engineering science, as a sort of reverse engineering that introduce products of unfamiliar technologies. [256]•. As synthetic biology and engineered biological systems represent unprecedented opportunities in energy production, and environmental remediation as well.

Biological organisms are complex systems characterized by collective behavior emerging out of the interaction of a large number of components (molecules and cells). ***In complex systems, even if the basic and local interactions are perfectly known, it is possible that the global (collective) behavior obeys new laws that are not obviously extrapolated from the individual properties***. Only an understanding of the dynamics of collective effects at the molecular and cellular scale allows answers to biological key questions. [257]•.

Key to solving these problems is the design and analysis of appropriate mathematical models for ***spatio-temporal pattern formation***. *Most of numerical representation of theses mathematical models employ both well-known* ***discrete techniques*** *such as diffusion-reaction limited algorithms, cellular automata, and Monte-Carlo and* ***continuum approaches.*** [258]•. Recently, it is realized that the role of cells in morphogenesis cannot be neglected. For example, living cells possess migration strategies that go far beyond the merely random displacements of non-living molecules (diffusion).

More evidence has been collected how populations of interacting and migrating cells can in a self-organized manner contribute to the formation of order in a developing organism.

The choice of modeling formalisms is important because it determines largely the model potential. An important guideline for the choice of models is *the* ***classical simplicity principle***. Thus, in classical models, simplicity most often resides in the number of variables and/or in the algebraic form of the interactions (linear versus nonlinear). [259]•.

Another important factor in the choice of modeling formalism relates to what can be observed in the model. In most classical biological and ecological models, the models are formulated in terms of observed variables. Such models can be called ***one-level models.***

In this chapter, the author is digging deeper to form a well-organized methodology to employ most recent mathematical and physical methods for biological systems simulation and modeling. Offering a focused insight into methods, computational tools, and applications within the microorganism scale that provide infinite potentials in application in architectural design in all aspects.

[1] Methods: simulation of microbial systems

Pattern formation in living systems has attracted much attention since the pioneering work of D'Arcy Thompson. In recent years, special attention has been given to patterns emerging from ***cell colony growth in hostile environments. These systems tend to exhibit complex growth patterns*** *when the growth is limited by the diffusion of a nutrient that is necessary for the growth of the cells*. The

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• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.**

• **[257] H. Hatzikirou, et al., Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations, (eds.), Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer, Berlin Heidelberg, 2010.**

• **[258] K. Krawczyk, et al., Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**

• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling, Bioinfnmutica Paduulaan 8-Utrecht, Netherlands.**

morphologies obtained from these living systems resemble that of many non-living systems like ***electrodeposition***, crystal growth and viscous fingers. [260]•.

This similarity between biological and non-living diffusion limited patterns has led to the hypothesis that the biological patterns could be explained with the same basic principles. ***In addition, pattern formation in microorganisms can be viewed as the result of the exchange of information between the microscopic objects (cell) and the macroscopic ensembles (the population).*** [258]•.

Physical parameters such as energy, temperature and compressibility combined with processes such as energy minimization and reaction-diffusion of chemicals control the evolution and properties of both living and nonliving materials. Thus, complex living organisms could be described simply by combining these classical physical concepts. ***The complexity arises in two ways: first as an emergent property of the interaction of a large number of autonomously motile cells that can self-organize. Second, cells have a complex feedback interaction with their environment, as*** *cells can modify their surroundings by e.g., secreting diffusible or non-diffusible chemicals*. Their environment in turn causes changes in cell properties (differentiation) by changing the levels of gene expression within the cell. [261]•.

As biological systems inherently have multiresolution aspects, ***continuum and discrete models*** are to be used both for covering these differential aspects of biological systems i.e., the spatio-temporal diversity of a biological system starting from molecular structures of a single organism, through its genetic features, metabolism, interaction with an environment, population growth, and finally, complex macroscopic behavior.[258]•.

For instance, the discrete models can be used most appropriately for modeling phenomena in which group behavior comes from microscopic interactions between independent objects and the objects and environment. These interactions can be expressed by analytic formula coming from basic principles or synthetic set of rules. A ***discrete cell-oriented approach*** is also required if the dynamic system behavior depends on fluctuations at the individual cell level.

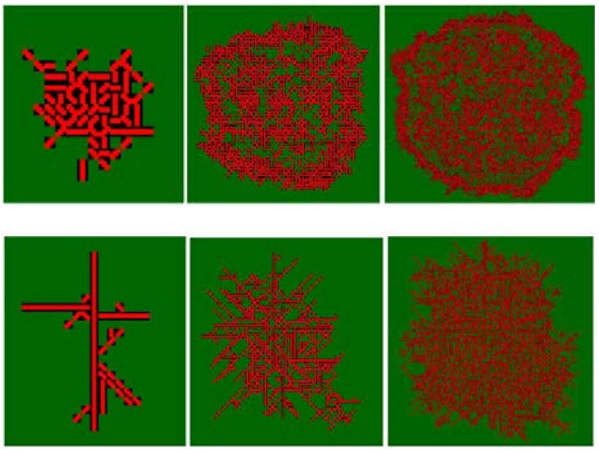

Figure. [58]. The snapshots representing three generations from simulations of complex non-linear morphology of bacterial colonies. Bacterium produce macroscopic patterns of various complexity. These non-linear patterns reflect different properties of the colonies including their adaptation features and fitness factors. The large-scale behavior comes from microscopic rules resulting from morphology of organisms and interactions of each individual with its neighborhood consisting of its physical environment and other individuals from the population. [258].

• **[260] P. Gerlee, A.R. Anderson, Stability Analysis of a Hybrid Cellular Automaton Model of Cell Colony Growth, Division of Mathematics, University of Dundee, Scotland, 2018.**

• **[258] K. Krawczyk, et al., Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**

•**[261] M. S. Alber,et al., On Cellular Automaton Approaches To Modeling Biological Cells.**

• **[258].**

[1.1] Intracellular physiology (gene expression/molecular reactions)

Quantitative descriptions of molecular interactions typically invoke the laws of physics and chemistry. The resulting models are thus *mechanistic as they describe the mechanisms that drive the observed behavior*. Each component of a mechanistic model represents some aspect of the system being studied; modifications to model components thus mimic modifications to the real system. However, mechanistic models can be contrasted with so-called descriptive models that seek only to summarize given data sets, which provide limited insight into system behavior. [256]•.

Network Science of Biological Systems. ***The essential molecular components of a cell are related by functional interdependencies of different nature (i.e., genetic) at different scales,*** making network modeling an essential tool for their modeling and analysis. On the one hand, ***the function of cells is the result of interacting proteins, which are responsible for the underlying modular and hierarchical organization into complexes, organelles and signal transduction pathways that build interactions***. [262]•.

Natural gene regulatory networks*:** Natural gene circuits can be roughly divided into two classes: ***sensory networks, which mount a response to a cell's current environmental conditions; and ***cell-fate decision*** (or developmental) networks, which cause cells to adopt persistent states***. Sensory networks*** *enhance a cell's survival by tailoring its behavior to suit the prevailing conditions*. ***Cell-fate decision*** *networks do not normally act under tight time-constraints; they often involve long cascades of interacting genes and complex feedback loops*, particularly positive feedback loops that lock in decisions. [256]•.

Biological cells interact with each other by two major means: local interaction by cell adhesion between cells in direct contact or between cells and their surrounding environment, and longer-range interactions such as signal transmission and reception mediated by a diffusing chemical field. [261]•.

- ***Cell adhesion is essential to multicellularity.*** *Experimentally, a mixture of cells with different types and quantities of adhesion molecules on their surfaces will sort out into islands of more cohesive cells within lakes of their less cohesive neighbors.* ***The final patterns, according to Steinberg's Differential Adhesion Hypothesis (DAH), correspond to the minimum of interfacial and surface energy. The DAH assumes that an aggregate of cells behaves like a mixture of immiscible fluids.*** *In vitro and in vivo experiments have confirmed the soundness of the analogy. Moreover, cell adhesion molecules, e.g. (controlling cell-cell adhesion) and (controlling cell-ECM (extra cellular matrix) adhesion); often serve as receptors to relay information to the cell to control multiple cell-signaling pathways****,*** *including those of cell growth factors. Their expression and modification relate intimately to cell differentiation, cell mobility, cell growth and cell death.* [261]•.

• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.**
• **[262] M. De Domenico, F. B. Kessler, Multilayer Network Modeling of Integrated Biological Systems, Network Science of Biological Systems at Different Scales A Review, Physics of Life Reviews, Volume 24, 2018.**
• **[256]**
• **[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**
• **[261]**

- ***Cell-to-Cell Communication*:** Gene networks operating in individual cells can communicate their states to one another by producing a signaling molecule that can pass from one cell to another providing an intercellular connection.

Bacterial quorum sensing*: Cell-to-cell signaling** is crucial to the development and proper functioning of all multi-cellular organisms. As in bacterial cells, cells use a multitude of signals to monitor their environment. ***Quorum sensing; a mechanism by which bacterial cells measure the local density of their population. Bacteria use this information to enhance their survival. (***One example is the formation of bacterial biofilms when cells reach sufficiently high-density.***). To implement quorum sensing, each cell communicates its presence by secreting a signaling molecule, called an **auto inducer**, into the local environment. These molecules are taken up by neighboring cells, and activate gene expression-including genes that lead to production of the auto inducer itself. *This positive feedback results in a switch-like response to changes in the local population density.* [256]•.

- ***Molecular reactions***: *Chemical reactions result from collisions of individual molecules.* ***On a molecular scale, reactions are thus rare events, and are difficult to predict.*** In many cellular processes, this molecular randomness is 'averaged out' over large numbers of reaction events, resulting in predictable system behavior. *In contrast, processes that depend on small numbers of molecules can be strongly affected by the randomness of biochemical events*. However, in some biological contexts, random behavior can be exploited for improved performance. A reaction network that comprises large numbers of reactant molecules will involve many simultaneous reaction events. In such cases, network behavior corresponds to the average over these events, and is well described by ***deterministic differential equation models.***

At the cellular level, randomness can be categorized into two categories: extrinsic noise, which refers to random variations that affect all processes in the cell equally, and intrinsic noise, which is driven by thermal fluctuations at the molecular level. In models of intracellular networks, extrinsic noise appears as randomness in the values of model parameters, and so can be directly incorporated into a differential equation-based framework. In contrast, treatment of intrinsic noise demands the adoption of a modelling framework that takes into account the randomness of the biochemical events that drive reaction dynamics. [257]•.

[1.2] Extracellular processes (spatial propagation = reproduction; nutrient search and uptake).

The most studied example of cell colony growth is the growth of bacterial colonies under low nutrient levels. Resulting in very complex shaped morphologies of the colonies. The main modelling approach that has been used is modeling growth via a system of ***reaction-diffusion equations***. These models are able to reproduce the observed patterns, ranging from ***Eden-like*** and ***dense branched morphologies*** to ***DLA-like*** patterns. Another approach is to model the bacteria as clusters of ***discrete walkers***, which obey dynamical rules. [260]•.

• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics.**
• **[257] H. Hatzikirou, et al., Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations, (eds.), Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer, Berlin Heidelberg, 2010.**
• **[260] P. Gerlee, A. R. Anderson, Stability Analysis of a Hybrid Cellular Automaton Model of Cell Colony Growth, Division of Mathematics, University of Dundee, Scotland, 2018.**

Fungal colonies also display complex patterns under diffusion-limited growth. Complex patterns with fractal morphologies have been observed for both multi-cellular filamentous growth, and for yeast like unicellular growth. These patterns have successfully been modelled using both **continuous and discrete techniques**. [260]•.

Complex growth patterns can emerge from simple model with minimal assumptions about the cell behavior. Integrating computational simulation and modeling in fungal biology analysis can be applied on highly varied scales. Some scientists focus on the microscopic events taking place inside the hyphal tip, while others explore the extent of fungal colonies covering immense surface areas, Spatial variation in model variables has also been explicitly studied at large scales, as macro-scale growth has also been modeled with focus on the production of fungal biomass by consumption of substrates. [263]•.

- ***Influence of the Microenvironment on Cell Migration: Active*** migration of cells is essential for a number of biological processes. Both in natural and artificial environments. In particular, the cellular microenvironment provides the substrate for cell migration. Environmental heterogeneity contributes to the complexity of the resulting cellular behaviors. As the cellular microenvironment can either enhance collective motion of cells or direct cell dispersion. [257]•
- ***Cell Migration Strategies:*** The cellular microenvironment is a highly heterogeneous medium. Cells move within their environment by responding to their surrounding's stimuli. In addition, cells change their environment locally by producing or absorbing chemicals and/or by degrading the neighboring tissue. This feedback establishes a dynamic relationship between individual cells and the surrounding substrate. One can distinguish two distinct strategies of cells responding to environmental stimuli: ***either the cells are following a certain direction and/or the environment imposes only an orientation preference***. ***Chemotaxis*** mediated by diffusible chemotactic signals provides a further example of directed cell motion in a dynamically changing environment. [257]•.

Cell motion through heterogeneous media involves phenomena at various spatial and temporal scales. These cannot be captured in a purely macroscopic modeling approach. In macroscopic models of heterogeneous media, diffusion is treated by using powerful methods that homogenize the environment by the definition of an effective diffusion coefficient. Continuous limits and effective descriptions require characteristic scales to be bounded and their validity lies far above these bounds. In particular, it is found that in motion through heterogeneous media, anomalous diffusion (sub-diffusion) describes the particles' movement over relevant experimental time scales, particularly if the environment is fractal; existing macroscopic continuum equations cannot describe such phenomena. [257•; 264•].

- ***Substrate uptake: In fungal strains, internal substrate is used to obtain external substrate by active transport across the plasma membrane (facilitated by the production and exudation***

• **[260].**
• **[263] S. Kelleter, Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of Coprinopsis cinerea, Master Thesis, University of Gottingen, Faculty of Forest Sciences and Forest Ecology, 2017.**
• **[257] H. Hatzikirou, et al., Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations.**
• **[257].**
• **[257].**
• **[264] M. Fricker, et al., Mycelial Networks: Structure and Dynamics, British Mycological Society Symposium Series r, 2008.**

of protons and other metabolites). The acquisition rate must therefore depend on the amount of internal substrate available to perform the active transport, external substrate available for absorption, and the hyphal surface area over which the absorption occurs. [264•; 265•]. This process is then related to the translocation mechanisms in order to distribute the uptake substrates where needed most.

Translocation: these could be categorized into two different translocation mechanisms which are responsible for nutrient reallocation in many fungal strains: ***simple diffusion and the active movement of intracellular metabolites from regions of local excess to regions of local scarcity***. [265]• only newly-formed hyphae (and associated hyphal tips) use active translocation, while older, established hyphae use diffusion as the major means of internal nutrient reallocation.[265]• These translocation mechanisms align with two distinct ***spatial propagation patterns of growth***: **exploration and exploitation**. ***The exploration phase is adopted in low-nutrient environments and features fast moving hyphal tips coupled with minimal branching, resulting in a sparse mycelial network. The exploitation phase is adopted in high-nutrient conditions and features slower moving hyphal tips and increased branching and anastomosis, resulting in a dense mycelial network***.

Boswell et al., (2007) propose that the exploration and exploitation phases are controlled predominantly by the translocation process. And that changes in the active translocation process *alone* account for the switch between exploration and exploitation phases. [265]• Mycelial systems tend to become more open with time as they become larger. Patterns are modified by the quantity and quality of the resource from which the mycelium is extending, nutrient concentration, and microclimate. [264]•.

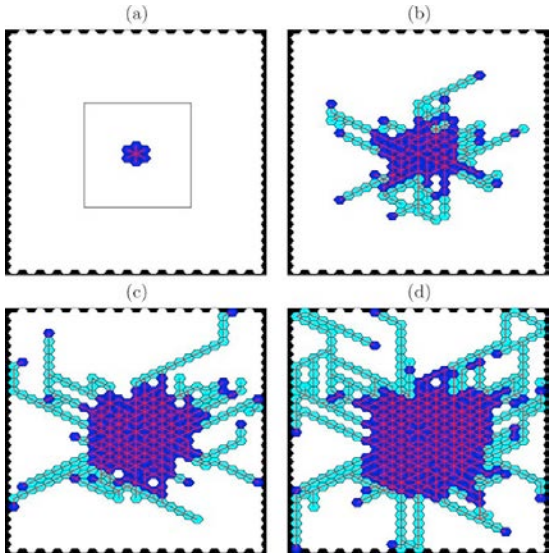

Figure. [59]. Fungal hyphal growth model in different substrate concentrations: The internal substrate distribution (blue) and model biomass network (red) is plotted for growth into a substrate-free region at times (a) $t = 0$, (b) $t = 0.1$, (c) $t = 0.2$, and (d) $t = 0.3$. The only external substrate in the system is confined to a central square region of the domain whose boundary is shown in (a). The dark blue and light blue cells respectively denote high and low levels of internal substrate. For purposes of improved visualization, the internal substrate status within each model hypha is represented by the color of the hypha's corresponding hexagonal cell. The model exhibits the exploration and exploitation methods of hyphal growth. [265].

• [264].
• [265] G. P. Boswell, et al., The Development of Fungal Networks in Complex Environments.
• [265].
• [265].
• [265].
• [264].

Persistent Mycelial Networks: 'Sit and Wait' Strategy: Arrival of new resources can result in reallocation of biomass, as fungal mycelial networks are connected they have a sort of communication, since hyphae maintain continuity with their immediate 'ancestors' and if contact is made with neighboring regions, theses hyphae can become connected via new formation of cross-links (anastomoses). ***This results both radially and tangentially in systems with many connected loops***. [264]•. Not only does the mycelium respond by changes to system architecture but also with physiological responses: there is highly coordinated uptake, storage and redistribution of nutrients throughout the network. Rates of translocation can be rapid, the largest fluxes being through cords interconnecting resources. Many factors, including the overall nutritional status of the mycelial system, and the distribution and quantity of colonized and newly encountered organic resources, affect the balance between, and the main sites of, uptake, storage and demand for carbon and mineral nutrients. [265]•.

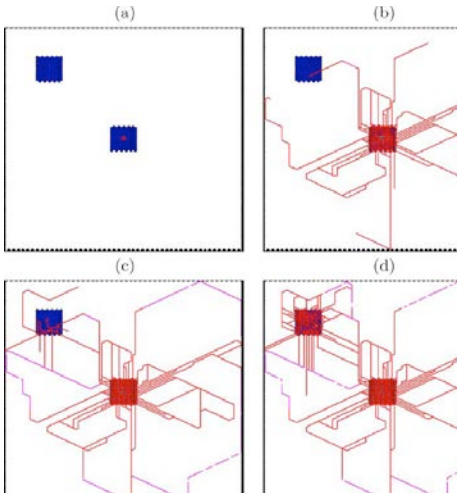

Figure. [60]. The model biomass (red) expands from what represents a localized food source (blue) into a domain that contains a distant resource but is otherwise empty of nutrients. The model biomass is shown at times (a) $t = 0$, (b) $t = 0.5$, (c) $t = 1$ and (d) $t = 1.5$.
the simultaneous use of "cell" and "bond" structures, allowed the explicit modelling of substrate movement through neighboring, but unconnected, hyphae. A similar modelling technique can be used to simulate other branching structures where the morphology is dependent on the status of material inside the developing network and the explicit modelling of anastomoses.

[265].•

In the study of fungal mycelia, scale is varied extremely: the indeterminate growth habit of fungi ensures that models may have to incorporate processes operating over scales ranging from the (sub) micron to the kilometer. Modeling is way more complex when modelling physical and nutritional heterogeneity of the host environment and the resultant diverse sensing and response events that media hyphal growth and accordingly determine network architecture. Thus, a meaningful, systems-based understanding requires the transfer of model information across varied scales. [266]•.

A common approach to modelling the large-scale spatio-temporal properties of fungal mycelia has been to model the fungus and growth-promoting substrates as continuous variables so that the model comprises a system of (non-linear) partial differential equations (PDEs).[265]•.

Another aspect of modeling fungal hyphae other than scale and nutrient searching strategies, is the hyphal branching patterns, two patterns of hyphal branching can be distinguished: ***apical (from the hyphal tip)*** and the more prevalent ***lateral branching (from the sides of the hypha). T***o model the physiology of this branching processes, a different approach on modeling hyphal tips is used it is called

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• **Ibid-[264] M. Fricker, et al., Mycelial Networks: Structure and Dynamics, British Mycological Society Symposium Series, r 2008.**
• **Op.cit-[265] G. P. Boswell, et al., The Development of Fungal Networks in Complex Environments.**
• **Ibid-[265]**
• **[266] F. A. Davidson, et al., Mathematical modelling of fungal growth and function, IMA Fungus, 2(1), 33-37, 2011.**
• **Op.cit.**

the ***Surface Stress Theory***, saying that the shape of fungal hyphae results from internal turgor pressure on the wall of the extension zone and is influenced by the wall elasticity and surface tension. In opposition to this model stands the ***vesicle supply center model***, where the mobile vesicle supply center coordinates the distribution of vesicles to the wall of the extension zone. When it moves from the center of a spore to the cell wall, it is said to cause a local bulging leading to germ tube formation. [267]•.

A mycelial network displays certain fractal properties. This was quantified by Ritz and Crawford (1990) who approximated the ***"fractal dimension" of mycelia*** at various stages of growth using the box-counting technique. The box-counting method is applied by super-imposing a square lattice over the triangular lattice and counting the number of squares containing any amount of model biomass. [265]•.

Other tools for analyzing fungal networks emerge from graph theory and statistical mechanics that are applicable to mycelial networks, a network is simply a set of nodes, or vertices, connected by a set of links. Weights, that define properties such as resistance to breakage or transport capacity, can be associated with either nodes or links, or both. The nodes of a fungal mycelium are the tips, branch points and fusions of hyphae or cords, while the links are the hyphae or cords themselves. *The sum of link weights per node, known as the node strength, is of greater interest, as calculating the node strength for link cross-sectional area could indicate which nodes are likely to be important in transport, from* the network that can be used to calculate the number of closed loops (cycles) in the network. This ***cyclomatic number is extremely important, for it indicates the number of alternate pathways among points in the network that determine both the resilience to damage and the capability of parallel flow***. [264]•.

Modelling of transport in the mycelium has been attempted using various approaches, including ***partial differential equations*** and ***autonomous agents***. Greater insights can be obtained by using an explicitly network-based approach into the analysis of transport. One way to achieve this is to ***calculate shortest path distances from each node to every other***. If the effective physiological distance, or transport resistance, from one end of a cord to the other is modelled as the cord length divided by its cross-sectional area, the shortest path from one node to another will be the route with the smallest sum of these distances. ***The shortest path is therefore effectively the path of least resistance in the same way that electricity will flow through each of a set of resistors in parallel.*** [264]•. ***Methods for solving current flow through networks of electrical resistors can in principle be used to model flow through mycelial networks, for example.*** [264]•.

- ***Functional-Structural Plant Modeling (FSPM)***. Structural elements of these models aim to accurately represent morphology while functional modeling focuses on metabolism and processes inside the organism. This type of model may lead to a better understanding of the development and functioning of complex organisms. [263]•.

• **[267] J. M. Halley, et al., Competition, succession and pattern in fungal communities: towards a cellular automaton model -OIKOS 70, 435-442, Copenhagen, 1994.**
• **Op.cit.**
• **[264] M. Fricker, et al., Mycelial Networks: Structure and Dynamics.**
• **Ibid.**
• **Ibid.**
• **[263] S. Kelleter, Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of Coprinopsis cinerea.**

It is obvious that fungal mycelia may be considered as a form of distributed intelligence. The rules which control the colony behavior will be local but lead to patterns on a large scale. This could be precisely represented by ***cellular automat to describe the spatial dynamics of physical, chemical or biological systems*** by means of the states of individual cells within a two-dimensional grid. Cells in the grid change states discretely according to a uniform and constant set of rules involving the states of the cells and their interaction. [267]•.

A study by G. P. Boswell et al., (2007) **presents a cellular automaton model** that allows detailed understanding of how events at the hyphal level are influenced by the nature of various environmental heterogeneities. Mycelial growth and function were simulated in a range of environments including homogeneous conditions, nutritionally heterogeneous conditions and structurally heterogeneous environments, the results of that study provided further understanding of the crucial processes involved in fungal growth, nutrient translocation and concomitant functional consequences. [265]•.

A novel property of the model system derived by **Boswell et al**, (2007), is the simultaneous use of a ***combination of "cell" models*** (which are used for modelling internal/external substrate and hyphal tips) and ***"bond"*** models (which are used for modelling active/inactive hyphae). *Cell models are ideal for modelling the movement of individual particles since each cell (or site) can take a value corresponding to the current state of that cell (for example, the presence or absence of a tip).* However, this cellular approach is not suitable for modelling the development of a network since adjacent cells need not to be connected. However, a ***bond-based approach*** explicitly models any connections between adjacent hyphae and thus can accurately model the formation of hyphae and internal substrate redistribution inside parallel hyphae showing various degrees of anastomosis. [265]•.

Figure. [61]. Simulated mycelial network expands from a nutrient source at the center of the domain and is shown after a representation of four days growth. Following a representation of two days growth, G. P. Boswell, et al., [265].

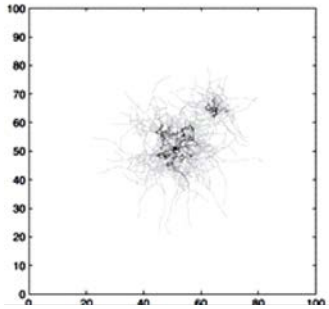
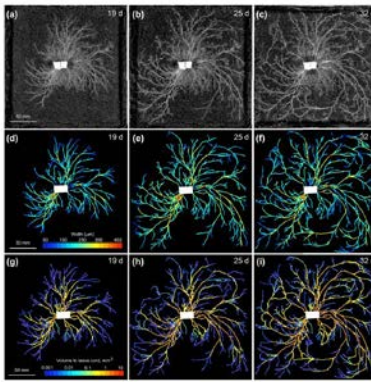


Figure. [62]. Network development and currents predicted by the mathematical model in *Phabaerochaete velutina*. (a) – (c) Network development in *P. velutina* after 19, 25 and 32 d. The image intensity of cords was used to estimate their thickness, enabling the production of the weighted, digitized networks (d) – (f). Are colour-coded to show the estimated thicknesses of all sections of all edges. Images (g) – (i) are colour coded according to the total volume that has passed through each cord, as calculated by our model. by L. Heaton. [266].



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• [267] J. M. Halley, et al., Competition, succession and pattern in fungal communities: towards a cellular automaton model.
• [265] G. P. Boswell, et al., The Development of Fungal Networks in Complex Environments.
• Ibid.
• [266] F. A. Davidson, et al., Mathematical modelling of fungal growth and function, IMA Fungus, 2(1), 33-37, 2011.

The ***bond-based approach*** is similar in essence to ***biased random walks***. Both result in the derivation of a ***master equation in which the rate of change of the value of a system variable at a given point is related via transition probability rates (movement probability rates), to the values of this variable at a number of neighboring points***. ***The discretization procedure allows certain key processes, including hyphal inactivation and reactivation, anastomosis and branching to be treated in a more detailed manner. These processes represent interactions of elements within the automaton*** (representing tips with hyphae, for example).

- The ***Neighbor-Sensing model*** simulates growth of fungal mycelia in three-dimensional space. The mycelium is represented as a tree-like structure. The virtual hyphal tips can branch, grow and alter their growth direction in response to different tropisms determined by the user. [263]•. ***The modelling process starts from the proposition that each hypha in the fungal mycelium generates a certain abstract field that (like physical fields) decrease with increasing distance***. ***Both scalar, and vector fields, are included in the models. The field(s) and its (their) gradient(s) are used to inform the algorithm that calculates the likelihood of branching, the angle of branching and the growth direction of each hyphal tip in the simulated mycelium.*** The growth vector is being informed of its surroundings so, effectively, the virtual hyphal tip is sensing the neighboring mycelium. This is why it is called the ***Neighbor-Sensing model***.

By simulating the mathematics of the control of hyphal growth and branching, the Neighbor-Sensing model provides the user with a way of experimenting with features that may regulate hyphal growth patterns during morphogenesis to arrive at suggestions that could be tested with live fungi. [268]•. The model was proposed by ***Audrius Meskauskas and David Moore in 2004***. *The key idea of this model is that all parts of the fungal mycelium have identical field generation systems, field sensing mechanisms and growth direction altering algorithms.* Under properly chosen model parameters, it is possible to observe the transformation of the initial unordered mycelium structure into various forms, some of them being very like natural fungal fruit bodies and other complex structures. [269]•.

- **Chemotaxis:** is another example of adaptation. Chemotaxis refers to motion induced by the presence of chemical species in the environment. **Microorganisms moves towards higher concentrations of nutrients-chemo attractants and away from toxins and other noxious substances chemo repellents.** *Chemotaxis by cells offer a mechanism for aggregation, either to a pacemaker or to a self-organized common center. If the cells secrete a chemoattractant, then a random fluctuation, which increases local cell density, will cause local chemoattractant concentration to increase, drawing in more cells and again increasing chemoattractant concentration in a positive feedback loop*. Eventually the cells will all move into one or more compact clusters (depending on the range of diffusion of the chemoattractant and the response and sensitivity of the cells). Cell movement in a ripple is approximately one-dimensional since the majority of cells move in parallel lines with or against the axis of wave propagation. [261]•.

• **[263] S. Kelleter, Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of Coprinopsis cinerea.**
•**[268] A. Meškauskas, et al., Simulating colonial growth of fungi with the Neighbour-Sensing model of hyphal growth. Mycological research, 108, 1241-1256, 2004.**
•**[269] A. Meškauskas, et al., Concerted regulation of tropisms in all hyphal tips is sufficient to generate most fungal structures, Mycological research, 108, 341-353, 2004.**
•**[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**

For instance, when E. coli cells are in a homogeneous environment with no preferred direction of motion, *each cell alternates between periods of running and periods of tumbling*. The resulting motion is a r***andom walk*** that samples the local environment. In contrast, when exposed to a gradient of chemoattractant (or chemo repellent) ***these cells bias their random walk by tumbling less frequently when moving in the 'good' direction and more frequently when moving in the 'bad' direction***. Once a cell finds itself again in a uniform environment, it returns to the original tumbling frequency, even if the homogeneous level of attractant (or repellent) is significantly different from before, the sensory mechanism adapts to the new nominal environment (on a time-scale of minutes). The cell is then tuned to respond to changes centered at this new nominal level. [261•; 267•].

[1.3] Environmental integration (bio sensing, bioluminescence, electrical bio catalysis)

Most biochemical networks are open-systems they exchange material with the outside environment and reach a steady state that involves a steady flow through the network. Such a state is called a dynamic equilibrium. [256]•. This focuses on biochemical systems generated by microorganisms that achieve an ecological value such as bio sensing for certain chemicals (toxins), bioluminescence or florescence and electrical bio catalysis for electricity generation.

- ***Biofilm formation***: ***biofilms*** exist in living organisms as well as environmental surfaces. In the modeling of the collective behavior of microorganisms forming biofilms, relevant space scales of centimeters might be represented by continuum mechanics in the form of the ***Navier-Stokes equations***. The ***discrete models*** are related to the smaller scales (hundreds of micrometers), which size depends on the volume of constituents such as fungus, viruses, bacteria, polysaccharides, etc. [258]•. T***he modeling of biofilm explores the relationship between pattern formation process and stochastic (and chaotic) micro-events on the organization of microbial communities***. There are many categories of both continuum and discrete models used for biofilm modeling. The classical model of continuum approach by Wanner and Gujer, (1986), ***mimics the growth of the biofilm's entities with differential equations***, ***another example represents the problem of nutrient consumption in biofilms, which is governed by a convection-diffusion transport equation in the liquid environment and by diffusion and reaction in the biofilm itself*** .[258]•. ***Growth of biofilm differs from growth of a conventional thin-film because the specimens have a special shape and size. They are much larger than individual atoms or molecules and move slowly, or not at all, over the substrate surface compared to the diffusion of atoms on substrates***. [258]•. Many of biological films are impossible to understand by using differential equations and discrete models. Therefore, ***heterogeneous continuum-discrete models*** were developed in which reaction diffusion limited aggregation models and continuum growth equations are combined together to mimic biofilm evolution. [258]•.

• **[261]**
•**[267] J. M. Halley, et al., Competition, succession and pattern in fungal communities: towards a cellular automaton model.**
• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.**
• **[258] K. Krawczyk, et al., Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**
• **[258]**
• **[258]**
• **[258]**

- ***Bio sensing:*** *biosensor is an integrated autonomous unit that combines biological molecules as the recognition element, which is the biological receptor or bio element corresponding to the target concentration.* [270]•*, with a physical transducer and provides quantitative or semi-quantitative analytical data.* The biosensor can be sensitive to both the nature and the toxicity of the pollutant. [271]•. **These sensing devices utilize whole cells, different configurations of antibodies, DNA, RNA, enzymes or receptor proteins as the recognition elements**. [272]•. ***A whole-cell biosensor is an analytic probe consists of a biological element, such as genetically engineered bacteria, antibody or antigen, integrated with an electronic component to yield a measurable signal***. ***As an example, whole-cell biosensor can be used as a real time environmental sensor, detecting the presence of hazardous chemicals***.

In whole-cell bio-sensing, changes in cellular metabolism, pH, and gene expression have been quantified as a response of the sensing elements to the presence of target molecules. [273]•. A variety of detection methods, suitable for a specific biological assay, has been developed for molecular detection. These methods include colorimetric, fluorescent, bioluminescent, and electrochemical detection. [274]•.

The electrochemical biosensor is a widely used technique offering high sensitivity and rapid detection. Electroactive species produced or consumed by microbes are monitored by conductimetric, amperometric, impedimetric, or potentiometric methods. Optical techniques are based on the measurement of fluorescent, luminescent, colorimetric, or other optical outputs from microorganisms. [275]•.

One of the most widely used intracellular sensing mechanisms is based on *the coupling between a transcriptional regulator and an inducible promoter* in response to different nutrient conditions, external toxicants, or communication signals [276]•. The interaction between the target molecules and transcriptional regulators activates or represses the expression of the reporter gene resulting in a measurable signal change in a concentration-dependent manner. ***This type of bio sensing would be employed in embedding microorganisms with certain qualities to achieve specific ecological functions in the built environment (interior and architectural elements)***. *That would be activated by specific threshold in the transducer (to provoke the interaction between the target molecules and transcriptional regulators) to activate a specific response (e.g. Activating luminescence activity in response for certain chemical concentration in interior space).*

In a similar manner ribosomal switches work, ribosomal switches are structured RNA domains, which detect molecules and regulate the expression of associated genes. [277]•. A riboswitch is typically consisted of an aptameric domain that undergoes a conformational change upon binding to the target molecule, resulting in the expression of a reporter gene [278]•. Since RNA is a key regulatory molecule in natural biological system and the design and engineering of RNA are relatively easy,

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•[270] F. Lagarde, N. Jaffrezic-Renault, Cell-based electrochemical biosensors for water quality assessment, Anal. Bioanal. Chem, 2011.
•[271] J. H. Lee, et al., A cell array-biosensor for environmental toxicity analysis. Biosens Bioelectron, 2005.
•[272] M. Park, et al., Microbial Biosensors: Engineered Microorganisms as the Sensing Machinery, Sensors 2013.
• [273] B. F. Pfleger, et al., Microbial sensors for small molecules: Development of a mevalonate biosensor. Metab. Eng, 9, 30–38 2007.
•[274] R. Daniel, et al., Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode, Biosensors and Bioelectronics, 24, 888–893, 2008.
• [275] L. Su, et al., Microbial biosensors: A review, Biosens. Bioelectron, 26,1788–1799, 2011.
• [276] S. Daunert, et al., Genetically engineered whole-cell sensing systems: Coupling biological recognition with reporter genes, Chem. Rev, 100, 2705–2738, 2000.
• [277] S. Topp, J. P. Gallivan, Emerging applications of riboswitches in chemical biology, ACS Chem. Biol, 5, 139–148, 2010.
• [278] M. N. Win, C. D. Smolke, A modular and extensible RNA-based gene-regulatory platform for engineering cellular function, Proc. Natl. Acad. Sci, 104, 14283–14288, 2007.

[279]•. Riboswitches with natural and synthetic RNA aptamers have been developed to sense temperature, metal ions, nucleic acids, small molecule, and proteins. [280]•.

- ***Bioluminescence:*** the bioluminescence phenomenon is not only influenced by many factors (O2, metals, and the carbon source used by the bacterial bio element): but also depends on the physiological state of the bio element, especially in a confined environment. Justifying a modeling of all the influencing parameters, modeling of the bioluminescence biological reaction [281]•, coupled by studying of the kinetics of bacterial growth, which influences the substrate consumption, the metal diffusion (inducer)[282]•, and the intensity of the bacterial bioluminescence [283]•, could provide a control tool over the occurrence of this phenomenon.

Bio sensing/ Bioluminescence: Various mathematical models have been developed for the bioluminescent detection scheme. The bioluminescent detection technique is widely used due to its high sensitivity, wide dynamic range, and relatively inexpensive instrumentation. [274]•. However, using a biosensor for a long time generates many changes in the growth of the immobilized bacteria and consequently alters the robustness of the detection. Emerging numerical tools in bacterial biosensors are more potent to be used as it overcomes the difficulty to measure experimentally in every condition the biosensor functioning during a long time (several days). [284]•. For instance, the numerical simulation of a biomass profile is made by coupling the diffusion equation and the consumption/reaction of the nutrients by the bacteria.

Microbial cell sensors have been constructed by genetically binding the Lux gene with an inducible gene promoter for toxicity testing. Genetic promoters have been utilized to act as very precise detectors of environmental toxins. ***The main function of the bio-reporters is to provide a detectable signal response correlated to the magnitude of the toxin dose***. [274•; 285•; 286•; 287•].

- ***Bio catalysis Reaction Kinetics:*** another aspect of environmental microbial integration behavior is the bio catalysis activity**.** To mathematically trace and model the reaction of substrate *kinetics consumption and microorganism's growth rate in MFCs, the* ***Monod kinetics equation****, is often used.* **The Monod equation is one of the most widespread analytical specific reaction rate expressions used either for microorganism growth or for substrate consumption**. **It expresses the dependence of the reaction rate in the following form: at high substrate concentration, the process is at its maximum rate, while at low substrate**

• [279] J. C. Liang, et al., Engineering biological systems with synthetic RNA molecules. Mol. Cell, 43, 915–926, 2011.
• [280] J. Sinha, et al., Reprogramming bacteria to seek and destroy an herbicide, Nat. Chem. Biol, 6, 464–470, 2010.
• [281] A. Roda, et al., A portable bioluminescence engineered cell-based biosensor for on-site applications, Biosens Bioelectron, 26(8):3647–53, 2011.
• [282] J. Huang, et al., Effect of free-cell growth parameters on oxygen concentration profiles in gel-immobilized recombinant Escherichia coli, Appl Microbiol Biotechnol, 33(6):619–23, 1990.
• [283] M. Affi, et al., Numerical design of a card and related physicochemical phenomena occurring inside agarose-immobilized bacteria: a valuable tool for increasing our knowledge of biosensors, Sensors Actuators B Chem, 138(1):310–7, 2009.
• [274] R. Daniel, et al., Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode, Biosensors and Bioelectronics, 24, 888–893, 2008.
• [284] M. Affi, et al., Numerical modeling of the dynamic response of a bioluminescent bacterial biosensor, Springer, Anal Bioanal Chem, 408:8761–8770, 2016.
• Op.cit.
• [285] E. A. Meighen, Bacterial bioluminescence: organization, regulation, and application of the lux genes, Faseb J, 7(11):1016–22, 1993.
• [286] J. M. Radovich, Mass transfer limitations in immobilized cells, Biotechnol Adv, 3(1):1–12, 1985.
• [287] C. Michan, et al., In vivo transcription of the Escherichia coli oxyR regulon as a function of growth phase and in response to oxidative stress, J Bacteriol, 181(9):2759–64, 1999.

concentrations the process becomes rate limited. *The Monod equation can be represented as:*

$$\mu = \mu_{max} \frac{[S]}{K_{[s]}+[S]}$$.[288]•.

Equation: [8]: The Monode equation for calculating reaction rate in microbial growth, or substrate consumption.

As μ microorganism growth rate [d^{-1}] , [s] is organic substrate concentration in the MFC's anodic compartment [mg-S L^{-1}], and K is half-saturation (Monod) constant [mg-S L^{-1} or mg-A L^{-1} or mg-M L^{-1} or mg-H^2 L^{-1}].

It is also common to describe the kinetics of substrate consumption using the "Haldane law" model. **This model illustrates a growth rate with possible substrate inhibitory effects at high concentrations, often called overloading:**

$$\mu = \mu_{max} \frac{[S]}{K_{[s]}+[S]+\frac{[S]^2}{K_i}}$$.[288].•

Equation: [9]: The "Haldane law" model equation for estimating microbial growth rate and possible substrate inhibitory effects at high concentrations.

Bio catalysis. Biofilm Modelling in MFC: One important aspect that also has to be taken into account in MFC modelling is the biofilm. Wanner and Gujer, (1986) linked the ***biofilm growth and composition with three main processes: (i) space limitation; (ii) substrate conversion, and: (iii) substrate diffusion***. An example of mixed composition of the biofilm was modelled in 2D and in 3D. The model was able to predict substrate consumption, concentration gradients, and the biofilm composition per biofilm position. The diffusion of several substrates and the growth of microorganisms were modelled using ***partial differential equations (PDEs).*** This complex modelling approach leads to large computational effort and numerous non-identifiable model parameters. [288]•.

An alternative approach to complex biofilm models is to assume that some of the biofilm's main processes are non-limiting, in order to use ***continuous mathematical functions*** and avoid the use of PDEs. A number of particular models that focus on the biofilm formation tackle the space limitation problem. One case includes the ***fluidized bed biofilm reactors (FBBRs) modelling***, ***in which the biomass is attached and grows in a fixed bed (biofilm around the bed particles) while substrate flows through this bed.*** A simplified description of substrate and product distribution within a biofilm (diffusion) can also avoid the use of PDEs, as shown by Rauch et al. (1999), with the assumption of a simplified layered biofilm structure. They assumed that substrate diffusion and biochemical conversion were decoupled, which allowed a simple relationship between substrate penetration depth and different homogeneous biofilm layers. [288]•.

For extensive study of bio catalysis behavior patterns in MFCs, the maximal ideal voltage and substrate conversion could also be subjugated to mathematical simulation and modeling. For the charge transport process, or electrochemical reactions, O'Hayre et al. (2006), proposed ***that the voltage predicted thermodynamically less some irreversible polarizations in the fuel cell could compute the fuel cell's voltage output. Generally, the thermodynamic cell voltage prediction can be computed***

• [288]
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for whole or partial limitations of the current by the rate at which the electro reactants are transported to the electrode surface. This can be described by the ***Nernst equation as follows;***

$$E_{thermo} = -\frac{\Delta G^0}{nF} + \frac{RT}{nF} ln \left[\frac{\prod reactant\ fugacity}{\prod product\ fugacity}\right].$$

Equation: [10]: The Nernest equation.

As E_{thermo} is the thermodynamic fuel cell maximum voltage [V], ΔG^0 is fuel cell's Gibbs free energy at a constant temperature [J], R is ideal gas constant [mL-H^2 atm K^{-1} mol-H^{2-1} or J K^{-1} mol-^{1}],and T MFC anode compartment temperature [K].

Since fuel cells operate at low pressures, the fugacity can often be approximated by the partial pressure of the components. Once the temperature and pressure of the system are constant, the fuel cell will present a constant thermodynamic maximum voltage. By neglecting irreversible processes occurring at open-circuit (fuel cell not connected to an external load), such as electrolyte crossover, the maximum thermodynamic voltage can be assumed to be experimentally equal to the open circuit potential ($E_{thermo} = E_{OCP}$). [288]•. Furthermore, ***the electricity produced in a fuel cell can be correlated with the consumption of a substrate in an electrode chamber during electrolysis through Faraday's law of electrolysis***:

$$\dot{m}_{substrate} = MM_{substrate} \frac{I_{cell}}{mF}.$$

Equation: [11]: Equation of Faraday's low of electrolysis.

As $\dot{m}$ is mass flow rate [kg s^{-1}], MM molar mass [kg mol^{-1}], I_{cell} fuel cell current [A], m number of electrons transferred per mol of mediator or per mol of hydrogen [mol-emolmed^{-1} or mol-e- molH^{2-1}].

Irreversible Voltage Losses : Once the fuel cell starts to deliver current to an external load, the output voltage drops from its maximum (E_{thermo}) due to irreversible losses. These losses are often separated in three major groups; Activation losses (due to activation energies and electrochemical reactions), Ohmic losses (due to resistance to the flow of ions in the electrolyte and electrode), and Concentration losses (due to mass transfer limitations). Therefore, an electrochemical balance that can be used to compute the output voltage of a fuel cell can be written as:

$$E_{output} = E_{thermo} - \eta_{act} - \eta_{ohmic} - \eta_{conc}.$$

Equation: [12]: Equation of output voltage of a fuel cell computed from electrochemical balance.

As E_{output} is fuel cell output voltage [V], E_{thermo} is the thermodynamic fuel cell maximum voltage [V], η_{act} activation over-potential [V], η_{ohm} ohmic over-potential [V], η_{conc} concentration over-potential [V].

Each of these polarizations has a different magnitude for different current density degrees. At low current densities, activation losses (η_{act}) are dominant due to reaction energy barriers at the electrode-electrolyte interface, which need to be overcome to start the reaction. At high current densities, reactant and product diffusion limitations lead to high concentration losses (η_{conc}). Finally, ohmic losses (η_{ohm}) increase linearly with current due to electron and ion conduction at the electrodes, electrolytes, and contact resistance across each material's interface, and interconnections to electrodes. [288]•.

• **Ibid**
• **Ibid**

Activation Losses :The activation losses at each electrode of a fuel cell are governed by the Butler-Volmer equation:

$$I_{cell} = i_0 A_{sur} \left[exp\left(\frac{\beta_1 mF\eta_{act}}{RT}\right) - exp\left(\frac{\beta_2 mF\eta_{act}}{RT}\right)\right].$$

Equation: [13]: Butler-Volmer equation for calculating activation losses at each electrode.

As i_0 is exchange current density in reference conditions [A m^2 -1], A_{sur} ,is the anode surface area [m^2], β is dimensionless reduction or oxidation transfer coefficient , m is number of electrons transferred per mol of mediator or per mol of hydrogen [mol-emolmed^{-1} or mol-e- molH2^{-1}], F is Faraday constant [A d mole^{-1}].

The electron transfer processes at the electrode-electrolyte interface determine the reduction (β1) and oxidation (β2) transfer coefficients. These coefficients are directly related to electrode reaction mechanisms and are difficult to identify. Thus, The Butler-Volmer equation can be further simplified once each reaction is assumed to occur in a one-step, single electron transfer. Under this assumption, the transfer coefficients are a function of a symmetric factor (*β1* = 1-*β* and *β2* = *β*), often assumed to be 0.5 for fuel cells. Therefore, for a symmetric reaction (*β* = 0.5). The Butler-Volmer equation can be represented as: [288]•.

$$\eta_{act} = \frac{RT}{\beta_m F} sinh^{-1}\left[\frac{I_{cell}}{2i_0 A_{sur}}\right].$$

Equation: [14]: Simplified Butler-Volmer equation of one step oxidation-reduction reaction for estimating activation losses.

Ohmic Losses: Resistance to the flow of electrons and ions during the fuel cell operation generates ohmic losses. These losses increase as the current flow augments and this linear relationship obeys Ohm's law, therefore ohmic losses can be described as: [288]•.

$$\eta_{ohm} = R_{int} I_{cell}.$$

Equation: [15]: Equation for calculating Ohmic losses.

Concentration losses contribute significantly to a decrease in cell potential, particularly at high current densities and low bulk reactant concentrations. These losses can be determined by the potential difference (*ΔE*) between the voltage at open circuit (bulk concentration, *Ei=0*) and the cell voltage at high current rates (*Ei-high*). Thus, the Nernst equation can be applied between the reactants concentrations in the bulk liquid and on the electrode surface, as:

$$\Delta E = \eta_{conc} = \frac{RT}{mF} ln\left[\frac{c_{Bulk}}{c_{surface}}\right].$$

Equation: [16]: Equation for calculating concentration losses.

[2] Tools: Analysis and Imaging of biological systems

Microbial systems is a rich and undiscovered source of design inspiration and implementation. Not just as formal inspiration (from microbial communities' patterns) but also as a behavioral intelligence schemes that would be applied in behavioral designs in built environment. To gain full insight of these systems there are tools for imaging and analysis of these microbial systems on various scales from cultures, cells, molecules, to atomic level. These imaging tools have exceeded the limits

• Ibid
• Ibid

of 2D and 3D imaging to the 4D (in time), thus discussing imaging and analysis tools would be divided into static and dynamic tools.

[2.1] Static imaging tools

The last twenty years have seen great advances in optical imaging with the ability to monitor biological phenomena with unprecedented resolution, specificity, dimensionality, complexity, and scale, all while maintaining viability and biological relevance: These advances have required the development of software to enable the acquisition, management, analysis, and visualization of the imaging data. [312]•. ***These novel imaging modalities, which are increasingly multi-parametric, rely heavily on computational approaches***. In the following section, the author will exhibit static imaging tools, dynamic imaging tools and computational platforms of biological imagery data acquisition.

Steady-State Behavior and Transient Behavior: Simulations of biological cells represent time-varying system behavior. Models of biological processes usually arrive, in the long run. Most commonly, models exhibit a persistent operating state, called a steady state; some systems display sustained oscillations. The time-course that leads from the initial state to the long-time (or asymptotic) behavior is referred to as the transient. [256]•.

In order to achieve biological imaging on micro scale, various types of microscopes could be used. These probes differ in their scale of magnification for biological organism; they also differ in the time scaling wither static or dynamic; and the technique of magnification. *Magnification is how many times the object is enlarged within the viewing lens. Moreover, Resolution is how detailed the object appears when viewed*. There are stereoscopes, compound microscopes, confocal microscopes, electron microscopes, etc. The specimen under observation determines the microscope needed. [289]•.

Imaging biological systems: *When considering biological system dynamics, the specific ordering and timing of individual processes become vital to determining the overall behavior of the integrated system. It is important to define where and how pathways interact, and to examine the functional effects of these interactions.* A wide range of dynamical properties including bistability, adaptation and oscillations exhibits the non-intuitive nature of non-linear systems. [290]•. ***This requires data from single cells and mathematical approaches to help comprehend their behavior. The ability to acquire spatial and temporal information in a single cell is fundamental, requiring non-invasive methods of measuring cellular processes in the same cell over time. The visualization of cellular processes in vivo enables the investigation of important biological questions such as understanding the function of genes and gene products, and how they regulate cellular processes*** through complex signaling networks. [291]•. Thus, it is essential to master imaging tools that is capable of providing the researcher and designer with full insight into cellular behaviors and functions, this is achieved by numerous types of microscopes within the optical, electron, and scanning probes, which are categorized in the following table:

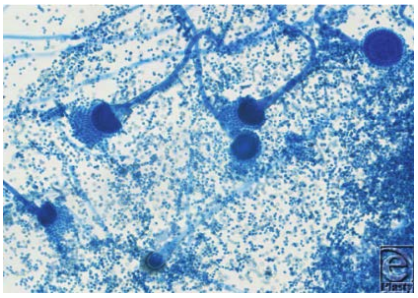
Category	Type	Description	Imaging

• [312] K. W. Eliceiri, et al., Biological Imaging Software Tools, Nat Methods, 2012.
• [256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.
• [289] https://sciencing.com/various-types-microscopes-biology-5949595.html.
• [290] J. J. Tyson, et al., Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell, Curr Opin Cell Biol, 2003.
• [291] D. Mullassery, et al., Single Live Cell Imaging for Systems Biology, Essays Biochem, 45, 121–133, 2008.

Optical microscope

Binocular stereoscopic microscope: is microscope that allows easy observation of 3D objects at low magnification. The stereoscope, also called the dissecting microscope and stereomicroscope is a light illuminated microscope that allows a three-dimensional view of a specimen. The image of the specimen is also lateral and upright. However, stereoscopes have lower power compared to compound microscopes. As images are only magnified up to about 100x. [289]•.

Compound: Like stereoscopes, compound microscopes are illuminated by light. They give a two-dimensional view of a specimen under observation but can have magnifications between 40x and 400x, with more powerful versions up to 2000x. Although the magnification can be high, resolution is limited by the wavelength of light. Compound microscopes cannot view detail less than 200 nanometers apart.[289]•



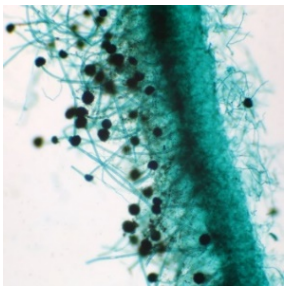


Image. [117]. Left, Light microscopy of *Aspergillus spp* with lacto phenol cotton blue stain

Image. [118]. Right, Microscope slide showing conidiophores of the brown mold, *Aspergillus*

[292•;293•]

Bright field microscope: A typical microscope that uses transmitted light to observe targets at high magnification. [294]•.

Polarizing microscope: A microscope that uses different light transmission characteristics of materials, such as crystalline structures, to produce an image.

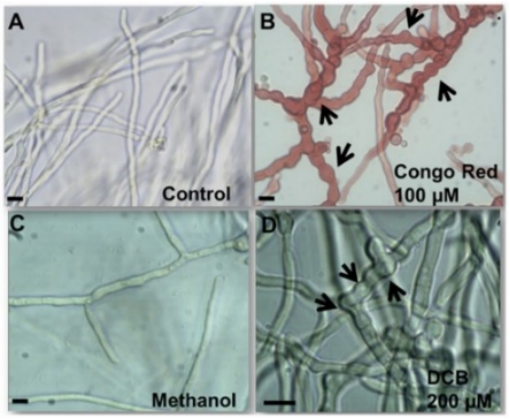


Image. [119]. Bright field microscopy pictures (40x in A, B and C, and 60x in D) of mycelium grown in liquid MM. The arrows point to aberrant structures present along the hyphae. Scale bar refers to 10 µm

[295•]

• [289].
• [289].
•[292]https://www.researchgate.net/figure/Light-microscopy-of-Aspergillus-spp-with-lactophenol-cotton-blue-stain-cultured-from-burn_fig1_261753683.
•[293] https://www.carolina.com/fungi-microscope-slides/aspergillus-conidiophores-slide-w-m/297872.pr.
•[294] Germination of conidia of Aspergillus niger is accompanied by major changes in RNA profiles-https://www.researchgate.net/figure/Germination-of-A-niger-conidia-as-observed-by-bright-field-A-and-fluorescence_fig1_235756427.
•[295] Sensitivity of Aspergillus nidulans to the Cellulose Synthase Inhibitor Dichlobenil: Insights from Wall-Related Genes' Expression and Ultrastructural Hyphal Morphologies-https://www.researchgate.net/figure/Bright-field-microscopy-pictures-40x-in-A-B-and-C-and-60x-in-D-of-mycelium-grown-in_fig2_258997623.

Phase contrast microscope: *A* microscope that visualizes minute surface irregularities by using light interference. It is commonly used to observe living cells without staining them.

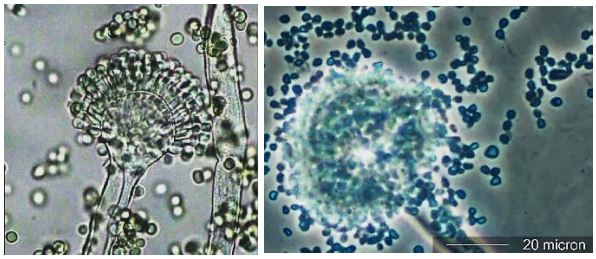


Image. [120]. Left, A microscopic picture by phase contrast microscope of fungal isolate *Aspergillus flavus*,
Image. [121]. Right, Transmitted Phase Contrast Illumination

.[296]• [297]•

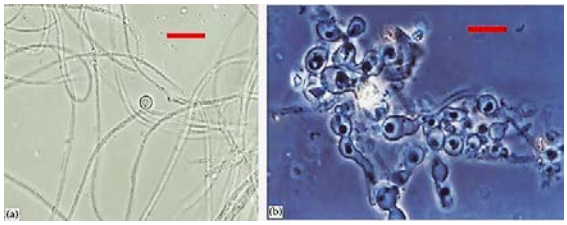


Image. [121, 122]. Photomicrographs of *Aspergillus terreus* (isolate # A 4634) showing (a) normal hyphae during growth at 1 bar/30 1C, and (b) abnormal hyphae with swellings during growth at 200 bar/5 1C. Bars represents 10 mm (b) was photographed under phase contrast.

[298]•

Differential interference contrast microscope: This microscope, similar to the phase contrast, is used to observe minute surface irregularities but at a higher resolution. However, the use of polarized light limits the variety of observable specimen containers.

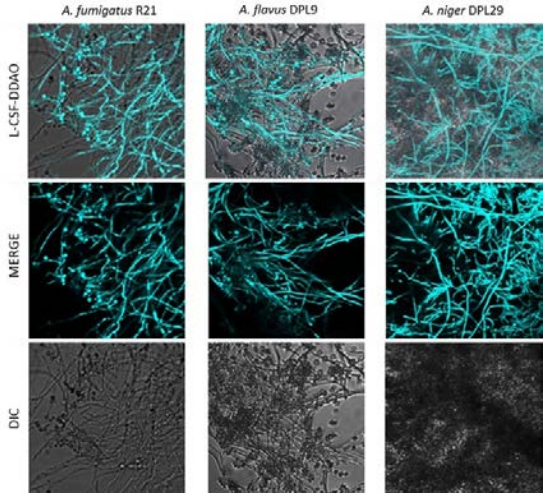


Image. [123]. Representative images of *A. fumigatus* strain R21, A. flavus strain DPL9, and *A. niger* strain DPL29 grown on glass slides labeled with L-CSF-DDAO visualized using confocal microscopy. (DIC: differential interference contrast).

[299]•

Fluorescence microscope: A biological microscope that observes fluorescence emitted by samples by using special light sources such as mercury lamps. When combined with additional equipment, brightfield microscopes can also perform fluorescence imaging.

•[296] Screening of Microorganisms Isolated from some Enviro-Agro-Industrial Wastes in Saudi Arabia for Amylase Production, https://www.researchgate.net/figure/A-microscopic-picture-by-phase-contrast-microscope-of-the-most-potent-fungal-isolate_fig1_317169982.
• [297] http://www.microlabgallery.com/gallery/Asper2.aspx.
•[298]https://www.researchgate.net/figure/Photomicrographs-of-Aspergillus-terreus-isolate-A-4634-showing-a-normal-hyphae_fig6_265865019.
• [299] M. H. Lee, et al., A novel, tomographic imaging probe for rapid diagnosis of fungal keratitis , Medical Mycology, 2017.

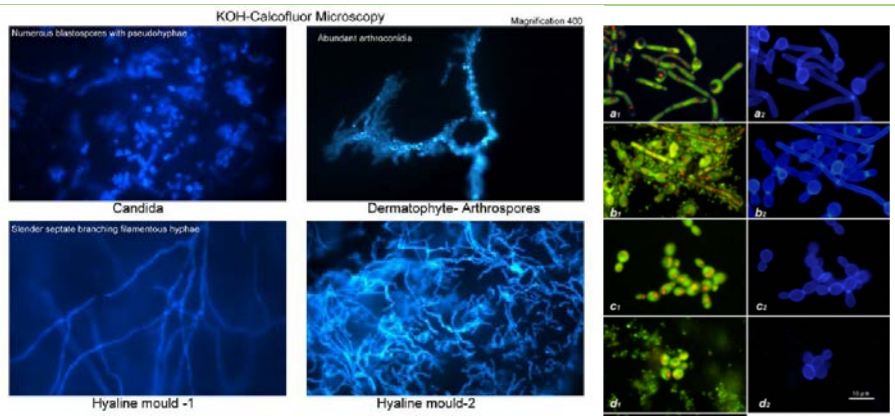


Image. [124]. Left, Fungal isolates with calcofluor white stain under fluorescence microscopy. Magnification 400X.

Image. [125]. Right, Vital staining of C. albicans by FUN1 (1) and calcofluor white M2R (2). (a) Hyphal form of control culture at 37°C; (b) hyphal form of culture at 37°C with *S. salivarius* K12; (c) yeast form of control culture at 30°C; (d) yeast form of culture with *S. salivarius* K12 at 30°C.

[300]• [301]•

Total internal reflection fluorescence microscope: A fluorescence microscope that uses an evanescent wave to only illuminate near the surface of a specimen. The region that is viewed is generally very thin compared to conventional microscopes. Observation is possible in molecular units due to reduced background light.

Laser microscope (Laser scanning confocal microscope): This microscope uses laser beams for clear observation of thick samples with different focal distances.

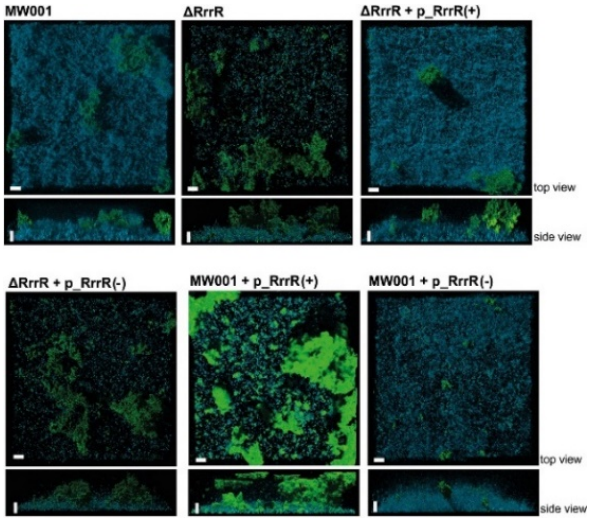


Image. [126]. Confocal laser scanning microscopy analysis of biofilm formation by the *S. acidocaldarius*.

[302]•

• **[300] S. Gurung, et al., Onychomycosis in two geographically distinct regions in India. https://www.researchgate.net/publication/235330794-Onychomycosis-in-two-geographically-distinct-regions-in-India, 2018.**

• **[301] A. A. Ishijima, et al., Effect of Streptococcus salivarius K12 on the In Vitro Growth of Candida albicans and Its Protective Effect in an Oral Candidiasis Model, Appl Environ Microbiol, 78(7):2190-9, 2012.**

• **[302] A. Orell, et al., A regulatory RNA is involved in RNA duplex formation and biofilm regulation in Sulfolobus acidocaldarius, Nucleic Acids Res, 18;46(9):4794-4806, 2018.**

[303]•

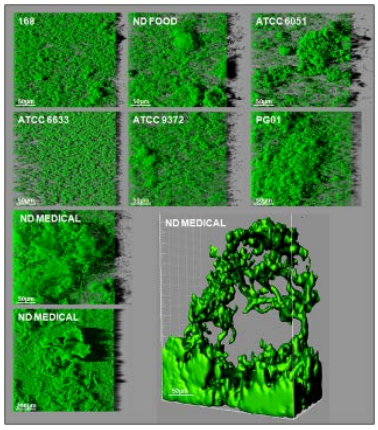


Image. [127]. Three-dimensional biofilm structures of seven *B. subtilis* strains. Images present a representative, aerial, 3D view of the 48h-biofilm structures obtained with the seven *B. subtilis* strains using a microplate system, obtained from confocal image series using IMARIS software (including the shadow projection on the right). One iso-surface representation of a particular ''beanstalk-like'' structure is also shown for *B. subtilis* Image from: Bridier A., et al., The spatial architecture of *Bacillus subtilis* biofilms deciphered using a surface-associated model and in situ Imaging.

Multiphoton excitation microscope: Intravital multiphoton microscopy: Focal excitation of fluorophores by simultaneous attack of multiple photons generates images with high spatial resolution. The use of near-infrared lasers for multiphoton excitation allows penetration of thicker specimens, **enabling biologists to visualize living cellular dynamics deep inside tissues and organs without thin sectioning**. **Moreover, the minimized photo bleaching and toxicity associated with multiphoton techniques is beneficial for imaging of live specimens for extended observation periods**. [304]•.

[305•]

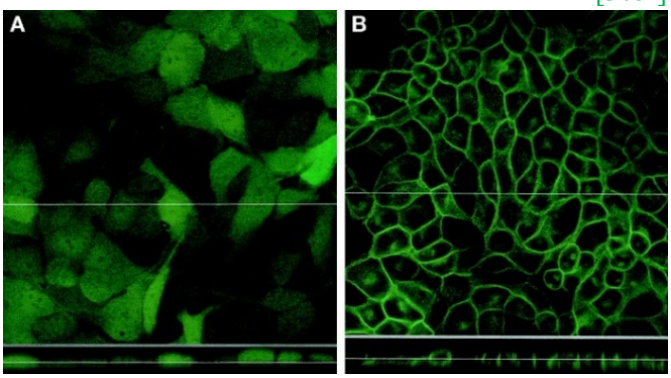


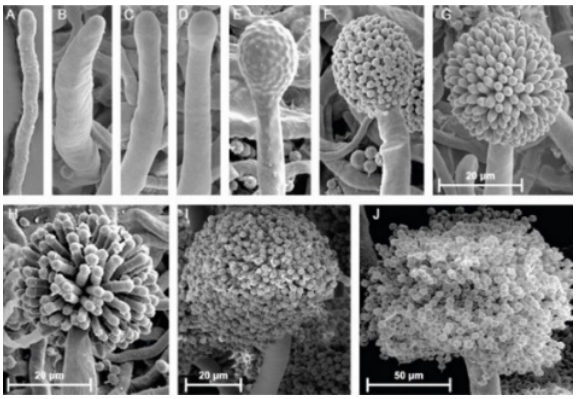
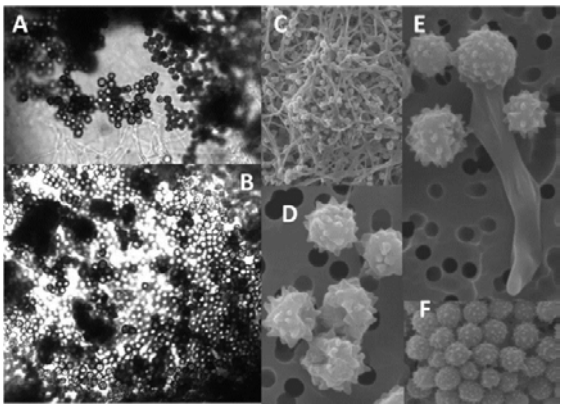
Image. [128]. Confocal images of MDCK-D1 cells expressing either GFP (A) or cP2Y11-GFP receptors (B). The upper images in each pair are fluorescence in the x-y plane. The small lower image is fluorescence of the z-plane, taken at the thin white lines bisecting the upper images. In the lower part of B, apical surface is upper surface (above the white line).

Structured illumination microscope: A high-resolution microscope with advanced technology to overcome limited resolution found in optical microscopes that is caused by the diffraction of light.

Electron microscope

Transmission electron microscope (TEM), scanning electron microscope (SEM), etc. The electron microscope could magnify objects to the 10,000-x range. A TEM works by focusing a beam of single-energy electrons strong enough to pass through a very thin specimen. The resulting images are then viewed through electron diffraction or direct electron imagining. The SEM works by scanning a sample's surface with an electron beam. This beam creates different signals, secondary electrons, X-rays, photons, and others, which all help characterize the sample. The signals are displayed

• **[303] http://www.bitplane.com/biofilms.aspx#prettyPhoto/0/11 ,2018**
• **[304] J. Kikuta, M. Ishii, Recent advances in intravital imaging of dynamic biological systems. J Pharmacol Sci. 2012;119(3):193-7. Epub 2012.**
• **[305] http://molpharm.aspetjournals.org/content/60/1/26.**

	on a screen that maps out the sample's material properties. [306]•. (Chapter 4, SEM imaging of Aspergillus sydowii NYKA 510, by the author). ***Environmental scanning electron microscopy (ESEM)*** is an imaging technique, which allows hydrated, insulating samples to be imaged under an electron beam. The resolution afforded by this technique is higher than conventional optical microscopy but lower than conventional scanning electron microscopy (CSEM). The major advantage of the technique is the minimal sample preparation needed, making ESEM quick to use and the images less susceptible to the artifacts that the extensive sample preparation usually required for CSEM may introduce. In some circumstances, it is possible to image live cells in the ESEM. [307]•.  **Image. [129]. Development of *A. niger* monitored by scanning electron microscopy.** [308]• **Image. [130]. *Aspergillus sydowii* conidiophores and hyphae from Australian coastal waters. (A and B) Light micrographs of spore masses from CPR silks. (C to F) Scanning micrographs of conidiophores and hyphae from CPR silks.**  [309]•
Scanning probe microscope (SPM)	***Atomic force microscope (AFM), scanning near-field optical microscope (SNOM), etc.*** This microscope scans the surface of samples with a probe and this interaction is used to measure fine surface shapes or properties.
Others	***X-ray microscope, ultrasonic microscope, etc***. [310]•.

• **[306] A. Sergei, et al., Ultrafast Electron Microscopy for Chemistry, Biology and Material Science, Journal of Analytical Sciences, Methods and Instrumentation, 2013.**
• **[307] J. E. McGregor, et al., Environmental scanning electron microscopy in cell biology.Methods Mol Biol. 2013.**
•**[308] https://www.researchgate.net/figure/Development-of-A-niger-monitored-by-scanning-electron-microscopy-The-vegetative_fig6_235763153.**
•**[309] Australian Dust Storm Associated with Extensive Aspergillus sydowii Fungal "Bloom" in Coastal Waters, https://www.researchgate.net/figure/Aspergillus-sydowii-conidiospores-and-hyphae-from-Australian-coastal-waters-A-and-B_fig2_261033408**
• **[310] https://www.keyence.com/ss/products/microscope/bz-x700/study/principle/002/index.jsp.**

Conventional microscopy methods have limitations in the dynamic analysis of biological processes, mainly since cells require chemical fixing and therefore observations are snapshots at single time points in non-physiological conditions, resulting in population-based rather than single cell data. [311]•.

Live cell imaging and computational modelling are compatible techniques, which allow quantitative analysis of cell dynamics. **Non-invasive imaging techniques, based on the use of various luciferases and fluorescent proteins,** ***trace cellular events such as gene expression, protein-protein interactions and protein localization in cells.*** Multiple parameters can be measured simultaneously in the same cell. Following acquisition using specialized microscopy**,** ***analysis of multi-parameter time-lapse images facilitates the identification of important qualitative and quantitative relationships***. [291]•.

Time-lapse fluorescence imaging facilitates the quantitative measurements of the dynamics of cellular processes with both high spatial and temporal resolution, and enables the real-time dynamics of biological processes to be observed.

The fluorescence detection requires only very short integration times of the order of milliseconds to seconds per frame. This enables the measurement of highly dynamic processes since time intervals between images can be fractions of a second. The limitations are dependent on the capabilities of the microscopy system, for example the sensitivity of the detectors used for acquiring individual images and the number of variables measured. ***The images generated are multi-dimensional, each single timeframe can be composed of multiple optical slices in the z-plane and generally include a transmission image plus an image for each different fluorescence compound used for each time point***. Thus, data may be collected in the X.Y.Z planes and at multiple wavelengths over time. There are likely to be many cells per field (20-50 with a 40x magnification objective lens – dependent on cell type), typical data extracted from a single cell in these images includes the cytoplasmic mean fluorescence and nuclear mean fluorescence. [291]•.

Image acquisition: Biological laboratories usually acquire images by measuring photon flux in parallel (using a camera) or sequentially (using a point detector and equipment that scans the area of interest). In most cases, image acquisition needs to be tightly synchronized with other computer-controllable equipment such as shutters, filter wheels, XY stages, Z-axis focus drives, and autofocus mechanisms (implemented in software or hardware). ***This automation is necessary in order to gather the appropriate information from a sample or to allow the unattended acquisition of large numbers of images in time-lapse series***, ***z-stacks, and multiple spatial locations in a large sample or multiple samples in a large-scale experiment***. Image acquisition software is therefore needed to communicate with these various components and coordinate their actions.

- ***Confocal microscope systems*** that require computer control to acquire an image are usually bundled with control software; several different independent software packages are available for ***wide-field microscope control*** and automation. Software compatibility with hardware is an essential consideration when planning a new microscope system. Several commercial software packages combine image acquisition and analysis***. These include Meta morph (Molecular***

• [311] R. D. Phair, T. Misteli, Kinetic modelling approaches to in vivo imaging. Nature Reviews Molecular Cell Biology, Dec; 2(12):898-907, 2001.
•[291] D. Mullassery, et al., Single Live Cell Imaging for Systems Biology, Essays Biochem. 45: 121–133, 2008.
• Ibid

Devices), Slide Book (3i), Image-Pro (Media Cybernetics) and Volocity (Perkin-Elmer). The obvious advantage of commercial image acquisition packages is that they provide a turnkey solution to all "standard" image analysis strategies (snapping individual images, taking time-lapse series, three-dimensional (3-D) stacks at multiple XY positions).

Ready dynamic data acquisition is almost useful for predetermined imagery data requirements, in other cases where researchers that have non-standard or frequently changing needs and equipment must often write their own code. This is possible by software development that is facilitated by ***toolkit environments such as LabVIEW*** (National Instruments) and ***MATLAB (Math works),*** which provide interfaces to a subset of available equipment and can be used to create a graphical user interface. ***Developing novel imaging technologies necessitates writing instrument control code, as the needs for these novel techniques simply could not be anticipated in existing software packages***. Examples of novel developments enabled by software written in research laboratories include structured illumination microscopy, super resolution microscopy and bessel beam microscopy. [313•; 314•].

Furthermore, two open-source software projects, ***µManager and ScanImage***, provide tools with more flexibility than commercial tools and greater ease of use than the toolkit environments. ***µManager*** mainly targets ***camera-based imaging****, although it is also used with scanning systems*. [315]•. It includes an easy-to-use interface that runs as an ImageJ plugin and enables researchers to design and execute common microscopy functions as well as customized image acquisition routines. µManager provides full control of the components of the light microscope such as cameras, stages, and filter wheels. The program can be used to collect multichannel data over space and time. [316]•.

ScanImage: provides a software framework to control ***laser-scanning microscopes*** and is used extensively for ***two-photon excitation microscopy***. [317]•. It implements most standard modes of image acquisition and basic automation and supports continuous *image acquisition synchronized to behavioral or physiological data*, the software framework is ***object-oriented and event-driven*** to promote extensibility, online analysis, and plugin development. [318]•.

Image analysis: after obtaining microscopy images, analysis tools are essential to convert microscopy images into quantitative data. [319]•, **and for designers it is more important as well to quantify the imagery data obtained in order to employ it in the mathematical modeling process of bio form, function , and behavioral patterns in bioactive designs.** In particular, image analysis is a necessary step for experiments where hundreds or thousands of images are collected by ***automated microscopy***, whether for screening multiple samples, collecting time or z-series data. In addition, image processing is important for many biological studies such as measuring changes in structures over time, or looking at non-spatial data such as fluorescence lifetime data. [320]•.

A vast number of image analysis algorithms and software packages have been developed for biological applications. The software packages differ in their intended application areas, the level of

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• [313] S. A. Jones, et al., Fast, Three-dimensional super-resolution imaging of live cells, Nat. Methods, 8:499–508, 2011.
• [314] T. A. Planchon, et al., Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination, Nat. Methods, 8:417–423, 2011.
• [315] A. Edelstein, et al., Computer control of microscopes using µManager, Mol Biol, 2010.
• [316] H. P. Lin, et al., Bimolecular fluorescence complementation analysis of eukaryotic fusion products, Biol Cell, 102, 9, 525-37, 2010.
• [317] T. A. Pologruto, et al., Scan Image: flexible software for operating laser-scanning microscopes, BioMedical Engineering OnLine volume, 2, 2003.
• [318] M. Linkert, et al., Metadata matters: access to image data in the real world, J Cell Biol, 189, 5, 777-82, 2010.
• [319] E. Glory, R. F. D. Murphy, Automated subcellular location determination and high-throughput microscopy, Cell, 12,1, 7-16, 2007.
• [320] J. R. Lakowicz, Principals of Fluorescence Spectroscopy, New York: Academic Press, 1999.

usability, and openness of the source code. *Most image-analysis software packages developed in academia are written to accomplish very specific tasks relevant to a research problem at hand*. Software exists that is designed solely for particular cell types, particular organisms, particular assay readouts, and particular imaging modalities.

The second category of image analysis software packages are those that can address a more general set of problems. They are typically modular and thus offer greater flexibility to multiple applications. Some commercial tools in this category include ***Meta Morph, Amira (Visage Imaging), Volocity, Imaris***, ***NIS-Elements, Slide Book, Image Pro Plus (Media Cybernetics) and ZEN (Zeiss),*** and are often offered by microscopy companies and sold together with imaging instrumentation. There are many open-source image analysis solutions, such as ***Bio Image XD, Icy, Fiji, Vaa3D, Cell Profiler, 3D Slicer, Image Slicer, Reconstruct, Fluo Render, Image Surfer, OsiriX, and IMOD***[2]. [321•; 322•;323•; 324•;325•;326•;327•; 328•; 329•; 330•]. For example, Fiji is tool of choice in analysis of electron microscopy data, while ***Icy offers unique features for behavioral analysis and cell segmentation and tracking, and Vaa3D is heavily together with Bio image XD offers the best facilities for 3-D visualization.***

In image analysis process, choosing between tools often comes down to preference for familiar interface and ease of use. All the open source software applications described previously utilize code from other applications in the form of libraries and in some cases: one platform can be even run inside another. Workflow systems offer more flexibility. They enable calling each application as components in the analysis pipeline allowing users to build their own virtual systems picking feature sets from any applications.

Image visualization: modern microscopy methods enable direct capture of n-dimensional data (several channels with data across three spatial dimensions and time). The spatial dimensions alone can be very large as the addition of a computer-controlled stage allows the researcher to perform step-and-repeat microscopy, imaging large regions of tissue (millimeters to centimeters) as a montage with sub-micron resolution [331•;332•]. Techniques such as ***serial section microscopy*** enable this montaging to extend to the axial dimension. [333]•.

These multi-dimensional imaging techniques allow direct comparison of labeled biological entities in full spatial and temporal context*. However, the benefits come at the cost of more complex data storage, visualization, and analysis needs. With modern software tools and contemporary desktop graphics hardware****, a three-dimensional multi-channel image cube can be projected onto the screen***

• **[321] P. Kankaanpää, et al., BioImageXD: an open, general-purpose and high-throughput image-processing platform, Nature Methods, volume 9, 683–689, 2012.**
• **[322] F. Chaumont, et al., Icy: an open bioimage informatics platform for extended reproducible research, Nat Methods, 9, 7, 690-6, 2012.**
• **[323] J. Schindelin, et al., An open-source platform for biological-image analysis, Nat Methods, 9, 7, 676-82, 2012.**
• **[324] H. Peng, et al., V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets, Nat Biotechnol, 28,4, 348-53, 2010.**
• **[325] A. E. Carpenter, et al., Cell Profiler: image analysis software for identifying and quantifying cell phenotypes, Genome Biol, 2006.**
• **[326] S. Pieper, et al., The NA-MIC Kit: ITK, VTK, pipelines, grids and 3D slicer as an open platform for the medical image computing community, Proceedings of the 3rd IEEE International Symposium on Biomedical Imaging: From Nano to Macro, Vol. 1, 698–701, 2006.**
• **[327] J. C. Fiala, Reconstruct a free editor for serial section microscopy, 218(Pt 1), 52-61, 2005.**
• **[328] Y. Wan, et al., Fluo Render: An Application of 2D Image Space Methods for 3D and 4D Confocal Microscopy Data Visualization in Neurobiology Research, IEEE Pac Vis Symp, 201-208, 2012.**
• **[329] D. Feng, et al., Stepping into the third dimension, J Neurosci, 27, 47, 12757-60, 2007.**
• **[330] A. Rosset, et al., OsiriX: an open-source software for navigating in multidimensional DICOM images, Digit Imaging, 17, 3, 205-16, 2004.**
• **[331] C.L. Tsai, et al., Robust, globally consistent and fully automatic multi-image registration and montage synthesis for 3-D multi-channel images, J. Microsc, 243, 154–171, 2011.**
• **[332] S. Reibisch, et al., Globally optimal stitching of tiled 3D microscopic image acquisitions, Bioinformatics, 25, 1463–1465, 2009.**
• **[333] S. Saalfeld, Elastic Volume Reconstruction from series of ultra-thin microscopy sections, Nat. Methods.**

at interactive speeds, allowing the user to examine the data from any chosen angle and zoom factor. The speed usually results from the exploitation of hardware graphics processing engines, and careful software optimization such as utilized in the Vaa3D software. [324]•. *When the entire image series can be loaded in computer memory, Vaa3D can be used to produce real-time 5D rendering.*

One obvious approach of Interactive 3-D visualization is hierarchical visualization, a method that combines both global and local 3-D rendering windows in memory and additional navigation window for large image files. [324]•.

Open source bio imaging libraries enables the assembly of various required imagery data acquisition and analysis computational processing clusters. Among open source bio imaging libraries, VTK and ITK. Both are designed as a collection of data processing units, filters that take input data and produce output data. These filters can be combined into processing pipelines that provide the flexibility and adaptability required by unexpected processing needs. ***VTK's focus on visualizing 2-D and 3-D images and geometrical meshes with a large variety of rendering techniques, together with 3-D widgets that facilitate user interactions with the objects being visualized***. *It also provides a collection of methods for performing information visualization, while in the other hand, ITK is a complementary library focused on actual data processing rather than visualization; ITK provides one of the largest collections of image analysis algorithms, in particular for image segmentation, image registration, image stitching, and feature extraction. The toolkit supports N-dimensional images, with particular emphasis in 2-D, 3-D and 4-D. ITK also provides support for a large variety of image file formats.* [324]•.

In addition, open CV is an open source library that provides a rich set of image analysis algorithms in the domain of computer vision, for native languages (C++, C, and Python). Open CV offers, feature extraction algorithms that can identify notable structures from images, feature matching and tracking algorithms that can follow moving objects in video sequences, and calibration algorithms for correlating objects from 3-D space with features that they project into the 2-D plane of an imaging sensor.

Workflow based tools allow researchers to flexibly and intuitively model data processing and analysis protocols and integrate a diverse array of tools without writing complex scripts or being constrained to application-focused, monolithic tools. Through intuitive user interface. "Visual programming", enabling access to a variety of functionality (e.g., image processing, data mining) while also giving access to multiple data sources (e.g., chemical, biological, textual).

[2.2] Dynamic imaging tools

***Dynamic behavior of reaction networks*:** To predict the time-varying changes in species' biochemical and biophysical processes, the rates at which the reactions occur must be known. The rate of a reaction depends on the concentrations of the reactants and the physio-chemical conditions (e.g. temperature, pH). [256]•. These demands ***Separation of Time-Scales and Model Reduction*,** when constructing a dynamic model, one must decide which time-scale to address. This choice is typically dictated by the time-scale of the relevant reactions and processes. The time-scales (time constants)

• **[324] H. Peng, et al., V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets, Nat Biotechnol, 28, 4, 348-53, 2010.**
• **Ibid**
• **Ibid**
• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.**

could be deduced from the reaction system. For nonlinear processes, characteristic time-scales are not as neatly defined as biological processes take place over a wide range of time-scales.

To model a system that involves processes acting on different time-scales, a primary time-scale must be chosen. Other time-scales are then treated as follows:

- Processes occurring on slower time-scales are approximated as frozen in time.
- Processes occurring on faster time-scales are presumed to occur instantaneously.

Usually, these time-scale separations are made during model construction; they often motivate the decisions as to which species and processes should be included in the model and which will be neglected. In other cases, existing models that incorporate separate time-scales can be simplified. This model reduction process approximates the original model with a model of reduced complexity. [256].•

Thus, biological dynamical imaging tools is far more complicated than static imaging, as it involves integration and combination, not just between specific type of microscopy chosen according to magnification scale and it's compatible software for imaging acquisition, visualization and analysis, but also: involving tools and methods that are developed specifically to a certain biological processes. It may use a time laps imaging approach, or it may utilize certain probes that are able to film directly reactions that occurs on different cellular scales. Thus, it could be specified that dynamic imaging is ***object based*** and it contains methods more than tools. For so, this research scope discusses the most prominent tools and methods of biological dynamic imaging:

- ***Optical microscopy using fluorescent probes***, such as green fluorescent proteins, these probes made it possible to visualize the processes occurring in vitro [334]•, despite the high temporal resolution of optical methods, their spatial resolution is usually limited by the wavelength to the range of 200 - 800 nm.
- ***Dynamic Transmission Electron Microscopy.*** [306]•.
- ***Ultrafast Electron Microscopy: (UEM) allows recording dynamic processes with temporal resolutions down to the femtosecond (10-15 s) level. The combination of sub-angstrom spatial and femtosecond temporal resolutions opens to investigation myriad fundamental, atomic-scale processes in fields that range from materials science to biology.*** [335]•.

Conventional TEMs are limited in temporal resolution by the millisecond shutter speeds of current compatible detectors as well as the current per unit time impinging on the CCD chip***. With UEM, the same detectors are used, but the exposure time is effectively shortened by illuminating the sample with a very brief packet of electrons. The electrons in this probe pulse are photoelectrons that are generated when the emitter in the TEM source is exposed to a laser pulse and subsequently accelerated in the conventional manner by an applied electric field into the optical column of the TEM.*** [335]•.

The UEM can be operated in either single-pulse or repetitive stroboscopic modes. The single-pulse mode is employed when studying processes that are non-reversible on the experimental time scale. For reversible processes, the stroboscopic mode can be used to achieve relatively high spatial

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• **Ibid**
• **[334] A. H. Zewail, J. M. Thomas, 4D Electron Microscopy. Imaging in Space and Time, Imperial College Press, London, 2010.**
• **[306] A. Sergei, et al., Ultrafast Electron Microscopy for Chemistry, Biology and Material Science, Journal of Analytical Sciences, Methods and Instrumentation, 2013.**
• **[335] D. J. Flannigan, O. Lourie, 4D ultrafast electron microscopy sheds light on dynamic processes from the micrometer to the atomic scale, Microscopy and Analysis, Nanotechnology Issue S7, 2013.**
• **Ibid**

and, especially, temporal resolutions. In this mode, the CCD shutter is left open, and the exposure is repeated many times to allow sufficient signal to accumulate on the detector. [334]•.

[336] •

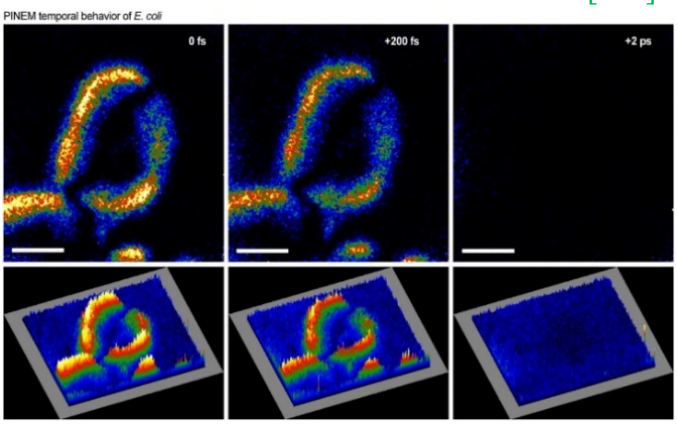

Image. [131]. Ultrafast PINEM imaging of a whole unstained and unfixed *E. coli* cell. Shown are three pseudo-color PINEM images (top row) and the corresponding three-dimensional surface plots (bottom row) of the same cell, but obtained at different points in time (0 fs, +200 fs, and +2 ps). Each image was acquired at a magnification of 53,000×, and all were filtered for noise removal. The contrast limits are set to the same range for each row of images. (Scale bars, 500 nm.)

[3] Tools: biological systems modeling (mathematical base /computational tool)

A mathematical model can be defined as a ***"simplification and an idealization***". The aim of mathematical models is the reduction of a complex biological system to a simpler mathematical system, which allows drawing conclusions on key properties of an organism. *Models can be used to compress a time frame, since a simulation model run on a computer can be used to quickly investigate events that take place over a long time*. [260]•.

In order to exploit mathematical modeling in bioactive design, it should be referred to the ecological theory; this theory comprises two disparate parts: *one **part is concerned with processes (behavior)*** (e.g. population dynamics). The other part is concerned with ***spatial patterns (forms, structures, materials)*** (e.g. community ecology) [259]•. The two parts have little in common: the dynamic models presuppose homogeneity and immediate information transfer between the different populations and *should therefore be considered as point models that do not have any spatial extension. The spatial models do not usually include dynamical processes*. Cellular-automaton models weld the two parts together by developing a theory of "***dynamic eco-morphology***" (or eco-morphological dynamics).

Dynamic systems: The dynamic system concept is a mathematical formalization for any fixed rule, which ***describes the time dependence of a point's position in its ambient space***. The concept unifies very different types of such rules in mathematics. Time can be measured by integers, by real or complex numbers or can be a more general algebraic object, losing the memory of its physical origin, and the ambient space may be simply a set, without the need of a smooth space-time structure defined on it. [337]•.

Real dynamic system :A *real dynamical system*, ***real-time dynamic system***, ***continuous time dynamic system*****, or *flow*** is a tuple (T, M, Φ) with T an open interval in the real numbers R, M

• **[334] A. H. Zewail, J. M. Thomas, 4D Electron Microscopy, Imaging in Space and Time.**
•**[336] D. J. Flannigan, et al., Biological imaging with 4D ultrafast electron microscopy, PNAS, 2010.**
• **[260] P. Gerlee, A.R. Anderson, Stability Analysis of a Hybrid Cellular Automaton Model of Cell Colony Growth.**
• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.**
•**[337] M. Giunti, C. Mazzola, Dynamical systems on monoids: Toward a general theory of deterministic systems and motion. Methods, Models, Simulations and Approaches Towards a General Theory of Change, 173-185, 2012.**

a manifold locally diffeomorphic to a Banach space (a **Banach space** is a complete normed vector space. Thus, a Banach space is a vector space with a metric that allows the computation of vector length and distance between vectors and is complete in the sense that a Cauchy sequence of vectors always converges to a well-defined limit that is within the space.) [338]•, and Φ a continuous function. If T=R the **system is *global***, if T is restricted to the non-negative reals the system is a ***semi-flow***. If Φ is continuously differentiable, the system is a ***differentiable dynamic system***. If the manifold M is locally diffeomorphic to R^n, the dynamic system is ***finite-dimensional*;** if not, the dynamic system is ***infinite-dimensional***.

Dynamic systems arise in the study of population genetics, ecology, and many other diverse fields *where one seeks to model the change in behavior of a system over time*. Several of the global features of dynamic systems such as attractors and periodicity over discrete time intervals, also occur in cellular automata, almost all dynamic systems share the following: • ***Homogeneity: all cell states are updated by the same set of rules;*** • ***Parallelism: all cell states are updated simultaneously;*** • ***Locality: the rules are local in nature***. [339]•.

[3.1] Dynamic Mathematical models

Biologists regularly make use of tangible 'real-world' models, such as model organisms, or they use conceptual models that take the form of verbal descriptions of systems, and are communicated by diagrams that illustrate a set of components and the ways in which they interact. These interaction diagrams play a central role in representing cellular processes. A drawback of these models is that they can be very vague in describing system behavior, especially when the interaction network involves feedback. Thus, using a mathematical description of the system would eliminate uncertainty in model behavior, by quantitative representation of each of the interactions in the diagram model. [256]•.

Investigation of mechanistic models follows two complementary paths. The more direct approach is ***model simulation***, or in ***silico experiments***: in which the model is used ***to predict system behavior*** (under given conditions). Simulations are carried out by numerical software packages; alternatively, models can be ***investigated directly***, yielding general insight into their potential behaviors. These model analysis approaches sometimes involve sophisticated mathematical techniques. The pay-off for mastering these techniques is an insight into system behavior that cannot be reached through simulation. ***While simulations indicate how a system behaves, model analysis reveals why a system behaves as it does***. This analysis can reveal non-intuitive connections between the structure of a system and its consequent behavior. [256]•.

Dynamic mathematical models aid biological investigation in a many ways. Constructing the dynamic mathematical model demands a critical consideration of the mechanisms that underlie a biological process as *a model recapitulates system behavior*; it concisely summarizes all of the data it was constructed to replicate. These model's Simulations can be carried often in seconds with no real cost. Moreover, the model behavior can be explored in conditions that could never be achieved in a laboratory with every aspect of its behavior observed at all time-points. [256]•.

•[338] S. Banach, (1932), Théorie des opérations linéaires, Monografie Matematyczne 1, Warszawa, 1932.
•[339] P. Y. L. Francesca, R. Nardi, Probabilistic Cellular Automata Theory, Applications and Future Perspectives, Complexity and Computation, Volume 27, Emergence, Complexity and Computation.
• [256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction.
• [256]
• [256]

Besides variables of state, dynamic mathematical models also include parameters. Model parameters characterize interactions among system components and with the environment. A change in the value of a model parameter corresponds to a change in environmental conditions or in the system itself. These values can be varied to explore system behavior under perturbations or in altered environments.

For gaining full understanding of the dynamic mathematical models, a study of the model's main characteristics are discussed as following:

- ***Linearity and nonlinearity*:** A relationship is called linear if it is a direct proportionality linearity allows for effortless extrapolation. Linear relationships involving more than two variables are similarly transparent. *A dynamic mathematical model* is called linear if all interactions among its components are linear relationships. However, this is considered a highly restrictive condition, and consequently linear models display only a limited range of behaviors. On contrary, ***nonlinear relations*** do not follow any specific pattern, thus, they are difficult to address with any generality. The nonlinearities that appear most often in biochemical and genetic interactions are ***saturations***, in which one variable increases with another at a diminishing rate, so that the dependent variable tends to a limiting, or asymptotic value. [256][•].
- ***Global and local behavior*:** Nonlinear dynamic models exhibit a wide range of behaviors. In most cases, a detailed analysis of the overall, global, behavior of such models would be overwhelming. Instead, attention can be focused on specific aspects of system behavior. In particular, *by limiting attention to the behavior near particular operating points*, taking advantage of the fact that, over small domains, nonlinearities can always be approximated by linear relationships. This local approximation allows application of linear analysis tools in this limited preview. The global behavior of systems is often tightly constrained by their behavior around few nominal operating points; local approximations are of particular use in biological modelling because self-regulating systems spend much of their time operating around specific nominal conditions. [256][•].
- ***Deterministic models and stochastic models*:** Mathematical model is called deterministic if its behavior is exactly reproducible. Although the behavior of a deterministic model is dependent on a specified set of conditions, no other forces have any influence, so that repeated simulations under the same conditions are always in perfect agreement. In contrast, stochastic models allow for randomness in their behavior. *The behavior of a stochastic model is influenced both by specified conditions and by unpredictable forces. Repeated stochastic simulations thus yield distinct samples of system behavior*. [256][•].
- **Memory and irreversible decision-making:** Adaptive *systems* are able to eventually ignore, or forget a persistent signal. The opposite behavior can also be useful; some systems remember the effect of a transient signal. This memory effect can be achieved by a ***bistable system***. An input that pushes the state from one basin of attraction to the other causes a change that persists even after the input is removed. ***The steady-state response of a bistable system is even more switch-like than the ultrasensitive behaviors***. Either the state is perturbed a little and then

[•] **Ibid**
[•] **Ibid**
[•] **Ibid**

relaxes back to its starting point, or it gets pushed into a new basin of attraction and so relaxes to the other steady state. ***This sort of decision-making mechanism is particularly suited to pathways in which a permanent yes/no decision must be made.*** For instance, when cells commit to developmental pathways, there is no meaningful 'intermediate' response-these are discrete (yes/no) decisions whose consequences persist for the lifetime of the cell. [256]•.

[3.1.1] Differential Equations

Numerical simulations of differential equation models are not as useful as analytic solution formulas, for two reasons. Firstly, an analytic formula is valid for all initial conditions, in contrast with, numerical simulations, as each numerical simulation must be generated from a particular initial condition. Secondly, the dependence on the model parameters can be easily discovered from an analytic solution formula (e.g. the time constants), unlike the case of numerical simulations, as no such insights are granted by the numerical simulation, in which the parameter values must be fixed, as computational exploration of different parameter values demands running multiple simulations. [256]•.

Many models of biological processes have employed PDEs (partial differential equations) ***to combine elements of random diffusive motion with biologically driven rules*** that generate motion that is more ordered. These models, however, *treat only local average densities of cells and do not include capturing the global interactions inherent in a population that moves as a collective unit. Nor do they include the discrete nature of cells and their non-trivial geometry and orientation.* [261]•.

[3.1.2] Cellular automata (CA)

This term was first defined and studied in the 1940s. [259]•. Cellular automata models have been proposed for a large number of biological applications for studying the emergence of collective macroscopic behavior emerging from the microscopic interaction of individual components, such as molecules, cells or organisms. CA model is based on a very limited set of rules but it shows the diversity of microbial growth behavior. CA is based on qualitative conclusions of the biology to design simple local evolution rules to examine the characteristics of microorganisms. This offers a theoretical CA model for the modeling and simulation of the complex system. [340•; 257•]. Based on the variability in the local dynamics, an "***interaction-module oriented***" cellular automaton modeling provides an intuitive and powerful approach to capture essential aspects of complex phenomena at various scales.

Cellular automata (CA) are systems consisting of a large set of basic discrete state elements interacting via a given set of local rules. The CA rules act locally but reveals in a global behavior of the entire system. This kind of global behavior typically cannot be predicted easily from the microscopic rules. ***Limitations of CA include their lack of biological sophistication in aggregating subcellular behaviors, the difficulty of going from qualitative to quantitative simulations, the artificial constraints of lattice discretization and the lack of a simple mechanism for rigid body***

• **Ibid**
• **Ibid**
• **[261] M. S. Alber, et al., On Cellular Automaton Approaches To Modeling Biological Cells.**
• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.**
• **[340] H. Men, X. Zhao. Microbial Growth Modeling and Simulation Based on Cellular Automata, Research Journal of Applied Sciences, Engineering and Technology, 2061-2066, Maxwell Scientific Organization, 2013.**
• **[257] H. Hatzikirou, et al., Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations, (eds.), Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer, Berlin Heidelberg, 2010.**

motion. In addition, interpreting simulation outcomes is not always as easy as for continuum equations. [261]•

However, cellular automaton models describe ***cell-cell and cell-environment interactions*** by phenomenological local rules, allowing simulation of a huge range of biological examples. [261]•.

As mentioned above, typical CA consist of discrete agents or particles, which occupy some or all sites of a regular lattice. These particles have one or more internal state variables (which may be discrete or continuous) and a set of rules describing the evolution of their state and position. Both the movement and change of state of particles depend on the current state of the particle and those of neighboring particles. These rules may either be ***discrete or continuous*** (In the form of ordinary ***differential equations (ODEs***)), ***deterministic or probabilistic***. Often the evolution rules apply in steps, e.g., a motion or transport step followed by a state change or interaction step. Updating can be ***synchronous or stochastic following Monte-Carlo model.*** At one extreme, the rules may approximate well-known ***continuous partial differential equations (PDEs)***; at the other, they may resemble the discrete logical interactions of simple ***Boolean computers.*** [261]•. ***Sophisticated flock models*** are an intermediate case. Thus, CA may produce very sophisticated self-organized structures. [256•; 259•].

Mathematically; a *cellular automaton* is a tuple (T, M, Φ), with T is a lattice such as the integers or a higher-dimensional integer grid, M is a set of functions from an integer lattice with one or more dimensions to a finite set, and Φ is a locally defined evolution function. The lattice in M represents the "space" lattice, while the one in T represents the "time" lattice.

As CA regular grid cells with each cell takes k different states, where k > 2, but not at once with the grid be n-dimensional (n -1), and the evolution of the cells takes place at discrete points in time.

This means that the state of each cell in the grid changes only at discrete moments of time, namely at time steps t. The time step t = 0 is usually considered as the initial step and therefore no changes at the state of the cells occur. [259]•.

All these characteristics make the simple cellular automata capable of complex behavior both in the formal sense (universal computation) and in the intuitive sense (generating patterns). *For elucidating this CA models; either trying to find the simplest rules, which produce a certain predefined behavior, or observing the behavior of a predefined set of cellular automata and classifying the behavior relative to the transition functions.* The two approaches are not independent; results of the second approach define behavior patterns, which can be studied by the first approach. [259]•.

Nevertheless, CA can be sophisticated enough that they can reproduce almost all commonly observed types of cell behavior. Philosophically, ***CA are attractive because their large-scale behaviors are completely self-organized rather than arising from responses to externally imposed signals***. Unlike other dynamic mathematical modeling that depends on the fact that an individual cell has no sense of direction or position, nor can it carry a road map that tells it where to go. *It can only respond to signals in its local environment*. Local environmental cues can provide direction and

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• **[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**
• **Ibid**
• **Ibid**
• **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction.**
• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.**
• **Ibid**
• **Ibid**

location information this would result in self-organized or externally generated cellular behavior. [340•; 261•].

The Self-reproduction or organization was first studied by ***von Neumann*** in combination with ***universal computability*** until Langton liberalized the criterion of genuine self-reproduction and presented a simple solution in which the self-description is stored in a dynamic loop instead of on a static tape. The Self-reproduction explanation presented by Langton can be generated in a much simpler way. By the ***"modulo prime***" transition function that implies that if the next state is equal to the sum of the neighboring states, then any initial configuration in any dimension, for any neighborhood will reproduce itself after a number of time steps depending on the size of the configuration. *Modulo prime rules initialized with a random configuration show self-similar patterns for which a fractal dimension can be determined*. [259]•.

Most cellular automata are ***irreversible***. Starting with the set of all possible initial configurations, and evolve to allow only a small subset of these configurations. In general, no global conservation laws hold in such universes. Special cellular automata which do have interesting conservation properties are particularly interesting specially: the class of ***reversible*** cellular automata. In this reversible automaton, every local configuration evolves into exactly one other local configuration, so that no information is lost and the computation can be run backwards. Starting from a structured initial configuration, ***entropy*** increases in such reversible models, but on running the automaton backward, and the initial configuration will reemerge from a seemingly random configuration. [259]•.

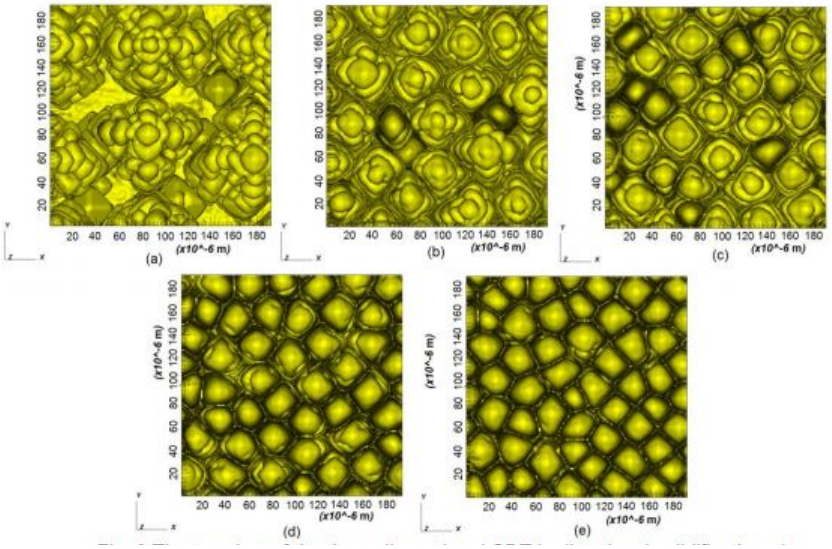
Cellular automata are in fact a special case of a wider class of formalisms, all poly automata consist of finite-state automata (cells) which are interconnected, which form each other's input and output neighborhoods, and which change their state synchronously. Distinguishing features of CA include:

1) Finite versus infinite number of cells (automaton versus space);
2) Uniform versus non-uniform (either with respect to automata or with respect to connections.);
3) Deterministic versus nondeterministic;
4) With or without external input/output;
5) Static versus dynamic (in static systems the number of cells and interconnections of cells, whether uniform or non-uniform, are fixed; in dynamic systems, cells and their interactions are generated as part of the behavior of a cell). [259]• However, synchrony is at variance with the localness of cellular automata, which is their major strength. To achieve synchrony a global clock is needed. Particularly in the case of a small number of states (the second major strength of cellular automata), synchrony can influence the qualitative behavior of the system considerably by imposing this global constraint. [259]•.

• **[340] H. Men, X. Zhao, Microbial Growth Modeling and Simulation Based on Cellular Automata, Research Journal of Applied Sciences, Engineering and Technology, Maxwell Scientific Organization, 2013.**
• **[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**
• **[259]**
• **[259]**
• **[259]**
• **[259]**

By varying the mentioned characteristics; four main classes of CAs emerges as follows:

1. ***Grid based CA***: In this case, identical automata are fixed in the nodes of a regular grid. The state of each automaton is synchronically updated according to a prescribed set of rules involving the states of the nearest neighbors of CA. [258]•.
2. ***Deterministic Eulerian automata***: They mimic the solution of partial differential equations and can model oscillations.
3. ***Lattice gas models***: In this class, the particles move on a discrete grid and collide in its nodes.
4. ***Solidifications models***: Particles are bounded and cannot move. These models simulate phase transition phenomena (e.g. s*olidification*, and *phase separation*). [258]•.



[341]•

Image. [132]. Top view of 3D cellular automaton in directional solidification.

Thus, Cellular automata can be studied from a number of different points of view.

1. ***As an architecture for fast computation.***
2. ***For the study of computational complexity.***
3. As ***pattern-recognition devices.*** Some classes of cellular automata can be used as powerful pattern filters: they recognize particular features embedded in much noise, Local pattern transformation methods and pattern segmentation methods can be regarded as cellular automata as well.
4. As ***modeling devices.***
5. As ***computation tools for independently formulated models.*** In this case, the cellular automaton is seen as an approximation. *In fact, every computer implementation of partial differential equations (PDEs) is a cellular-automaton simulation*: where necessity, time and space are discretized and so are the variables. Therefore, such models fall into the formal definition of cellular automata, although not into their typical image as the number of states is large, and the transition function takes a specific algebraic form.

• **[258] K. Krawczyk, et al., Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**

• **[258]**

• **[341] L. Wei1, et al., Low artificial anisotropy cellular automaton model and its applications to the cell-to-dendrite transition in directional solidification, Materials Discovery, 2016.**

6. As ***models of actual physical phenomenon.*** Traditionally most physical phenomena are modeled in terms of continuum models. [259]•.

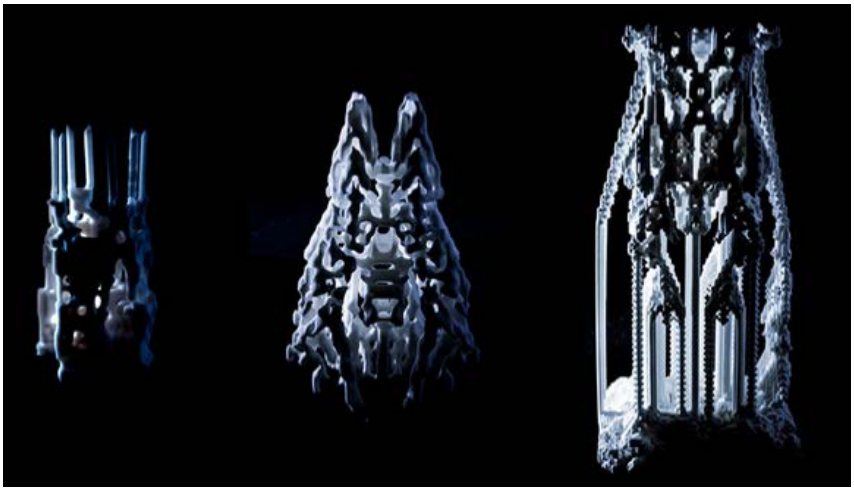


Image. [133]. Physical models of different cellular automata investigated in (AADRL –cellular automata workshop).

AA DRL cellular automata workshop. Computational architecture 2013, aimed to explore cellular automata theory and practice. To define the strategies of control the self-organizing system and to find the directions of usage in architectural design. Employing implementation of the algorithm for space tessellated into truncated octahedrons instead of usual cubic voxels. The study developed an algorithm that analyzes the system and cellular automata based on the statistical data decides how to change the input parameters (rules of growth or initial generation) and modifies the geometric structure of the system given the strategies that are necessary for the achievement of design (frame search, combining in clusters , etc.).

Cellular automata as ***"paradigm systems."*** Paradigm systems are not designed to simulate particular observed phenomena, but rather they embody certain concepts. Thus, a given cellular automaton defines its own universe. The behavior of such a universe can be compared to the observed (hypothesized) behavior of another universe (e.g. "reality"). These paradigm systems include:

(1) Symmetries and symmetry breaking,
(2) Conservation laws (in particular in reversible cellular automata.),
(3) A conspicuous arrow of time,
(4) order parameters and ergodicity,
(5) Relaxation to chaos (e.g. chemical turbulence). [259]•.

According to Wolfram, linear cellular automata were classified in terms of patterns observed in their time evolution into four classes, these emerged from the study of a set of linear cellular automata with varying number of states (k) and varying extent of neighborhood (r) and was initialized with a random configuration of zeros and ones. These four classes are:

One: Evolution to a *uniform state* independent of the initial configuration.

• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.**
• **Op.cit.**

Two: Evolution to a set of *separate simple stable or periodic structures*, the position of these structures depends on the initial configuration, but local changes in the initial configuration propagate over a limited area only.

Three: Evolution to *chaotic patterns* both in time and in space. A change in initial configuration eventually spreads over the entire automaton. *These chaotic patterns are similar to chaotic behavior studied in the theory of dynamical systems under the influence of strange attractors*. Such chaotic patterns give rise to a type of unpredictability that depends on the impossibility of describing completely the initial configuration (the configuration of an infinite cellular automaton, or the initial state as a real number of sufficient precision).

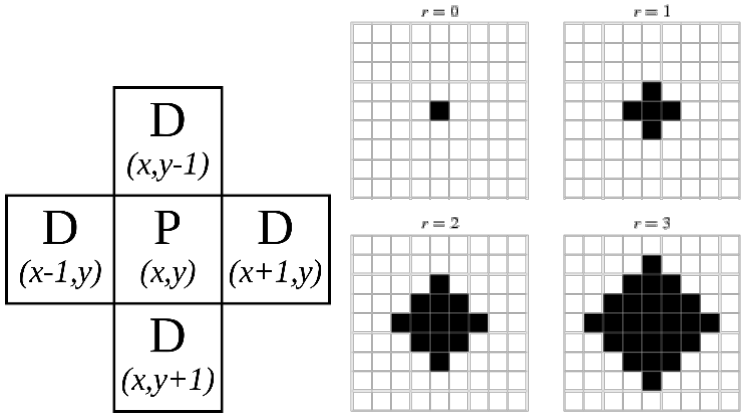
Four: Evolution to *complex localized structures*. In contrast to the situation in class 2 automata, the information propagation is not limited. The cellular automata is capable of universal computation fall into this class. [259]•.

In addition, according to Sante et al. (2010) a classification of CA transition rules was proposed as follows:

- Transitions can only occur based on the states of neighboring cells.
- Based on potentials or probabilities altered by the environmental status of a cell.
- Pattern development rules, which adjust the states based on shape or the existence of a network.
- Rules use computational intelligence methods to determine the rules from prior system behavior. *Typical are Case-Based Reasoning, neural networks, and data mining*.
- Rules use fuzzy logic and uncertainty reasoning. [259]•.

It is also worth mentioning that cellular-automata models incorporate a quite different criterion of simplicity: the number of variables is huge. However, the number of states of each of the variables is typically small (thus allowing for a qualitative rather than a quantitative description), for each cell, a set of cells called its neighborhood is defined relative to the specified cell. Regarding the two dimensional CA, the two most common types of neighborhood that are mainly considered are:

- **Von Neumann neighborhood** is classically defined on a two-dimensional square lattice and is composed of a central cell and its four adjacent cells. [342•; 343•; 344•].



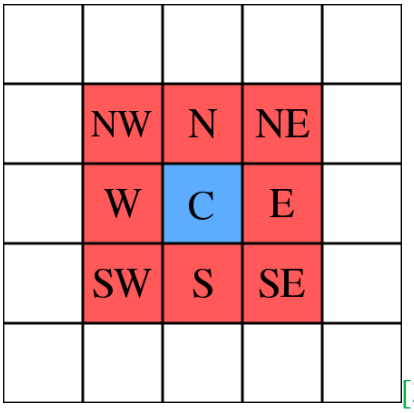
[345]•

Figure. [63]. Von Neumann neighborhoods for ranges r=0, 1, 2, and 3

• **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.**
• **Ibid**
• **[342] T. Toffoli, N. Margolus, Cellular Automata Machines: A New Environment for Modeling, MIT Press, p. 60, 1987.**
• **[343] A. Ben-Menahem, Historical Encyclopedia of Natural and Mathematical Sciences, Volume 1, Springer, p. 4632, 2009.**
• **[344] J. N. Wilson, G. X. Ritter, Handbook of Computer Vision Algorithms in Image Algebra (2nd ed.), CRC Press, p. 177, 2000.**
• **[345] http://mathworld.wolfram.com/vonNeumannNeighborhood.html.**

The von Neumann neighborhood of a cell is the cell itself, and the cells at a Manhattan distance of 1. (a form of geometry in which the usual distance function or metric of Euclidean geometry is replaced by a new metric in which the distance between two points is the sum of the absolute differences of their Cartesian coordinates. also known as rectilinear distance, snake distance, city block distance, Manhattan distance, and LASSO.). [346]•. The concept can be extended to higher dimensions, for example forming a 6-cell octahedral neighborhood for a cubic cellular automaton in three dimensions. [347]•.

-**Moore neighborhood** is defined on a two-dimensional square lattice and is composed of a central cell and the eight cells, which surround it (the same cells with the von Neumann neighborhood together with the four other adjacent cells of the central cell). *The idea behind the formulation of Moore neighborhood is to find the contour of a given graph*. For instance, ***Conway's Game of Life*** uses the Moore neighborhood. The concept can be extended to higher dimensions, for example forming a 26-cell cubic neighborhood for a cellular automaton in three dimensions, as used by 3D Life.



[348]•

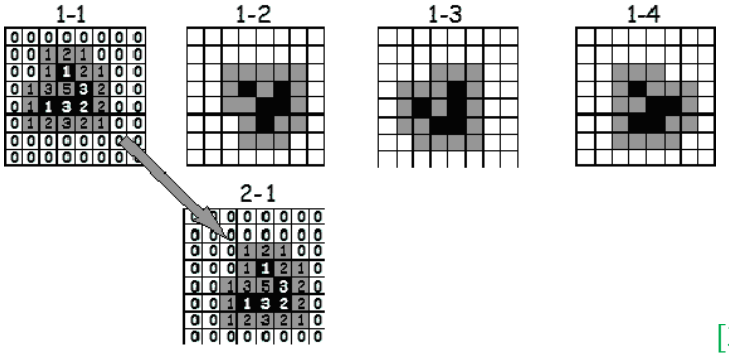
Figure. [64]. The Moore neighborhood is composed of nine cells: a central cell and the eight cells, which surround it.

In two dimensions, the number of cells in an *extended* Moore neighborhood, given its range r is: $(2r + 1)^2$. The evolution of the cells demands the definition of a cell state, the neighboring cells as well as the local transition function. [349]•.

- ***Conway's Game of Life (GoL)*** is a two-dimensional CA with binary states. The neighborhood considered is Moore neighborhood and the two states that each cell can adopt is alive and dead (or `1' and `0', respectively). The local transition rule uses the states of all cells in the neighborhood, during the directly preceding time step, to determine the new state of the central cell in the neighborhood. More specifically, the following transitions between states can occur either, when a cell is dead at time t and precisely three of the eight neighbors are alive as the cell adopts the state alive at time t+1. Or, when a cell is alive at time t and one or more than three of the eight neighbors are alive, in this case the cell adopts the state dead at time t+1. If none of the two aforementioned cases is true, the local rule dictates that the cell retains its

• **[346] P. E. Black, Manhattan distance, in Dictionary of Algorithms and Data Structures, 2006.**
• **[347] R. Breukelaar, T. H. Bäck, Using a Genetic Algorithm to Evolve Behavior in Multi-Dimensional Cellular Automata: Emergence of Behavior, Proceedings of the 7th Annual Conference on Genetic and Evolutionary Computation (GECCO '05), New York, NY, USA: ACM, pp. 107–114, 2005.**
• **[348] http://www.wikiwand.com/en/Moore_neighborhood.**
• **[349] M. A. Tsompanas, et al., Towards implementation of Cellular Automata in Microbial Fuel Cells, PLoS ONE, 12, 5.**

previous state. It is suggested that the inherent complexity of GoL is due to the fact that its transition rule is non- monotonic and nonlinear.



[350]•

Figure. [65]. A simple "glider" in Conway's Game of Life (GOL).

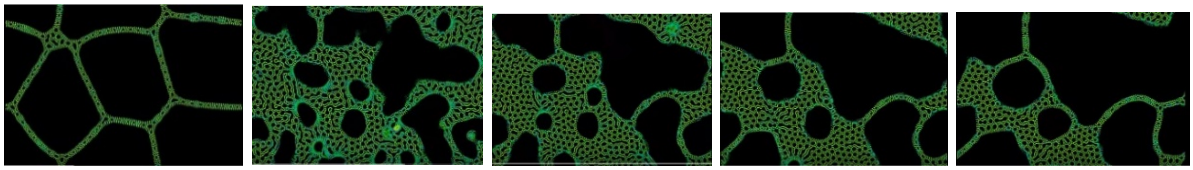


Figure. [66]. Generalized Conway Game of Life – time laps [351]•

A continuous version of GoL***, is the discrete-time, continuous spatial automaton*** with the same behavior as GoL***. In continuous spatial automata, the cells and their states form a continuum***. ***A similar behavior to GoL in a continuous field can be realized with a local rule implemented by a non-monotonic function of the population density in the neighborhood***. That is assuming the function will be graphically represented in two dimensions, namely the input or the population density in x-axis and the output or the next state of the cell in y-axis. Keeping in mind the rules of the original GoL, the continuous local rule function has to be continuously increasing in the space and continuously decreasing in the space. [349]•.

The application of the GoL rules to realize functions that can be translated as global computations:

A global computation behavior could be found in an arrays of MFCs, as they could, in principle, act as massive-parallel computing devices with local connectivity between elementary processors. [349]•. A study by Tsompanas et al., (2017) modeled a Conway's Game of Life CA that exhibits an enormously rich spectrum of patterns. Each cell of the Game of Life CA is realized using two MFCs. The MFCs were linked electrically and hydraulically. The model is verified via simulation of an electrical circuit demonstrating equivalent behaviors. The design is a first step towards future implementations of fully autonomous biological computing devices with massive parallelism.

A similar application is using configurations based on MFCs that realize computing units; to reproduce conventional binary logic gates using MFCs, ***with the goal of constructing non-silicon multi-valued logic processing units***. Designs of hydraulic and electrical interconnections are suggested for building three basic logic gates (AND, OR and NOT) that can be combined to assemble universal gates, hence circuits capable of universal computation. [349]•. Apart from basic logic gates,

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•[350] https://www.researchgate.net/figure/A-simple-glider-in-Conways-Game-of-Life-GOL-The-GOL-universe-is-a-two-dimensional_fig6_257811192- INTERLUDE - Time, Dynamics, Life, and the Emergence of Complexity, 2013.

• [351] https://sourceforge.net/projects/smoothlife/files.

• [349] M. A. Tsompanas, et al., Towards implementation of Cellular Automata in Microbial Fuel Cells, PLoS ONE, 12, 5.

• Ibid

• Ibid

more complicated computational abilities were exhibited with the appropriate interconnection of a small number of MFCs, namely a simplified ***Pavlovian learning model, the symbiotic mix of natural biological cells, such as the anodophiles, and artificial systems, like electrodes, actuators, pumps and chemical solutions, were used to simulate a learning cycle. Finally, the amount of physicochemical parameters that can be externally manipulated and affect the performance and, thus, the outputs of the biofilms in MFCs is enormous. This fact can justify the utilization of MFCs for more Complex computational schemes than the ones suggested up to this date***. [349]•.

- ***Random walk:*** The term **random walk** was first introduced by Karl Pearson in 1905.[352]• A **random walk** is a mathematical object, known as a stochastic or random process that describes a path that consists of a succession of random steps on some mathematical space such as the integers. An elementary example of a random walk is the random walk on the integer number line, which starts at 0 and at each step moves +1 or −1 with equal probability. ***Examples include the path traced by a molecule as it travels in a liquid or a gas, that can be approximated by random walk models, even though they may not be truly random in reality,*** random walks have applications to many scientific fields including ecology, computer science, physics, chemistry, and biology. ***Random walks explain the observed behaviors of many processes in these fields, and thus serve as a fundamental model for the recorded stochastic activity***. [353•; 354•].

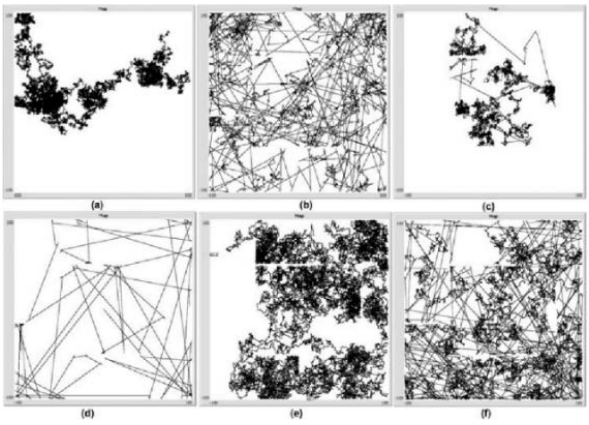
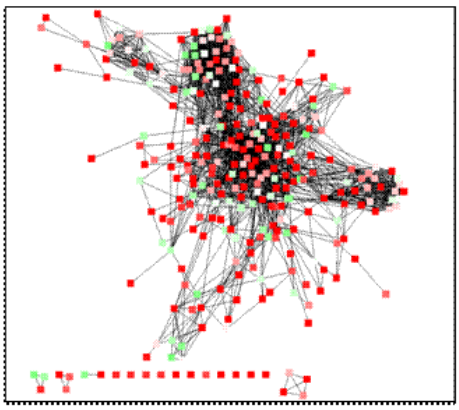


Figure. [67]. Plots showing simulated random walks: (a) Brownian walk, (b) Lévy walk; (c) Adaptive switching behavior between Lévy walk and Brownian walk; (d) Intermittent strategy using regime 1; (e) Sequence of knots as a random walk; (f) Composite random walk.

[355]•

• **Ibid**
• **[352] K. Pearson, The Problem of the Random Walk, Nature, 72, 1865, 294, 1905.**
• **[353] E. Wirth, Pi from agent border crossings by NetLogo package, Wolfram Library Archive, 2015.**
• **[354] E. Wirth, et al., measure landscape diversity with logical scout agents. ISPRS – International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, 2016.**
•**[355] https://www.semanticscholar.org/paper/A-Composite-Random-Walk-for-Facing-Environmental- C. A. Piña-et al., A Composite Random Walk for Facing Environmental Uncertainty and Reduced Perceptual Capabilities, ICIRA, 2011.**



[356]•

Figure. [68]. Novel Cyto-scape plug-in, GPEC, identifies putative genes likely to be associated with specific diseases or pathways. In the plug-in, gene prioritization is performed through a random walk with restart algorithm, a state-of-the art network-based method, along with a gene/protein relationship network.

There are various different types of random walks, which can differ in several ways. The term itself most often refers to a special category ***of Markov chains or Markov processes*** (A **Markov chain** ***is a stochastic model describing a sequence of possible events in which the probability of each event depends only on the state attained in the previous event***. [357•; 358•]. A **Markov process** is sometimes characterized as "***memorylessness***"). [359•; 360•]. ***A process satisfies the Markov property if one can make predictions for the future of the process based solely on its present state independently from the full process history, but many time-dependent processes are referred to as random walks, with a modifier indicating their specific properties***. Random walks can also take place on a variety of spaces: including graphs, the integers or the real line, in the plane or in higher-dimensional vector spaces, on curved surfaces or higher-dimensional Riemannian manifolds, and also on groups finitely generated. ***The time parameter can also be manipulated. In the simplest context, the walk is in discrete time that is a sequence of random variables indexed by the natural numbers***.

However, it is also possible to define random walks which take their steps at random times, and in that case the position X_t has to be defined for all times. Specific cases or limits of random walks include the ***Lévy flight*** (is a random walk in which the step-lengths have a probability distribution that is heavy-tailed. When defined as a walk in a space of dimension greater than one, the steps made are in isotropic random directions. The term ***"Lévy flight" was coined by Benoît Mandelbrot***. [361]•.

Later researchers have extended the use of the term "Lévy flight" to include cases where the random walk takes place on a discrete grid rather than on a continuous space, and diffusion models such as ***Brownian motion.***

- ***Lattice random walk:*** A popular random walk model is that of a random walk on a regular lattice, where at each step the location jumps to another site according to some probability distribution. In a ***simple random walk***, the location can only jump to neighboring sites of the lattice, forming a lattice path. In ***simple symmetric random walk*** on a locally finite lattice, the probabilities of the location jumping to each one of its immediate neighbors are the same. The best studied example is of random walk on the *d*-dimensional integer lattice (hyper cubic

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• **[356] D. H. Le, Y. K. Kwon, GPEC: A Cytoscape plug-in for random walk-based gene prioritization and biomedical evidence collection, Computational Biology and Chemistry, Volume 37, Pages 17-23, 2012.**
• **[357] P. A. Gagniuc, Markov Chains: From Theory to Implementation and Experimentation. USA, NJ: John Wiley & Sons, pp. 1–235, 2017.**
• **[358] Markov chain; Definition of Markov chain in US English by Oxford Dictionaries", Oxford Dictionaries, English. Retrieved, 2017-12-14.**
• **[359] R. Serfozo, Basics of Applied Stochastic Processes, Springer Science & Business Media, p. 2. 2009, 2017.**
• **[360] Y.A. Rozanov, Markov Random Fields, Springer Science & Business Media, p. 58. 2012, 2017.**
• **[361] B.B. Mandelbrot, The Fractal Geometry of Nature. Freeman, New York, 1982.**

lattice). [362]•. If the state space is limited to finite dimensions, the random walk model is called ***simple bordered symmetric random walk*** and the transition probabilities depend on the location of the state, because on margin and corner states the movement is limited. [363]•.

In higher dimensions, the set of randomly walked points has interesting geometric properties. As a discrete fractal is obtained, that is, a set which exhibits stochastic self-similarity on large scales. On small scales, sharpness could be observed; resulting from the grid on which the walk is performed.

The trajectory of a random walk is the collection of points visited, considered as a set with disregard to *when* the walk arrived at the point. In one dimension, the trajectory is simply all points between the minimum height and the maximum height the walk achieved. [362]•.

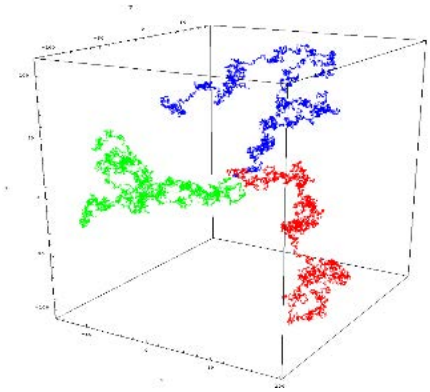
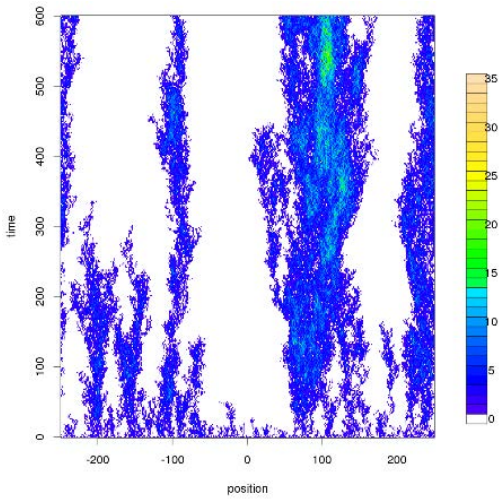


Figure. [69]. Three random walks in three dimensions.

- ***Branching random walk:*** according to probability theory, a branching random walk is a stochastic process that generalizes both the concept of a random walk and of a branching process. At every generation (a point of discrete time), a branching random walk's value is a set of elements that are located in some linear space, such as the real line. Each element of a given generation can have several descendants in the next generation. The location of any descendant is the sum of its parent's location and a random variable. [364]•.

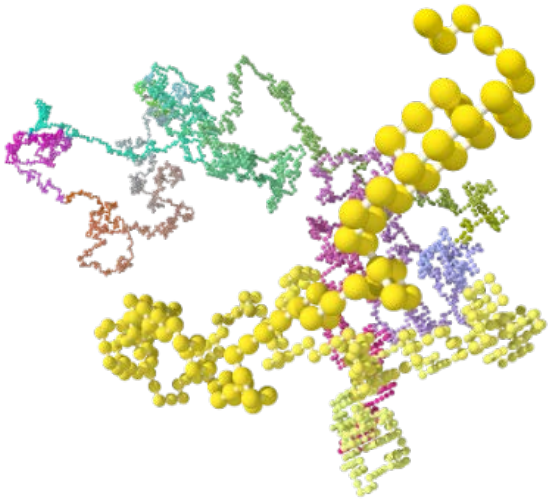


[365]•

Figure. [70]. Self -catalytic branching random walks.

• **[362] P. Révész, Random walk in random and non random environments, World Scientific "Pólya's Random Walk Constants", 1990. 2016-11-02**
• **[363] M. Kohls, T. Hernandez, Expected Coverage of Random Walk Mobility Algorithm, 2016.**
• **Op.cit.**
• **[364] https://en.wikipedia.org/wiki/Branching_random_walk.**
• **[365] https://www.probstat.math.uni-mainz.de.**

- ***Self-avoiding walk (SAW)***: is a sequence of moves on a lattice path that does not visit the same point more than once. A self-avoiding polygon (SAP) is a closed self-avoiding walk on a lattice. *SAWs were first introduced by the chemist Paul Flory* ***to model the real-life behavior of chain-like entities such as solvents and polymers.*** [366]•, ***whose physical volume prohibits multiple occupation of the same spatial point.*** In computational physics a self-avoiding walk is a *chain-like path with a certain number of nodes, typically a fixed step length and it does not cross itself or another walk. A system of self-avoiding walks satisfies the excluded volume condition. In higher dimensions, the self-avoiding walk is believed to behave much like the ordinary random walk. SAWs play a central role in the modelling of the topological and knot-theoretic behavior of thread- and loop-like molecules such as proteins*. [367•; 368•].



[369]•

Figure. [71]. Typical conformation of a 5000-step self-avoiding walk on the 3D simple cubic lattice, generated by N. Clisby's highly efficient SAW-tree algorithm. The actual study used chains of up to 34 million steps. © Nathan Clisby (2016).

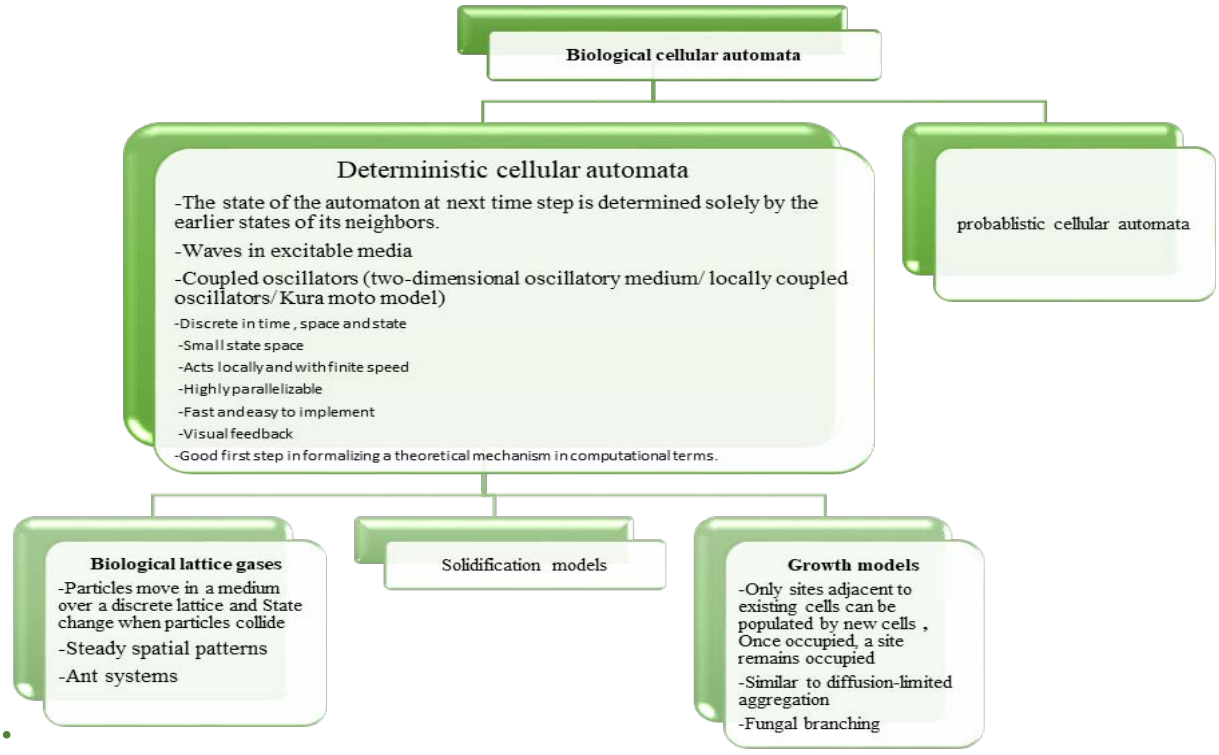
The properties of SAWs cannot be calculated analytically, so numerical simulations are employed. The pivot algorithm is a common method for ***Markov chain Monte Carlo simulations*** for the uniform measure on *n*-step self-avoiding walks. *The pivot algorithm works by taking a self-avoiding walk and randomly choosing a point on this walk, and then applying a symmetry operation (rotations and reflections) on the walk after the* n^{th} *step to create a new walk. Calculating the number of self-avoiding walks in any given lattice is a common computational problem. There is currently no known formula for determining the number of self-avoiding walks, although there are rigorous methods for approximating them.* [368]•.

One of the phenomena associated with self-avoiding walks and 2-dimensional statistical physics models in general is the notion of universality, that is, independence of macroscopic observables from microscopic details.

• **[366] N. Madras, G. Slade, The Self-Avoiding Walk, Birkhäuser, 1996.**
• **[367] G. F. Lawler, Intersections of Random Walks. Birkhäuser. 1991.**
• **[368] N. Madras, A. D. Sokal, The pivot algorithm- A highly efficient Monte-Carlo method for the self-avoiding walk. Journal of Statistical Physics, 50, 109-186, 1988.**
• **[369] N. Clisby, B. Dünweg, High-precision estimate of the hydrodynamic radius for self-avoiding walks, Physical Review E, 2016.**
• **Op.cit**

- ***Quantum walks (QW)***: are the quantum extension of the classical random walk with a quantum walker. When compared to the classical case they exhibit very different properties due to the interference of the walker with itself. This is manifested in the faster spreading of the walker (ballistically) throughout a topology (i.e. 1D line, 2D lattice) when compared to a classical walker which moves diffusely. A QW on the one-dimensional line with the walker initially localized at a central position evolves in time to a probability distribution with two parts moving ballistically away from the origin. [370]•. Theoretically, QWs have been shown to be universal for quantum computation, forming an implementation of the ***Grover search algorithm***, and other search algorithms. They have also been used to model transport processes such as quantum heat transport, and percolation. ***QWs come in two different forms; continuous-time and discrete-time quantum walk (CTQW & DTQW)***. *CTQWs are described as a collection of coupled oscillators, and evolve under the Schrodinger equation. In DTQWs the walkers have an internal state, called a coin state, and evolve iteratively by the discrete application of two unitary operations, the coin operator and the step operator.*

Biological cellular Automata



[371]•

Diagram. [9]. Categorization of Biological cellular automata models.

Probabilistic biological cellular automata

Probabilistic Cellular Automata (PCA) are interacting discrete stochastic dynamical systems used as a modeling tool for a wide range of natural and societal phenomena, they are the extension obtained when the rules for updating conventional CA are allowed to be random. New values of each automaton are chosen according to probability distributions determined by the configuration of its neighborhood.

• [370] C. S. Hamilton, et al., Driven Discrete Time Quantum Walks, IOP Publishing Ltd and Deutsche Physikalische Gesellschaft, 2016.
• [371] G. B. Ermentrout, L. Edelstein-Keshet, Cellular automata approaches to biological modeling, J Theor Biol, 160, 1, 97-133, 1993.

Usually, this updating is parallel or *synchronous,* all cells are simultaneously updated independently of each other, and neighborhoods are finite sets. However, the notion of PCA is understood in a rather general sense that includes partially asynchronous dynamics and not necessarily finite neighborhoods. [339]•. The probabilistic component turns PCA into flexible computing tools for complex numerical constructions, and realistic simulation tools for phenomena driven by interactions among a large number of neighboring structures. PCA are, therefore, useful for the study of key issues of statistical mechanical and mathematical physics, such ***as phase transitions***, ***meta-stability***, percolation, and transport theory.

On the mathematical side, PCA have a rich mathematical theory that leads to a better understanding of the role of randomness and synchronicity in the evolution of large systems. [339]•.

They are also naturally adapted as multiscale simulation frameworks for studying natural systems or large interconnected network structures systems and processes in life involving systems characterized by high levels of complexity and low level of reproducibility, even under extremely controlled conditions, due to inherent randomness or experimental limitations.

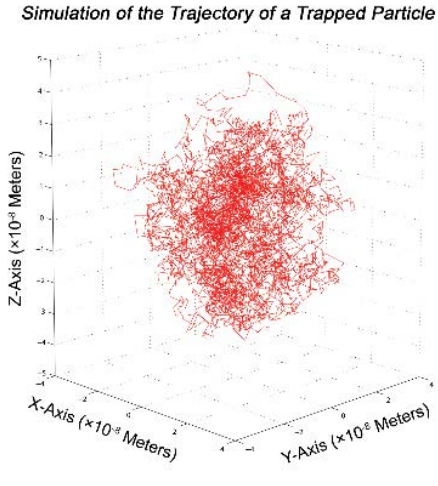
PCA dynamics belong to the category of ***non-equilibrium lattice models***. Thus, the definition of the PCA usually involves a notion of *neighborhood* defined as vertices separated by a maximum given number of links. ***However, PCA theory focuses, particularly, in phenomena taking place during the evolution toward equilibrium***; there are three scenarios that lead to typical non-equilibrium issues addressed through PCA. The first one is ***meta-stability*** refers to the appearance of obstacles delaying convergence. In some instances, these obstacles are related to the emergence of non-trivial collective behavior manifested as phase transitions. These statistical mechanics phenomena are also related to some highly challenging optimization issues. The second scenario is ***epidemiology***, which addresses the issue of survival vs extinction in large interacting populations. The third scenario is ***wildfires that*** illustrates the study of dynamic percolation phenomena. [339]•.

Mathematically, ***PCA are systems of Markov chains*** interconnected through a network, which typically is a lattice, or a finite subpart of it. These Markov chains evolve in a parallel but coupled fashion, in which the distribution of future states of each chain depends on present states of neighboring chains. This coupling of transition probabilities is, however, local, ***and this makes PCA appealing as algorithms for high-performance computing***, distributed computing, and simulations. [339]•.

- ***Brownian motion***: Or **pedesis**, named after the botanist Robert Brown, it describes the random motion of particles suspended in a fluid resulting from their collision with the fast-moving molecules in the fluid. [372]•. This pattern of motion typically alternates random fluctuations in a particle's position inside a fluid sub-domain with a relocation to another sub-domain. Each relocation is followed by more fluctuations within the new closed volume. *This pattern describes a fluid at thermal equilibrium, defined by a given temperature. Within such fluid, there exists no preferential direction of flow as in transport phenomena*. More specifically the fluid's overall linear and angular momenta remain null over time. The many-body interactions that yield the Brownian pattern cannot be solved by a model accounting for

• **[339] P. Y. L. Francesca, R. Nardi, Probabilistic Cellular Automata Theory, Applications and Future Perspectives, Complexity and Computation.**
• **Ibid**
• **Ibid**
• **Ibid**
• **[372] R. Feynman, The Brownian Movement, The Feynman Lectures of Physics, Volume I, pp. 41-1, 1964.**

every involved molecule. In consequence, only ***probabilistic models*** applied to molecular populations can be employed to describe it. Another, pure probabilistic class of models is the class of the ***stochastic process models.*** There exist both simpler and more complicated stochastic processes, which in extreme may describe the Brownian motion. [372]•.



[373]•

Figure. [72]. Simulation for Brownian motion within a harmonic potential allows for particle trajectory simulations for trapped microspheres suspended in a medium.

- ***Diffusion equations***: These equations yield an approximation of the time evolution of the probability density function associated to the position of the particle going under a Brownian movement. The approximation is valid on short timescales. The time evolution of the position of the Brownian particle itself is best described using ***Langevin equation*** ***(****Langevin equation is a* ***stochastic differential equation*** *describing the time evolution of a subset of the degrees of freedom. These degrees of freedom typically are* ***collective (macroscopic) variables changing only slowly in comparison to the other (microscopic) variables of the system****. The fast (microscopic) variables are responsible for the stochastic nature of the Langevin equation.*) [374•; 375•], ***an equation which involves a random force field representing the effect of the thermal fluctuations of the solvent on the particle***.

The displacement of a particle undergoing Brownian motion is obtained by solving the diffusion equation under appropriate boundary conditions. This shows that the displacement varies as the square root of the time (not linearly), at very short time scales (*On long timescales, the mathematical Brownian motion is well described by a Langevin equation. (On small timescales, inertial effects are predominant in the Langevin equation*.), however, the motion of a particle is dominated by its inertia and its displacement will be linearly dependent on time: $\Delta x = v\Delta t$. So the prompt velocity of the Brownian motion can be measured as $v = \Delta x/\Delta t$, when $\Delta t < \tau$, where τ is the momentum relaxation time. [376]•.

- ***Narrow escape:*** The **narrow escape problem** is an omnipresent problem in biology, biophysics and cellular biology which has the following formulation: a Brownian particle

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• **Ibid**
•**[373]https://www.mathworks.com/matlabcentral/fileexchange/44733-simulation-for-brownian-motion-within-an-harmonic-potential.**
•**[374] P. Langevin, Sur la théorie du mouvement brownien [On the Theory of Brownian Motion]. C. R. Acad. Sci. Paris, 146, 530–533, reviewed by D. S. Lemons, A. Gythiel, Paul Langevin's 1908 paper "On the Theory of Brownian Motion", Am. J. Phys, 65, 1079, 1997.**
•**[375] http://mathworld.wolfram.com/MooreNeighborhood.html-http://cell-auto.com/neighbourhood/moore.**
• **[376] T. Li, et al., Measurement of the instantaneous velocity of a Brownian particle, Science, 328, 5986, 1673 -1675, 2010.**

(ion, molecule, or protein) is limited to a bounded domain by a reflecting boundary, except for a small gap through which it can escape. The narrow escape problem is that of calculating the mean escape time. This time diverges as the gap shrinks, thus rendering the calculation a singular perturbation problem.

Deterministic biological cellular automata

- ***Lattice gas:*** LGCA represent a class of CA whose structure facilitates mathematical analysis, in which the particles select from a finite number of discrete allowed velocities (channels). During the interaction, particles appear, disappear or change their velocity state. During the transport step, all particles simultaneously move in the direction of their velocity. ***LGCA can model a wide range of phenomena including the diffusion of ideal gases and fluids, reaction-diffusion processes and population dynamics.*** Often the overall macroscopic behavior of the system can very well be approximated if averages over larger spatial scales are considered. [377]•. The emergent collective behavior, e.g. ***spatio-temporal pattern formation*** in a LGCA shows up in the macroscopic limit, which can be derived from a theory of statistical mechanics on a lattice. ***In biological applications, LGCA treat cells as point like objects with an internal state but no spatial structure.***

In a lattice-gas model, implementing movement of individuals is straight forward, by having separate channels for each direction of movement and imposing an exclusion principle. The movement steps are alternated with interaction steps, in which processes affecting, e.g., birth and death can be implemented. [377]•. Furthermore, the update rule is split into two parts, which are called *interaction and propagation*, respectively. The interaction rule of LGCA can be compared with the update rule for CA in that it assigns new states to each particle based on the states of the sites in a local neighborhood.

After the interaction/collision step, the state of each node is propagated to a neighboring node. The discrete structure of the LGCA facilitates the implementation of complicated environments without any computational problems, as LGCA are examples of parallel algorithms.

A further complex model is the CPM (cellular Potts model) is a more complex probabilistic CA with Monte-Carlo updating, in which a cell consists of a domain of lattice sites, thus describing cell volume and shape more realistically. This spatial realism is important when modeling interactions dependent on cell geometry. [377]•.

Applications of LGCA-based models in biology: LGCA are used for modeling ***large groups of living elements that often exhibit coordinated polarized movement. This polarization usually occurs via alignment***, where individuals democratically align their direction and velocity with those of neighbors of the same type, rather than by aligning under the control of a single leader cell or in response to externally supplied signals. This self-organized local alignment states multiple descriptions. [261]•. *Mogilner and Edelstein-Keshet, realized that they could model such phenomena more realistically using* ***integro-differential partial differential equations*** *to account for the effects of neighbor interactions on each member of the population. In 1997, Cook et al. described* ***spatio-angular***

• **[377] A. Deutsch, S. Dormann, Cellular Automaton Modeling of Biological Pattern Formation Characterization, Applications and Analysis, Mathematical Biosciences, 200, 118–123, Birkhäuser, 2006.**
• **Ibid**
• **Ibid**
• **[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**

self-organization *(the tendency of polarized cells to align to form chains) using a LGCA model based on a simplified integro-differential model in diffusion-limited aggregation (DLA), furthermore, Ben-Jacob and Shapiro have shown that DLA has extensive applications to* ***bacterial colony growth in gels where nutrient or waste diffusion is slow.*** [261]•.

Using lattice gas in modeling intra cellular biological behavior could be considered non-efficient as when modeling cell–cell interactions the lattice-gas method cannot incorporate any detail below the level of the complete cells. Interestingly, a cellular automaton-like approach does incorporate this detail. In this approach, an ensemble of cellular automaton sites encodes a biological cell, and the microscopic rules are describing expansion or retraction of the cell. The total cell volume is monitored, and cellular expansion and retraction depend on deviations from a target volume. An example of the success of the method is a simulation of morphogenesis in the slime mold Dictyostelium ***disodium***. [377]•.

For models of discrete particles, ***Lattice Boltzmann (LB) models*** deal with continuous distribution functions which interact locally and which propagate after collision to the next neighbor node. LB models can be inferred as mean-field approximations of LGCA in a mean field approximation, all-spatial correlations are removed from the system. A straightforward way to simulate mean field is to randomize the automaton after each time step. [257•; 378•].

The mean-field (Boltzmann) equation describing a given LGCA model arises under the assumption that the probability of finding two cells at specific positions is given by the product of corresponding single particle distribution functions. This analysis could particularly improve understanding of short and long-time behavior. [257]•.

The lattice-gas method is potentially valuable for a bottom–up approach, in particular to study new dynamical possibilities if individuals that are allowed to move in a spatial domain with local interactions. Particularly, lattice-gas cellular automata (LGCA) can model the interplay of cells with themselves and their heterogeneous environment. These models describe interaction at a cell-based (microscopic) scale. ***As cell-based models*** are ***required to extract the organization principles of interacting cell systems down to length scales of the order of a cell diameter in order to link the individual (microscopic) cell dynamics with a particular collective (macroscopic) phenomenon***. [257]•.

• ***Swarming***: Swarming and flocking are a class of ***collective self-organization*** that emerges from a multitude of simultaneous local actions rather than following a global guide. Swarming occurs in a wide variety of fields, including animal aggregation, ***bacteria colonies, social amoebae cell migration***, fish or bird flocking and insect swarming. Swarming patterns all share one feature: the apparent haphazard autonomous activities of a large number of particles (organisms or cells), on a larger scale, reveal a remarkable unity of organization, usually including synchronized non-colliding, aligned and aggregate motion. Most models, however, only measure the density distribution, i.e. they look for nearly constant density in the center of the swarm and an abrupt density drop to zero at the edge. [261]•.

• **Ibid**
•**[377] A. Deutsch, S. Dormann, Cellular Automaton Modeling of Biological Pattern Formation Characterization, Applications and Analysis, Mathematical Biosciences, 200, 118–123, Birkha¨user, 2006.**
•**[257] H. Hatzikirou, et al., Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations.**
• **[378] J.Kroc, et al., Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer-Verlag, Berlin Heidelberg, 2010.**
• **Op.cit.**
• **Ibid**
• **[261] M. S. Alber, et al., On Cellular Automaton Approaches to Modeling Biological Cells.**

Many artificial-life simulations produce extremely similar emergent characteristics, where simple local rules are:

1) **Collision Avoidance**: Avoid collisions with nearby flock mates,
2) **Velocity Matching**: Attempt to match velocity with nearby flock mates,
3) **Flock Centering**: Attempt to stay close to nearby flock mates; give rise to complex global behaviors.

Most swarming models are of molecular dynamics type, with all particles obeying the same equations of motion and residing in a continuum rather than a lattice. Particles have no memory of their behavior except for their current velocity and orientation. Particles are also self-propelled since they move spontaneously without external forces, unlike non-living classical particles whose motions results from external forces. [261]•.

Unfortunately, particle interactions cannot always be interpreted by an interaction potential or force, this is due to the vague understand of the interactions between particles, or because their actions may depend in a complex way on the internal states and history of the particles. In this case, phenomenological rules are more appropriate. In such cases, CA models are perfect for studying swarming as a collective behavior arising from individual local rules.

- ***Growth models:*** A growth or population model is a type of mathematical model that is applied to the study of population dynamics. Providing a controllable way of understanding how numbers change over time or in relation to each other. Many patterns can be noticed by using population modeling as a tool. [379]•. Ecological population modeling is concerned with the changes in parameters such as population size and distribution within a population. This is due to interactions within and with the environment, individuals of their own species, or other species. [380]•.

- **Branching systems**
  - ***L systems***: Lindenmayer systems were considered as a mathematical theory of plant development. Originally, they did not include enough detail to allow for comprehensive modeling of higher plants. The emphasis was on plant topology, that is, the neighborhood relations between cells or larger plant modules. The principal concept of L-systems is rewriting. Rewriting is a technique for defining complex objects by successively replacing parts of a simple initial object using a set of *rewriting rules* or *productions*. [269]•. The classic example of a graphical object defined in terms of rewriting rules is the *snowflake curve,* proposed in 1905 by *Koch.*

Mandelbrot restates the L-system construction in steps as follows: beginning with two shapes, an initiator and a generator. The generator is an oriented broken line made up of N equal sides of length r. Thus, each stage of the construction begins with a broken line and consists in replacing each straight interval with a copy of the generator, reduced and displaced to have the same ends as those of the interval being replaced. A similar array-rewriting mechanism is the cornerstone of Conway's *game of life.*

• **Ibid**
• **[379] D. Worster, Nature's Economy, Cambridge University Press, 398–401, 1994.**
• **[380] M. Uyenoyama, R. Singh, The Evolution of Population Biology, Cambridge University Press, 1–19, 2004.**
•**[269] A. Meškauskas, et al., Concerted regulation of tropisms in all hyphal tips is sufficient to generate most fungal structures, Mycological research, 108, 341-353, 2004.**

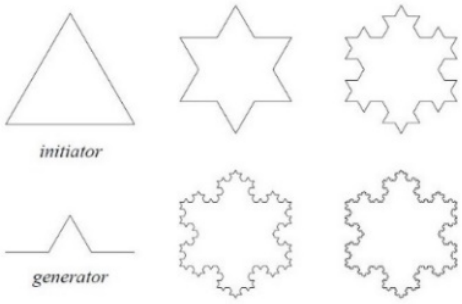
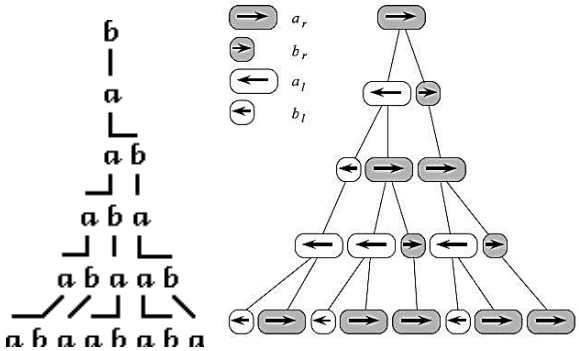


Figure. [73]. Construction of snowflake curve [381]•

In L-systems, method of applying productions are applied in parallel and simultaneously replace all letters in a given word. This reflects the biological motivation of L-systems. ***Productions are intended to capture cell divisions in multicellular organisms, where many divisions may occur at the same time.*** Parallel production application has an essential impact on the formal properties of rewriting systems. For example, there are languages, which can be generated by context-free L-systems (called OL-systems). [382•; 383•].

- **DOL-systems:** is the simplest class of L-systems, they are deterministic and context-free, strings are built of two letters *a* and *b*, which may occur many times in a string. Each letter is associated with a rewriting rule. The rule $a \rightarrow ab$ means that the letter *a* is to be replaced by the string *ab*. The rewriting process starts from a distinguished string called the axiom. It consists of a single letter *b*. In the first derivation step the axiom *b* is replaced by *a* using production $b \rightarrow a$. In the second step *a* is replaced by *ab* using production $a \rightarrow ab$. The word *ab* consists of two letters, both of which are *simultaneously* replaced in the next derivation step. Thus, *a* is replaced by *ab*, *b* is replaced by *a*, and the string *aba* results. In a similar way, the string *aba* yields *abaab, which* in turn yields *abaababa*, then *abaababaabaab*, and so on. [383]•.



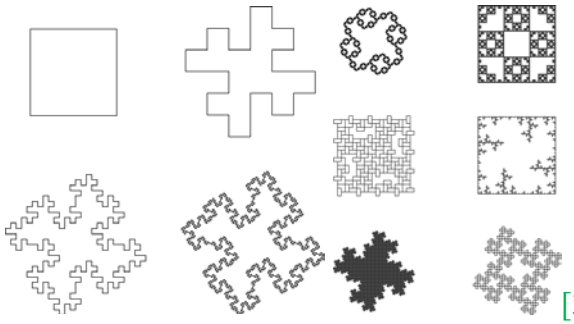
[383]•

Figure. [74]. Left, example of a derivation in a DOL-system. Right, development of a filament (*Anabaena catenula*) simulated using a DOL-system. The formalism is used to simulate the development of a fragment of a multicellular filament such as that found in the blue-green bacteria *Anabaena catenula* and various algae. The symbols *a* and *b* represent cytological states of the cells (their size and readiness to divide). The subscripts *l* and *r* indicate cell polarity, specifying the positions in which daughter cells of type *a* and *b* will be produced. [383]•.

• **[381] M. Rani, et al., Variants of Koch curve: A Review, National Conference on Development of Reliable Information Systems, Techniques and Related Issues (DRISTI) 2012 Proceedings published in International Journal of Computer Applications, (IJCA), 2018.**
• **[382] P. Prusinkiewicz, et al., L-systems: from the Theory to Visual Models of Plants, 2nd CSIRO Symposium on Computational Challenges in Life Sciences, CSIRO Publishing, 1996.**
• **[383] http://algorithmicbotany.org/papers/abop/abop-ch1.pdf**
• **[383]**
• **[383]**
• **[383]**

In order to model higher plants, a more sophisticated graphical interpretation method of L-systems is needed. Hogeweg and Hesper used L-systems primarily to determine the branching topology of the modeled plants. The geometric aspects, such as the lengths of line segments and the angle values, were added in a post-processing phase. The results of Hogeweg and Hesper were subsequently extended by Smith, who demonstrated the potential of L-systems for realistic image synthesis.

Szilard and Quinton proposed a different approach to L-system interpretation in 1979. They concentrated on image representations with rigorously defined geometry, such as chain coding, and showed that outstandingly simple DOL-systems could generate the intriguing, convoluted curves known today as fractals. [383]•.



[383]•

Figure. [75]. Left, generating a quadratic Koch island, four approximations of the quadratic Koch Island taken from Mandelbrot's book, example reveals a close relationship between Koch Constructions and L-systems. The initiator corresponds to the axiom and the generator corresponds to the production successor. L-systems specified in this way can be perceived as coding for Koch constructions. Right, a sequence of Koch curves obtained by successive modification of the production successor. [383]•.

Interestingly, there are two modes of operation for L-systems with turtle interpretation, the edge rewriting and node rewriting using terminology borrowed from graph grammars. In the case of edge rewriting, productions substitute figures for polygon edges, while in node rewriting, productions operate on polygon vertices. Both approaches rely on capturing the recursive structure of figures and relating it to a tiling of a plane. Although the concepts are illustrated using abstract curves, they apply to branching structures found in plants as well. [383]•.

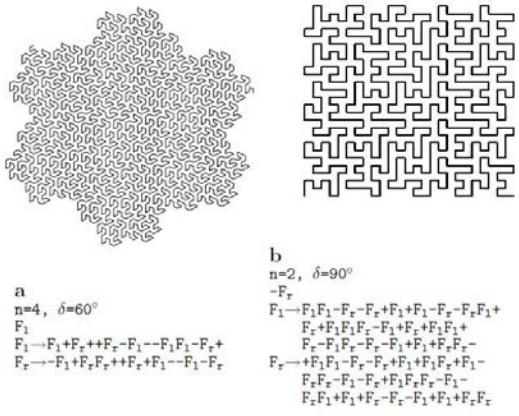


Figure. [76]. Examples of space-filling, self-avoiding, simple and self-similar curves, which can be thought of as finite, self-avoiding approximations of curves that pass through all points of a square generated by edge-rewriting L-systems: (a) hexagonal Gosper curve, (b) quadratic Gosper curve or E-curve

[383]•

• [383]
• Ibid
• Ibid
• Ibid
• Ibid

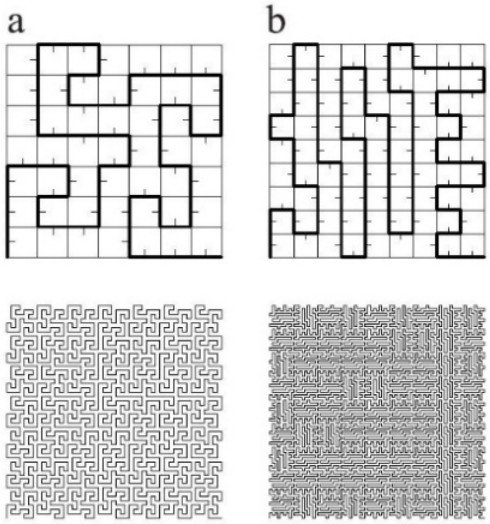


Figure. [77]. Examples of curves generated on the square grid using edge replacement: (a) a square recurve (grid size 7 × 7), (b) an E-tour (grid size 9 × 9).

- ***Axial trees:*** A rooted tree has edges that are labeled and directed. The edge sequences form paths from a distinguished node, called the root or base, to the terminal nodes. In the biological context, these edges are referred to as branch segments. A segment followed by at least one more segment in some path is called an internode. A terminal segment with no succeeding edges is called an apex. An axial tree is a special type of rooted tree. At each of its nodes, at most one outgoing straight segment is distinguished. All remaining edges are called lateral or side segments. A sequence of segments is called an axis if the first segment in the sequence originates at the root of the tree or as a lateral segment at some node, if each subsequent segment is a straight segment, and the last segment is not followed by any straight segment in the tree. Together with all its descendants, an axis constitutes a branch. A branch is itself an axial (sub) tree. Axes and branches are ordered. The axis originating at the root of the entire plant has order zero. An axis originating as a lateral segment of an n-order parent axis has order n+1. The order of a branch is equal to the order of its lowest-order or main axis. [383][•].

Axial trees are purely topological objects. The geometric connotation of such terms as straight segment, lateral segment and axis should be viewed at this point as an intuitive link between the graph-theoretic formalism and real plant structures.

- ***Stochastic L-systems:*** All plants generated by the same deterministic L-system are identical with an artificial regularity. To prevent this effect, it is necessary to present specimen-to-specimen variations that will preserve the general aspects of a plant but will modify its details. Variation can be achieved by randomizing the turtle interpretation, the L-system, or both. Randomization of the interpretation alone has a limited effect. While the geometric aspects of a plant -such as the stem lengths and branching angles- are modified, the principal topology remains unchanged. In contrast, stochastic application of productions may affect both the topology and the geometry of the plant. [383][•].
- ***Context-sensitive L-systems:*** Assemblies in OL-systems are context-free as they are applicable regardless of the context in which the prototype appears. However, production application may still depend on the prototype's context. This effect is useful in simulating interactions between

[•] Ibid
[•] Ibid

plant parts, ***due for example to the flow of nutrients or hormones***. 2L-systems use productions of the form, al < a > ar → χ, where the letter "a" (called the strict predecessor) can produce word "χ" if only "a" is preceded by letter "al" and followed by "ar". Thus, letters "al" and "ar" form the left and the right context of "a" in this production. Productions in 1L-systems have one-sided context only; consequently, they are either of the form, al < a → χ or a > ar → χ. OL-systems, 1L-systems and 2L-systems belong to a wider class of IL-systems, also called (k,l)-systems. In a (k,l)-system, the left context is a word of length "k" and the right context is a word of length "l".[383]•.

Fungal hyphae rearranging subsequently changing their position and orientation could be modeled with parametric L-systems. For instance, providing rotation symbols with time-dependent or conditional parameters could enable subsequent rotation of a branch and every subordinate module. The length or diameter of existing hyphae could similarly be changed. Modeling hyphal aggregation, the accumulation and coalescing of hyphae to form mycelial cords or complex structures like fruiting bodies could be feasible using relational growth grammars (RGGs) in GroIMP. Their advantage over L-systems, which are designed specifically for the modeling of plant topology, is the representation of general graphs and their dynamics via graph rewriting instead of string rewriting. [263]•.

Using L-systems is considered suitable and applicable for simulating fungal colony architecture, especially ***when researching the production of enzymes, the influencing factors can be built in quickly and the amount of enzymes produced be set as a target variable***. In this way, metabolite production can be investigated and quantified, leading to a better understanding of fungal growth and possibly providing benefits for numerous applications including protection of the environment. [263]•.

- **Parametric L-systems:** As explained previously, despite the ability of *L*-systems with turtle interpretation to generate a variety of interesting objects, from abstract fractals to plant-like branching structures, theses systems' modeling power is quite limited. Consequently, the representation of a simple plant module may require a large number of symbols. Problems become even more obvious while simulating changes to the modeled structure over time, since some growth functions cannot be expressed conveniently using L-systems .In order to solve similar problems, Lindenmayer proposed that numerical parameters be associated with L-system symbols. This idea was illustrated by referring to the continuous development of branching structures and diffusion of chemical compounds in a non-branching filament of *Anabaena catenula*. [383]•.

Parametric L-systems operate on *parametric words*, which are strings that are p*arametric* of *modules* consisting of *letters* with associated *parameters*. The letters belong to an *alphabet "V",* and the parameters belong to the set of *real numbers "R"*. A module with letter $A \in V$ and parameters $a1, a2, ..., an \in R$is symbolized by A ($a1, a2, ..., an$). Every module belongs to the set $M = V \times R*$, where

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• **Ibid**
• **[263] S. Kelleter , Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of Coprinopsis cinerea, Master Thesis, University of Gottingen, Faculty of Forest Sciences and Forest Ecology, 2017.**
• **Ibid**
• **[383] http://algorithmicbotany.org/papers/abop/abop-ch1.pdf**

$R*$ is the set of all finite sequences of parameters. The set of all strings of modules and the set of all nonempty strings are denoted by $M* = (V \times R*)*$ and $M+ = (V \times R*)+$, respectively. [383]•.

- ***Parametric 2L-systems:*** A context sensitive extension is necessary to model information exchange between neighboring modules. In the parametric case, each component of the production predecessor (the left context, the strict predecessor and the right context) is a parametric word with letters from the alphabet "V" and formal parameters from the set Σ. Any formal parameters may appear in the condition and the production successor. [383]•. Parametric 2L-systems provide a convenient tool for expressing developmental models that involve diffusion of substances throughout a *heterocysts* organism.

[3.1.3] Agent –based

In agent-based modeling (ABM), a system is modeled as a collection of autonomous decision-making entities called agents. Each agent individually assesses its situation and makes decisions based on a set of rules. Agents may perform various behaviors appropriate for the system they represent.

Repetitive competitive interactions between agents are a feature of agent-based modeling, which relies on the power of computers to explore dynamics out of the reach of pure mathematical methods.

Agents may be capable of evolving, allowing unanticipated behaviors to emerge. Sophisticated ABM sometimes incorporates ***neural networks, evolutionary algorithms***, or other learning techniques to allow realistic learning and adaptation. [384]•. Agent-based simulation is popular as a modeling approach; as it represents directly individual entities and their interactions. In comparison with variable-based approaches using structural equations, or system-based approaches using differential equations, agent-based simulation offers the possibility of modeling individual heterogeneity, representing explicitly agents' decision rules, and situating agents in space. It allows modelers to represent in a natural way multiple scales of analysis, the emergence of structures at the macro level from individual action, and various kinds of adaptation and learning, none of which is easy to do with other modeling approaches. [385]•.

One of the serious drawbacks of agent objects approach is a large memory load they required. In software independent agent systems, the agents – implemented as a special kind of object - perform a similar code and are aware of the existence of the others agents. The agents communicate (interact) with each other by means of communication layers defined as the properties of the environment in which they operate. [258]•.

ABMs combine game theory, complex systems theory, evolutionary programming, and stochastic modeling. In ecology and biology, and are termed "individual-based models. [386]•. Normally ABM consists of:

- Agents specified at specific model scales (granularity) and types,
- Decision-making heuristics, often informed by censuses and surveys in the real world,
- Learning or adaptive rules,

• **Ibid**
• **Ibid**
• **[384] E. Bonabeau, Agent-based modeling: Methods and techniques for simulating human systems, PNAS, vol. 99, 2002.**
• **[385] N. Gilbert, AGENT-BASED MODELS, SAGE Publications, Inc, UK, 2007.**
• **[258] K. Krawczyk, et al., Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**
• **[386] S. MATSUURA, Growth and Colony Patterning of Filamentous Fungi, Biology, Published, 2000.**

- A procedure for agent engagement, for example, move, interact,
- An environment that can both influence and be impacted by the agents. [386]•.

Agents: are either separate computer programs or, more commonly, distinct parts of a program that are used to represent cells. They are programmed to react to the computational environment in which they are located. This environment is a model of the real environment in which the real cells operate. A *crucial feature of agent-based models is that the agents can interact, that is, they can pass informational messages to each other and act based on what they learn from these messages such as the observation of another agent or the detection of the effects of another agent's actions.* [384•; 387•].

Agents can represent any entity of interest, such as a *molecule, cell or multicellular organism*, and each independently follows a prescribed set of rules. In a biological setting, these rules are often *encoded as genetic circuits that drive cellular responses to particular stimuli*. By simulating the behavior of these virtual populations in realistic environments, it is possible to gain an understanding of how low-level cellular rules lead to the emergence of collective population-level behaviors. [388]•.

Agent based models have been used to explore the intricate structures of different intra-cellular reactions at the level of the colony as an area for future morphogenetic engineering applications. This demonstrated the ability for physical interactions alone to lead to the emergence of complex population-level features. In addition to the study of colony substructure during normal growth, agent-based models have also been used to explore the self-organization of cells at high-cell densities. In addition, to develop rules controlling growth that guide the emergence of desired colony morphologies. [388]•.

The Environment: The environment is the virtual world in which the agents act. It may be an entirely neutral medium with little or no effect on the agents, or in other models, may be as carefully crafted as the agents. *In these spatial models, the agents have coordinates to indicate their location.*

Another option is to have no spatial representation at all but to link agents together into a network in which the only indication of an agent's relationship to other agents is the list of the agents to which it is connected by network links. This approach, also called ***companion modeling*** involves building a *multi-agent system in close association with informants selected* **.**There can be a direct correspondence between the computational agents in the model and real-world cells, which makes it easier to design the model and interpret its outcome than would be the case with an equation-based model.

The gain of agent-based models in freely defining agent properties and interactions soon turns out to be a drawback, because this way arbitrary pattern is generated and it is difficult to choose the right values in a high dimensional parameter space. To minimize these problems, there are two ways:

- ***to closely link the agent's properties to experimentally observed data,***

• Ibid
• [384] E. Bonabeau, Agent-based modeling: Methods and techniques for simulating human systems.
• [387] H. V. D. Parunak, et al., Agent-Based Modeling vs. Equation-Based Modeling:-A Case Study and Users' Guide, International Workshop on Multi-Agent Systems and Agent-Based Simulation, MABS 1998: Multi-Agent Systems and Agent-Based Simulation, pp 10-25, Lecture Notes in Computer Science book series, (LNCS, volume 1534).
• [388] T. E. Gorochowski, Agent-based modelling in synthetic biology, Essays in Biochemistry, 60, 325–336, 2016.
•Ibid

- To apply methods that allow aggregating the agent dynamics, to formally derive the systems dynamics. [389]•. This provides a firm relation between agent's features and systems feature's and may reveal also the role of certain (control) parameters.

Advantages of Agent-Based Modeling. The benefits of ABM over other modeling techniques can be captured in three statements:

- *ABM captures emergent phenomena; ABM generates emergent phenomena from the bottom up, it raises the issue of what constitutes an explanation of such a phenomenon.*
- *ABM provides a natural description of a system*; In many cases, ABM is most natural for describing and simulating a system composed of ''behavioral'' entities.
- ABM is flexible.

ABM is mostly used when *individual behavior is nonlinear, cannot be clearly defined through aggregate transition rates, are intractable by equations, and can be characterized by thresholds.* In this case, a*ctivities are a more natural way of describing the system than processes, by employing "if-then" rules, or nonlinear coupling*. Individual behavior exhibits memory, path-dependence, non-markovian behavior, or temporal correlations, including learning and adaptation. For modeling this behavior, agent interactions ought to be heterogeneous and able to generate network effects.

Aggregate flow equations usually assume global homogeneous mixing, but the topology of the interaction network can lead to significant deviations from predicted aggregate behavior. [260•; 267•].

Aggregate differential equations tend to smooth out fluctuations, not ABM, which is important because under certain conditions, fluctuations can be amplified which means that the system is linearly stable but unstable to larger perturbations. [390]•.

Brownian agents were developed to facilitate describing the dynamics of agents in a stochastic manner, similar to the Langevin approach of Brownian motion. This allows obtaining on the macroscopic level a ***closed-form partial differential equation for the density***, However, The dynamics in most real systems, is much more complicated. Agents are not simple random walkers, they respond to information in their environment, follow chemical gradients, and can contribute to generating information at the same time, etc. Further, agents do not behave the same all the time. Instead, they may have different modes of activity each of which corresponds to a particular behavior. To cope with these features, ***Brownian agents*** are described by internal degrees of freedom and their environment is modeled as an adaptive landscape, or effective potential, which can be modified by the agents while responding to the information provided. Further, transitions between the agent's internal degrees are possible, dependent on internal or external conditions. [339•; 391•]. This leads to Active Brownian Particles.

Active Brownian particles *are Brownian particles with the ability to generate a self-consistent field, which in turn influences their further movement and physical and chemical behavior. This non-*

• [389] M. Birbaumer, F. Schweitzer, Agent-Based Modeling of Intracellular Transport, European Physical Journal B, vol. 82, no. 3-4, pp. 245-255, 2011.

• [260] P. Gerlee, A.R. Anderson, Stability Analysis of a Hybrid Cellular Automaton Model of Cell Colony Growth, Division of Mathematics, University of Dundee, DD1 4HN, Scotland, 2018.

• [267] J. M. Halley, et al., Competition, succession and pattern in fungal communities: towards a cellular automaton model, OIKOS, 70, 435-442, Copenhagen, 1994.

• [390] F. Schweitzer, Active Brownian Particles: Artificial Agents in Physics, Stochastic Dynamics, pp 358-371, 2007.

•[339] P. Y. L. Francesca, R. Nardi, Probabilistic Cellular Automata Theory, Applications and Future Perspectives, Complexity and Computation.

• [391] J.L. Schiff, Introduction to Cellular Automata, 2005.

linear feedback between the particles and the field generated by themselves results in an interactive structure formation process on the macroscopic level. Within a discrete approximation, the particles can be described as ***Active Walkers***, which have been used to simulate a broad variety of pattern formations in complex systems. [390]•.

Individual-based models (ABM) are considered as a modern and flexible tool to describe ***self-organization in complex systems,*** provided by active ***Brownian particles***. Different examples interpret the broad variety of applications, ranging from ***physico-chemical pattern formation***, to ***self-assembling networks*** and collective search strategies. The consideration of biological features, such as replication or internal energy depots, extents the description of active Brownian particles towards an artificial agents model. [390]•.

The interaction between the active Brownian particles can be described as a *non-linear and indirect communication process*. [390]•, which all particles are involved in. Communication is based on the exchange of information, and therefore needs a medium, in the model of active Brownian particles, this medium is described as a space and time dependent field *h(r,t).* With respect to the particles, the communication consists of three processes:

- "Writing": The particles locally generate information by contributing to the field.
- "Reading": The particles locally receive information by measuring the gradient of the field.
- "Acting": The particles locally change the direction of their movement based on both the response to the information received and unpredictable circumstances by means of the field *h(r,t),* the information generated is stored and distributed through the system via diffusion. On the other hand, the information can also fade out, expressed by a decay of the field. Communication can be considered here as a special type of *global coupling* between the particles, which feeds back to their individual actions.

- ***Learning***

Agent-based models are able to simulate learning at both the individual and population levels learning can be modeling in any or all of three ways:

- ***Individual learning*** in which agents learn from their own experience;
- ***Evolutionary learning***, in which the population of agents learns because some agents decease and are replaced by better agents, leading to improvements in the population average;
- ***Population (social) learning***, in which some agents imitate or are taught by other agents, leading to the sharing of experience gathered individually but distributed over the whole population.[389]•.

ABM vs. EBM: In many domains, agent-based modeling competes with equation-based approaches that identify system variables and evaluate or integrate sets of equations relating these variables. Both approaches simulate the system by constructing a model and performing it on a computer.

• **[390] F. Schweitzer, Active Brownian Particles: Artificial Agents in Physics, Stochastic Dynamics, pp 358-371, 2007.**
• **Ibid**
• **Ibid**
• **[389] M. Birbaumer, F. Schweitzer, Agent-Based Modeling of Intracellular Transport.**

- ***(Model components): The first fundamental difference between ABM and EBM is in the relationships on which one focuses attention.*** The difference is in the form of the model and how it is executed. In agent-based modeling (ABM), the model consists of a set of agents that encapsulate the behaviors of the various individuals that make up the system, and execution consists of emulating these behaviors. In equation-based modeling (EBM), the model is a set of equations, and execution consists of evaluating them. Thus, "simulation" is the general term that applies to both methods, which are distinguished as ***(agent-based) emulation and (equation-based) evaluation.*** **[387]•.**
- ***Individuals vs. density****:* A system is made up of a set of interacting individuals. Some of the observables of interest may be defined only at the system level, while others may be expressed either at the individual level or as an aggregate at the system level (e.g., location of an organism vs. the density of organisms per unit space of habitat).
- ***The difference between ABM and EBM is the fundamental relationships among entities that they model***: Both approaches recognize two kinds of entities: *individuals and observables*, each with a temporal aspect. Individuals are bounded active regions of the domain. In some domains, the boundaries that set individuals apart are physical, in other domains; the boundaries may be more abstract. They are called "active regions" because they refer to the individuals as having behaviors. **[387]•.**
- ***Observables are measurable characteristics of interest: They may be associated either with separate individuals or with the collection of individuals as a whole.*** In general, the values of these observables change over time. In both kinds of models, these observables are represented as variables that take on assignments. Each of these sets of entities invites to articulate the relationships that unify it and show how those relationships predict the behavior of the overall system through time. **[387]•.**

- EBM begins with a set of equations that express relationships among observables. Since it is often easier to formulate closed-form equations using such quantities. the evaluation of these equations produces the evolution of the observables over time. These equations may be algebraic, or they may capture variability over time (as in system dynamics) or over time and space (partial differential equations, or PDE's). It is recognized that these relationships result from the interlocking behaviors of the individuals, but those behaviors have no explicit representation in EBM.

- ABM begins, to define agent behaviors in terms of observables accessible to the individual agent, which leads away from reliance on system-level information. Not with equations that relate observables to one another, but with behaviors through which individuals interact with one another. These behaviors may involve multiple individuals directly or indirectly through a shared environment. The modeler pays close attention to the observables as the model runs, and may value an account of the relations among those observables, but such an account is the result of the modeling and simulation activity, not its starting point. The modeler begins

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• [387] H. V. D. Parunak, et al., Agent-Based Modeling vs. Equation-Based Modeling:-A Case Study and Users' Guide, International Workshop on Multi-Agent Systems and Agent-Based Simulation, MABS, 1998.
• Ibid
• Ibid

by representing the behaviors of each individual, and then turns them loose to interact. Direct relationships among the observables are an output of the process, not its input. In other words, the evolution of system-level observables does emerge from an agent-based model, but the modeler is not as likely use these observables explicitly to drive the model's dynamics as in equation-based modeling. [387]•.

- ***Physical vs interaction space***: ABM's make it easier to distinguish physical space from interaction space. In many applications, physical space helps define which individuals can interact with one another. [387]•. Both ABM's and EBM's can be validated at the system level, by comparing model output with real system behavior. However, ABM's can be validated at the individual level, since the behaviors encoded for each agent can be compared with local observations on the actual behavior of the domain individuals. A balancing consideration is that the code needed to represent an agent's behavior in ABM is often longer and more complex than a typical equation in an EBM, and thus potentially more susceptible to representational error.
- ***Model structure:*** *In many cases, simulation of a system is part of a larger project whose desired outcome is a control scheme that more or less automatically regulates the behavior of the entire system. The agents in an ABM correspond one-to-one with the individuals in the system being modeled, and their behaviors are analogs of the real behaviors. These two characteristics make agents a natural locus for the application of adaptive techniques that can modify their behaviors as the agents execute, to control the emergent behavior of the overall system*. The migration from simulation model to adaptive control model is much more straightforward in ABM than in EBM. [387]•.
- ***Representation resolution & manipulation***: In many domains, ABM's give more realistic results than EBM's, for manageable levels of representational detail. The PDE model may be much too complex for reasonable manipulation and comprehension, on the other hand, EBM's based on simpler formalisms than PDE's may yield less realistic results regardless of the level of detail in the representation. [387]•. In contrast, with ABM models that emulate the behavior of individual atoms and can be developed for arbitrary dimensions. ABM are more accurate both qualitatively and quantitatively than the mean field approximation as they are inherently local. It is natural to let each agent monitor the value of system variables locally, without averaging over time and space thus without losing the local characteristics that can determine overall system behavior. ***ABM is most appropriate for domains characterized by a high degree of localization and distribution and dominated by discrete decisions. EBM is most naturally applied to systems that can be modeled centrally, and in which the dynamics are dominated by physical laws rather than information processing.*** [387]•.

- ***ABM vs. cellular automata:*** Cellular automata and agent-based models have both represented complex adaptive systems approach in modeling. In this approach, models are

• **Ibid**
• **Ibid**
• **Ibid**
• **Ibid**
• **Ibid**

microsimulations, run at the atomic level, and aggregate behavior emerges as a consequence of large numbers of agent interactions. CA and ABM share their individual basis. *In CA, the modeled entities are cells that remain static while spatial and other processes move across or through them. In ABM, the agents can move in space, interact with each other directly, and interact with other agent types*. In both cases, a large number of independent autonomous lowest level actors create the overall landscape. [386•; 389•].

- ***ABM model combinations***

ABM + EBM : The two approaches can be combined within an individual agent in an ABM, behavioral decisions may be driven by the evaluation of equations over particular observables, and one could implement an agent with global view whose task is to access system-level observables and make them visible to local agents, thus driving an ABM with system level information. Furthermore, while agents can embody arbitrary computational processes, some equation-based systems (those based particularly on PDE's), are also computationally complete. The decision between the two approaches must be made case by case based on practical considerations.

ABM+ cellular automata: By combining cellular automata with agent objects, computational and memory requirements of agent–based systems can be decreased, simultaneously increasing flexibility of a typical cellular automata model.

The most recent research on agent-based models has demonstrated the need for combining agent-based and ***complex network-based models***. This has included a desire for models with reusable components, tools for proof of concept and design, descriptive agent-based modeling for developing descriptions of agent-based models by means of templates and complex network-based models, and a need for better validation. [387]•.

- ***Genetic algorithms and evolutionary design***

Optimization is a primary tool, needed to tackle the unsolvable or hard problems. It could be described as the process of modifying the inputs or characteristics of a device, or as a mathematical process to obtain minimum or maximum of the output. The input to the optimization process is the *cost function*, objective function or *fitness function* is the output of the system. In trial and error optimization, the processes affect the output without knowing about the constraints, responsible to produce the output. [392]•.

One- dimensional optimization contains one variable and a problem having more than one variable requires multi-dimensional approach. As the number of dimensions increases, the process of optimization becomes difficult. ***Dynamic optimization*** is time dependent, unlike the static optimization. The static problem is difficult to solve for finding the best solution. ***Discrete variable optimization*** contains only a finite number of possible values, whereas continuous variables have an infinite number of possible values. Variables often have limits or constraints. Constrained optimization incorporates variable equalities and inequalities into the cost function, whereas unconstrained

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• **[386] S. Matsuura, Growth and Colony Patterning of Filamentous Fungi, Biology, 2000.**
• **[389] M. Birbaumer, F. Schweitzer, Agent-Based Modeling of Intracellular.**
• **[387] H. V. D. Parunak, et al., Agent-Based Modeling vs. Equation-Based Modeling:-A Case Study and Users' Guide, International Workshop on Multi-Agent Systems and Agent-Based Simulation, MABS, 1998.**
• **[392] R. Malhotra, N. Singh, Y. Singh, Genetic Algorithms: Concepts, Design for Optimization of Process Controllers, Computer and Information Science, Vol. 4, 2011.**

optimization allows the variable to take any value. A constrained optimization problem can be converted into unconstrained one through the transformation of variables. [392]•.

Evolution is any process of change occurring with time. In terms of life sciences, *evolution is a change in gene frequency in a population*. The genes are the fundamental physical and functional units of heredity, they are made up of DNA, many genes constitute a chromosome and each organism in turn has many chromosomes.

The mechanisms of evolution are ***natural selection, mutation, recombination, genetic drift and gene flow***, Natural selection is the principle mechanism that causes evolution, this was expressed as a general law by Darwin and Huxely (1859), as quoted:

"IF there are organisms that reproduce, and, IF offspring inherit traits from their progenitor, and, IF there is variability of traits, and, IF the environment cannot support all members of a growing population [393]•, THEN those members of the population with inferior traits will die out, and, THEN those members with better traits will thrive."

Natural selection can be subdivided into two types: ***Ecological selection, and Sexual selection.***

Ecological selection takes place in situations where inheritance of specific traits is solely determined by ecology. While ***Sexual selection*** is the theory that states that competition for mates, between individuals of the same sex, drives the evolution of certain traits. *Natural selection* occurs only when the individuals of a population have diverse characteristics. Natural selection ceases to operate when the population does no longer have any genetic variation. For evolution to continue, mechanisms that increase the genetic diversity are necessary. Mutation, recombination and gene flow are the mechanisms that increase the diversity in the population so that evolution can proceed onwards [393]•

Genetic drift is the mechanism that acts in conjunction with natural selection and changes the characteristics of the species over a period of time. This is a stochastic process and is caused by random sampling in the reproduction of offspring. Like natural selection, genetic drift changes the frequencies of alleles but decreases the genetic variations.

Gene flow is the transfer of genes from one population to another. Migration into or out of a population may be responsible for a significant change in the gene pool frequency.

Recombination is the process by which the combination of genes in an organism's offspring differs from that of its parents. Recombination results in a shuffling of the genes. It is considered as a mechanism of evolution because it adds new alleles to the gene pool.

Mutations are permanent changes to the genetic material of a cell. The process of mutation introduces new genetic variations and this facilitates the process of evolution. In essence, Genetic algorithms have all the features of these evolution mechanisms. If the details of all the mechanisms of biological evolution are understood and implemented in genetic algorithms then the efficiency of GAs will increase many fold.

Evolutionary searching strategies. Known also as ***Darwin strategy***, in this strategy, the ensemble of N searchers is divided into different subpopulations, each characterized by a fitness E_l. As for biological species, *replication* and *mutation* of the members of the subpopulations are allowed. In the average, only subpopulations with a fitness above the mean fitness, $E_i > (E)$, grow; therefore the

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• **Ibid**
• **[393] P. Kumar, et al., Improved genetic algorithm inspired by biological evolution, Soft Comput, 11:923–941, Springer-Verlag, 2007.**
• **Ibid**

replication rate is assumed proportional to the fitness. The fitness E_i of the subspecies "i" can be chosen to be the negative of the potential U_i indicating that the subspecies, which has found the better minimum in the potential landscape, also has the higher replication rate. ***The second element of the Darwin strategy, mutation, means that the searchers by chance can be transferred into a state with a better or worse fitness.*** The mutation rates are usually assumed to be symmetric, since there are no directed mutations. [388•; 390•].

Genetic algorithms originated from the studies of cellular automata conducted by Holland, since the idea of genetic algorithms was introduced by Holland in the early 1970s, GAs have been applied to several optimization problems. [393]• .***Genetic algorithms (GAs) are randomized, parallel search algorithms that model the principles of natural selection that leads to evolution. The success of natural selection provides proof of the viability to use an evolutionary process as a model for design***. Like natural selection, GAs is a robust search method requiring little information to search effectively in large and poorly understood search spaces.

The GAs begin with random initialization of the population. The transition from one generation of population to the next takes place by application of the genetic operators: ***selection, crossover and mutation***. The *selection* operator selects chromosomes in the population for reproduction. The fitter the chromosomes, the greater number of times it is likely to be selected for reproduction. The *crossover* operator randomly chooses a locus and exchanges the sub sequences before, and after, that locus between two chromosomes to create two offspring.

Encoding Technique in Genetic Algorithms (GAs): Encoding techniques in genetic algorithms are problem specific, which transforms the problem solution into chromosomes. Various encoding techniques used in genetic algorithms are binary encoding, permutation encoding, value encoding and tree encoding.

- ***Binary encoding****: It* is the most common form of encoding in which the data value is converted into binary strings. Binary encoding gives many possible chromosomes with a small number of alleles.
- ***Permutation encoding****:* It is best suited for ordering or queuing problems. In permutation encoding, every chromosome is a string of numbers in a sequence.
- ***Value encoding****:* It can be form of number, real number on characters to some complicated objects. Value encoding is technique in which every chromosome is a string of some values and is used where some more complicated values are required.
- ***Tree Encoding****: It* is best suited technique for evolving expressions or programs such as genetic programming. In tree encoding, every chromosome is a tree of some objects, functions or commands in programming languages. [392]•.

There are no specific directions for using the type of encoding scheme in the specified problem rather; it depends upon the applicability and the requirements of the problem.

• [388] T. E. Gorochowski, Agent-based modelling in synthetic biology, Essays in Biochemistry, 2016.
• [390] F. Schweitzer, Active Brownian Particles: Artificial Agents in Physics, Stochastic Dynamics, pp 358-371, 2007.
• [393] P. Kumar, et al., Improved genetic algorithm inspired by biological evolution.
• [392] R. Malhotra, et al., Genetic Algorithms: Concepts, Design for Optimization of Process Controllers, Computer and Information Science, 2011.

Types of mutation: Molecular studies have shown that mutations include not only nucleotide substitutions but also important processes as gene duplication and recombination. Mutations are considered the driving force of evolution, where less favorable ones are removed by the process of selection and the favorable ones tend to propagate from generation to generation, thereby improving the fitness of individuals in the population. The various types of mutation can be broadly put into three categories namely:

- ***Point mutations***: These are changes in the single DNA nucleotides. A point mutation may consist of the *deletion* of a nucleotide, the *insertion* of additional nucleotide or the *substitution* of one nucleotide for another.
- ***Large mutations:*** These mutations involve a whole gene at a time. Various types of large mutation that are implemented in the GAs are *deletion*, *inversion, insertion* and *gene duplication*.
- ***Chromosomal mutations:*** These are very large-scale mutations, involve whole chromosomes or a piece of them, and can alter many genes at a time in that chromosome. They are an important source of new genetic material.

Based on Goldberg (1989) studies, the ***multi-objective evolutionary algorithms (MOEAs)*** evolved including, Initial MOEAs, such as the ***multi-objective genetic algorithm (MOGA)***, ***the non-dominated sorting genetic algorithm (NSGA)*** and the ***niched pareto genetic algorithm (NPGA)***. These consisted of two primary steps: (1) The fitness of a solution determined using its dominance within the population and, (2) The diversity among solutions preserved using a niching strategy. [393]•.

As a result of this a number of advanced algorithms emerged the ***strength pareto evolutionary algorithm (SPEA)***, the ***pareto archived genetic algorithm (PAES)***, and the ***non-dominated sorting genetic algorithm II (NSGA-II)***. In further attempts to improve the quality of the solutions and to obtain well spread solutions of the Pareto Front, algorithms with dynamic population size were developed by Tan et al. (2001). Adaptive mutation rates were implemented to further accelerate the search for optima and to enhance the ability to locate optima accurately.

[3.1.4] Complex systems

A system consists of a set of components; anything that is not one of those components is part of the 'external environment'. In certain contexts, a system is defined exclusively in terms of its interaction with this 'outside world,' and is then called an input-output system. [256]•. A system qualifies as complex if the overall behavior of the system cannot be intuitively understood in terms of the individual components or interactions. A defining feature of complex systems is that the qualitative nature of their behavior can depend on quantitative differences in their structure. That is, behavior can be altered by insignificant changes in system features. Two essential features of complex systems are nonlinear interactions and feedback loops. Feedback can be classified as negative or positive:

- ***Negative feedback*** is exhibited when system components inhibit their own activity. These feedback loops generally stabilize system behavior; they are the key feature of self-regulation. However, that instability and oscillations can arise when there is a lag in the action of a negative feedback loop.

•[393] P. Kumar, et al., Improved genetic algorithm inspired by biological evolution.
• [256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics.

- ***Positive feedback*** is typically associated with unstable divergent behavior. However, when constrained by saturation effects, positive feedback can serve as a mechanism to 'locked in' a system's long-term behavior thus allowing a cell to retain a memory of past conditions. [256•; 259•].

John Holland suggested defining condition for identifying complex systems and complexity that was termed Emergence. Emergence has been criticized as a subjective criterion to identify complexity but is said to exist in a system when new and unpredicted patterns or global-level structures arise as a direct result of local-level procedures. The structure or pattern that emerges cannot be understood or predicted from the programmed or assumed behavior of the individual units alone. [394]•.

The main concepts in complexity theory are the dynamical systems; they exist in three aggregate phases: ***chaos, stability, and complexity***. In ***chaos***, no obvious rules, structures, or even heuristics applied, in ***stability***, behavior is linear or can be modeled by polynomials, that is, and the change is differentiable and solvable with differential equations, equilibrium theory, and optimization. The third is ***Complexity,*** is marked by periods (time) or sub regions (space) of both stability and chaos. A system can move from one aggregate behavior state to another (a phase change), but each behavior type is robust (resilient) against perturbation to some degree.

Multilayer Networks: a Framework for Integrated Biological Systems

Distinct complex networks provide a fair description of isolated networked systems, consisting of static units, which are related by a single type of relationships. Such systems are named ***single-layer networks*** or, simplex networks. However, biological systems exhibit a higher level of complexity, with interdependencies within and across different networks that can also vary over time. ***Multilayer network models*** provide a powerful representation of such systems and allow for the integration of multiple types of interactions among biological units of different types, while reducing loss or aggregation of available information. In this framework, each network is encoded into a different layer of the system, while layers can be coupled each other in a complex way, to resemble complex interaction patterns observed in biology.

Despite the success of multilayer network modeling and analysis in systems biology, some methodological challenges are still to be tackled to build consistent, replicable and reproducible representations of multi-omics, connectomics and intercellular interactions. Alternatives approaches have been investigated based on the development of objective null models based, for instance, on ***random matrix theory*** **or** ***problem-specific topological principles***. [262]•.

- ***System Dynamics***

In the system dynamics approach to modeling, one creates a model that expresses the temporal cause-and-effect relationships between variables, but agents are not represented directly. System dynamics is able to handle direct causal links; it is often convenient to represent a system dynamics model with a diagram in which arrows represent the causal links between variables.

System dynamics is based on the evaluation of sets of simultaneous differential equations, each of which calculates the value of a variable at the next time step given the values of other causal variables

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• Ibid
• [259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling.
• [394] K. C. Clarke, Cellular Automata and Agent-Based Models 62, Handbook of Regional Science, pp 1217-1233, 2013
• [262] M. De Domenico, F. B. Kessler, Multilayer Network Modeling of Integrated Biological Systems, Network Science of Biological Systems at Different Scales A Review, Physics of Life Reviews, 2018.

at the current time step. Software such as Stella and Net-Logo can help with drawing the diagrams and execute the simulation by computing these equations. [395•; 396•]. In comparison with agent-based modeling, the system dynamics approach deals with an aggregate, rather than with individual agents, it is also hard to represent agent behaviors that depend on the agent's past experience, memory, or learning in a system dynamics model. On the other hand, because they deal with aggregates, the system dynamics approach is good for topics where there are large populations of behaviorally similar agents. [387]•.

[3.2] Computational tools for biological data simulation

As exhibited through this chapter, mathematical modelling and computational simulations form an essential part of the design process. They enable large-scale in silico investigations into the robustness of specific designs, help to identify key parameters, and can filter out designs that are likely to be non-functional. This reduces the costly and time-consuming laboratory work required to develop a functional system. [388]•. Since, a model is a simplification with the aim to better understand, quantify or visualize complex systems like organisms, these models out worth the advantages of experimental studies, as models have higher speed of simulation, and infinite possibilities of varying parameters of experimentations. [263]•.

The important factor when choosing a modelling tool is the ease of use and accessibility to non-programmers. At present, the majority of agent-based tools require some basic level of programming experience in order to define a working model. One of the major reasons that users are required to program models is that they often need to implement features (e.g. agent rules) that have never been used before. Programming languages are highly expressive and offer the simplest way to provide the greatest functionality to a user. Some tools do attempt to aid new users by providing simpler languages with which to define agent rules and environmental features, but they still require a significant investment of time to learn. [388]•.

- ***The Synthetic Biology Open Language (SBOL)*** is used to aid the exchange of *genetic design information* and definition of biochemical models. Furthermore, the integration of tools designed to efficiently model the reaction networks inside cells. In addition to, the application of *whole-cell models to provide detailed behavioral responses* with accurate simulations. [388]•.
- Many agent-based modelling tools provide features of direct relevance to synthetic biology . Three of the most widely used agent-based frameworks are ***Net-Logo***, ***Repast*** and ***the Flexible Large-scale Agent Modelling Environment (FLAME)***. These are all general-propose frameworks that provide minimal built-in functionality. They allow for extensive customization of agent behaviors and the environment itself. Repast and FLAME are also designed to produce highly scalable simulations that can be automatically optimized to run on systems ranging from desktop computers to high-performance computer clusters. The

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• **[395] http://www.iseesystems .com.**
• **[396] http://ccl.northwestern.edu/netlogo.**
• **[387] H. V. D. Parunak, et al., Agent-Based Modeling vs. Equation-Based Modeling: A Case Study and Users' Guide, International Workshop on Multi-Agent Systems and Agent-Based Simulation, MABS, 1998.**
• **[388] T. E. Gorochowski, Agent-based modelling in synthetic biology, Essays in Biochemistry, 60 325–336, 2016.**
• **[263] S. Kelleter, Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of *Coprinopsis cinerea*, Master Thesis, University of Gottingen, Faculty of Forest Sciences and Forest Ecology, 2017.**
• **[388] T. E. Gorochowski, Agent-based modelling in synthetic biology, Essays in Biochemistry, 60 325–336, 2016.**
• **Ibid**

requirement on a user to implement complex cellular traits (e.g. growth, replication and movement) as well as the environmental physics necessary to capture movement and interactions of cells means that such frameworks are generally only suitable for highly specific problems where customized implementations of many processes are necessary. [388]•.

- The ***Agent Cell software*** implements the entire ***chemotaxis biochemical network*** *of bacteria and* provides a physically realistic three-dimensional environment for cellular movement. The tool includes a fully stochastic simulator for the biochemical reaction networks within each cell.
- The ***Rapid Cell software*** also simulates a population of motile *bacteria cells*, but within a simplified two-dimensional environment. It employs a hybrid simulation approach. This mixes algebraic and differential equations to model the fast and slow reactions respectively. This significantly reduces the computational demands, allowing for up to 1million cells to be simulated on a standard desktop computer, with results that still accurately match experimental observations. [388]•.
- The ***BacSim*** software is an agent-based tool to study ***biofilm growth*** and asses the role of heterogeneity within these populations. *Biologically verified rules relating to substrate uptake, metabolism, maintenance and growth are implemented within each cell, and simulations take place in an environment that allows for the diffusion of substrates.*
- ***iDynoMiCS*** implements a more detailed three-dimensional environment than *BacSim*; including many improvements such as ***pressure fields to enable the contraction or spreading of biofilms over time, and more realistic fluid behavior of the extracellular matrix***. This model has been used to test the effect of physical and biological factors on biofilm growth and ***the role of quorum-sensing inhibition*** as a way to disrupt their structures. [388]•.
- The ***Organism software*** allows for standard ordinary differential equation (ODE) models of general ***biochemical reaction networks*** and mechanical rules within and between cells.
- The ***BSim*** software provides features for physically realistic three-dimensional environment that implements ***Brownian motion***, ***diffusive chemical fields***, and the ability to include multiple forms of agent within a single simulation. Agent dynamics can take many forms with simulators provided for ODEs, delay differential equations (DDEs) and general rule-based dynamics. BSim also provides a broad range of example simulations that can be adapted and combined to tackle a wide range of agent-based modelling tasks.
- The ***BNSim*** software provides stochastic simulators that implement solvers for ***stochastic differential equations (SDEs).*** BNSim is also optimized to accelerate simulations through the efficient use of multi-core processors. [388]•.
- ***Cell Modeller*** software is designed to study the formation of ***synthetic biofilms*** and makes use of Open CL (a high-performance computing library) to enable the efficient simulation of colonies containing more than 30000 cells. Through the implementation of ***novel parallel algorithms*** that can rapidly compute the collisions and forces between cells. For agent dynamics, Cell Modeller provides simulators for both rule-based programs and ODE equations.

• **Ibid**
• **Ibid**
• **Ibid**
• **Ibid**

- The ***gro software*** makes use of its own high-level specification language called 'gro' to define simulation parameters and agent rules. This language is designed to simplify the expression of high-level rules, while still being capable of implementing any ***chemical reaction network or gene regulatory model***.
- The ***cell-based chaste*** software is able to simulate cell populations using ***lattice-based, cell-center or vertex-based models*** for ***cell position and connectivity in one-, two- or three-dimensional environments.*** Furthermore, to account for changes ***in cell–cell adhesion, which affects tissue structure***, the laws governing forces between cells can be modified. Detailed cell-cycle models are embedded within each cell. Complex boundary conditions can also be accommodated. Simulators are included for a full range of deterministic and stochastic models that can supplement existing cellular models of behavior.
- ***CompuCell3D*** software is suitable for ***tissue-based systems*** and uses a ***cellular Potts model (CPM)*** for cell growth. This approach allows for highly complex cell morphologies and has been successfully used to capture the growth of many different types of tissue. CompuCell3D offers the novel ability to use Systems Biology Markup Language (SBML) models to control ***cellular behaviors***. It also includes highly optimized parallel implementations of simulators and an entire set of supporting applications to simplify the development of large models. [388]•.

The execution of models at these scales requires the adoption of efficient parallelizable algorithms and high-performance computing architectures. A shift to parallel computing architectures has already taken place in ***molecular dynamics*** simulations, leading to huge leaps in the speed and scale of problems that can be solved. Some attempts have also been made to use this approach for ***synthetic biology applications***, as in Cell Modeller, that exploits graphics processing units (GPUs) to accelerate simulations, but these optimizations often come at the cost of limiting the range of possible agent behaviors and the complexity of the virtual environment. While several of the general purpose modelling frameworks (as FLAME and Repast) do support these types of large-scale simulation, they also lack the biologically relevant built-in features that are critical for the efficient development of synthetic biology-related simulations.

To alleviate some of these computational difficulties, attempts have also been made to employ alternative forms of modelling. Hybrid approaches in which an agent-based model is combined with continuous models has been shown to significantly reduce the computational demands of some forms of simulation. As in dynamic network-based models that could be used to simplify the virtual environment, while still ensuring that interactions between cells are fully captured. It is likely that a single tool could eventually encapsulate the functionality of all of these, as in Chaste and BSim that are built around a 'plug-n-play' architecture where simulations are built from a set of available modules, besides the possibility of users defining their own modules from scratch. [388]•.

[4] Tools: synthetic biology hybrid design

Synthetic biology aims to control natural systems and develop new synthetic systems with useful prescribed behaviors. [388]•. It also aims to enable the more rational design of novel functionalities.

This has resulted in the engineering of cells able to perform complex computations and acting as biosensors.

• Ibid
• Ibid
• Ibid

Due to the ability to observe and measure many diverse aspects of individual cells, much of the modelling in synthetic biology to date has focused on intracellular dynamics (i.e. capturing changes in the rates of ***transcription and translation, and variations in the concentrations of chemicals, mRNAs and proteins over time***). However, there is growing realization that the robustness of natural biological systems is often derived from collective population-level features that extend beyond individual cells. [388]•.

*** *Synthetic biology hybrid design*** is a design methodology proposed in the current study; that combines the synthetic biology tools' control over biological function, with complex systems design, in order to reach the integrated hybrid that enables ***selective biological behavioral systems that achieves different ecological functions in space***. The main tool of this method is DNA manipulation in order to promote selective behaviors rules implemented in each individual cell, and inhibit none recommended others. DNA manipulation also supports homogenizing ***bioactive architecture***; for its living and nonliving components. (it is important to point out the difference between the two terms describing the embedding of organisms in architectural,* the term ***bioactive*** (growth in time) while the term ***biological*** describe utilizing materials and devices that could be produced by living organism but it doesn't exhibit vital signs (non-living)). This hybridization is achieved by binary toolkit; synthetic biology tools (DNA manipulation), and computational modeling.

Computation by Gene Regulatory Networks

The initial discovery of gene regulatory networks prompted an analogy to the human-made technology of electrical circuits, and thus lead to the term ***'genetic circuit.'*** This analogy can be made explicit by treating ***promoter/transcription-factor interactions*** (the building blocks of gene networks) ***as logic gates*** (the building blocks of computational electrical circuits). [388]•.

A discretization process can be applied to the continuously varying concentrations of transcription factors in a gene network. This abstraction results in a binary description of gene activity: at a given time-point, each gene is either 'on' (expressing above threshold) or 'off' (expressing below threshold).

Dynamic models that describe two-state (on/off) behaviors are called ***Boolean models***. (Boolean models are often used to describe gene regulatory networks, and are particularly useful for addressing large networks). Using the Boolean framework, all signals take either the value 1 (HIGH) or 0 (LOW).

Applying this notion to the concentration of a transcription factor provides, as an example, the comparison between repression of expression and a *digital inverter*, promoters that are regulated by multiple transcription factors can be represented by multi-input logic gates. In first case, the binding of either promoter is sufficient to drive expression, so the promoter acts as an OR gate. In the second case binding of both activators is necessary to drive expression; this implements AND gate logic. Promoters that are regulated by two distinct repressors can be classified in a similar way: if either repressor suffices to inhibit repression, the promoter acts as a NOR (i.e. NOT-OR) gate, while if repression only occurs if both repressors are bound, then a NAND (i.e. NOT-AND) logic applies. This implies a potential application in different ecological systems embedded in architecture, as for programming the real gene expression Boolean models to work as logic gates in specific functions; such as bio sensing for toxins.

• **Ibid**
• **Ibid**

Another crucial distinction between electrical circuits and gene circuits is the manner in which the specificity of the interconnections is achieved. In an electrical circuit, all connections employ the same signal. In contrast, the signal carriers for gene circuits (transcription factors) are mixed together in a single compartment. Unwanted interconnections are avoided through chemical specificity (of the protein-DNA binding surfaces). This reliance on chemical specificity allows complex networks to operate on tiny spatial scales. [390•; 388•].

[5] Redefinition, manifestation and discussion

From the previous review (chapter 1,5), it is obvious that embedding microorganisms in design as living active component, is a specialized branch of the wider umbrella of biodigital design theory, the main differences between this part and its whole is referred to the following:

1. *Biodigital design: is mainly concerned about learning from nature, and emerging nature in design process, either by extracting nature rules and applying them to design intelligence (the way of performance & behavior) in formal generation, functional adjustment or both, or by embedding natural elements or products, but not necessarily alive or bioactive.*
2. *Bioactive design: apart from paradox names of different recent projects, the term " living" in this context does precisely mean "alive and performing active vital processes", including different physiological processes that leads to growth, evolution and morphogenesis emerging from the embedded bio-element to the host design elements; achieving a hybrid creature composed from harmonized and symbiotic living and nonliving elements; thus a necessary classification of this nascent sub theory could be:*

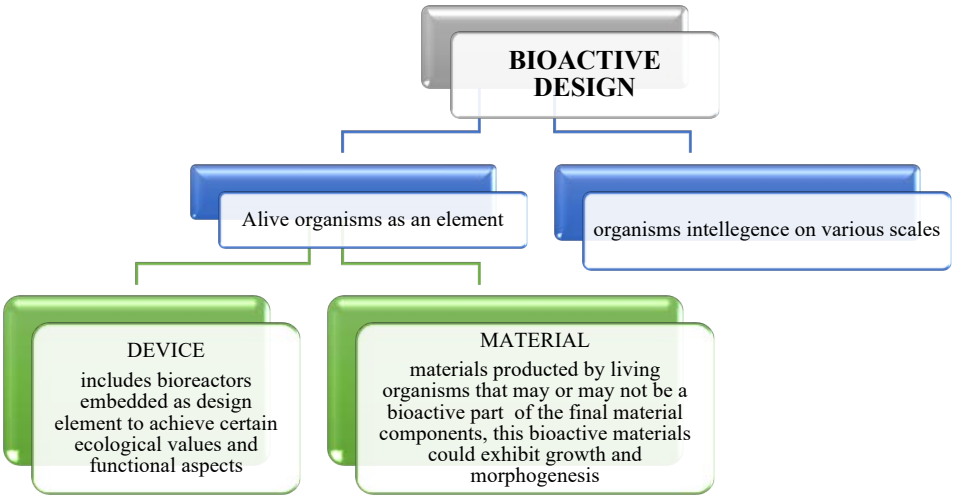

Diagram. [10]. Proposed methodology of BioActive Design, for embedding microorganisms in architectural design, developed by the author.

Although both biodigital & bioactive design shares almost same tools of biological data acquisition and computational design tools, they may differ in methodology, due to the morphogenesis aspect of the hybrid design that includes functional and formal criteria that supports the function/ form/ time/ space, continuous propagation and variation which demands advanced

• **[390] F. Schweitzer, Active Brownian Particles: Artificial Agents in Physics, Stochastic Dynamics, pp 358-371, 2007.**
• **Op.cit**

complex systems that support this growth. (These criteria will be exhibited extensively in following chapter).

Design theory	BioActive Design	Bio Digital Design
Similarities	Tools: • Computational Design tools for simulation & Modeling • Biological Data acquisition: including imaging & microscopy. • Biological Data processing & bioinformatics. • Mathematical modeling for biological data. • Biological behavioral data simulation & modeling. Morphogenesis: unlimited growth in space, form, function ,and time	
Differences: • *Methodology* • *Scale* • *Ecological added value*	1.**Bioactive** design adopts methodology that support variation and probability factors that result from the continuous growth and transformation in the space /time / function and form - Bottom up feedback loop - Includes automated adaptation & morphogenesis 2.Bioactive design scale focuses on Microorganisms and DNA manipulation, focusing on active living organisms. 3.Achieving ecological value is a must: production or purification (Bioelectricity, biodegradation, bio sensing, etc.) .	1.**Bio digital** design adopt a wider methodology depending on extracting nature rules and behavioral patterns; that may support manipulation in form /space /function / time, separately (one up to 3 parameter per and according to each design case). - Bottom up methodology. 2. Bio digital design scale include any natural element in the widest scope (not necessarily active alive). 3.achieving ecological value is optional

E-1.1) bioactive vs. biomimicry /bio inspired

Design Theory Deference	Bioactive	Bio mimicry /Bio inspired
	• ***Inspiration level***: biological processes in living biological systems (form, function, and behavior), configuration, transformation, and morphogenesis in time is mandatory (dynamical models). • ***Methodology***: bottom up feedback loop, complex systems loop. • ***Tools***: biological microscopy, imaging, mathematical dynamical	• ***Inspiration level***: vary between three levels; first, simple formal mimicking of natural elements and organisms (not necessarily biological), second, behavioral mimicking of organisms (not necessarily dynamic or morphogenetic) and third, mimicking the ecosystem. • ***Methodology***: top down or bottom up methodology. • ***Tools***: no specific tools for source of inspiration data, or design generation (could vary from necked eye to sophisticated probes and from manual

	models, and computational design tools are mandatory. • ***Ecological added value***: mandatory. • ***Morphogenesis***: unlimited Growth and morphogenesis in space, form, function, and time.	design generation methods to computational methods). • ***Ecological added value*** is optional • ***Morphogenesis:*** design paradox with or without form and function shifting in limited configurations (within predefined physical boundaries).

E-1.2) bioactive vs. environmental /Green architecture

Design theory	**Bioactive**	**Environmental /Green Architecture**
Similarities	**Objectives: Ecological effect:** in both design trends, achieving ecological aspect is mandatory, it only varies in scale of application and tools of design and application, bioactive design moves more towards maximum integration of ecology self-sufficient systems in design, it also employs living organisms as an integrated part of built environment. In contrast with environmental design that may aim to achieve ecological effect without the use of natural element, bio systems or biomaterials (e.g. solar energy panels, wind energy fixtures, etc.).	
Differences	***Level of inspiration***: microbiological systems. ***Level of integration***: embedded living microorganisms. ***Methods***: bottom-up feedback loops, complex systems. ***Tools***: mathematical dynamical tools for simulation & modeling (e.g. cellular automata, agent based). and computational tools.	***Level of inspiration***: ecological systems. ***Level of integration***: from mechanistic to natural. ***Methods***: top down, bottom up, feedback loops. ***Tools***: computational design tools for simulation & modeling (environmental simulation with parametric software).

E-1.3) bioactive vs. responsive /interactive (according to systems /behavior)

Design Theory	***Bioactive***	***Interactive***	***Responsive***
Similarities	***System behavior:*** Bioactive design shares the same methodology in designing systems behavior with responsive design; in regard of designing feedback loops and modeling systems' behavior. The only difference between the two theories is that the bioactive design modeling of systems' behavior is far more complicated due to dealing with microorganisms that depend on sophisticated biological processes of stochastic dynamical nature; that makes the design of system's behavior complex and include a factor of uncertainty (chaos). Thus, the designer and biologist play key role in bioactive design, simulating, predicting, modeling and opening functional, formal, and spatial and time margins for chaos to guaranty sufficient performance of the system in all cases. Unlike interactive design, as designing system response to certain type of stimuli is much simpler in terms of computational modeling. ***Methodology: all*** three theories share the bottom-up feedback loops methodology and in some cases complex systems for modeling systems behavior.		

	Tools: the three theories share computational modeling and simulation tools.		
Differences: • *Inspiration* • *Objective* • *Tools* • *Morphogenesis*	• Microbiological systems behavioral and formal inspiration. • Ecological added value. • Biological data acquisition, Dynamic mathematical methods, computational simulation /modeling tools. • Growth and morphogenesis in space, form, function, time.	• Open. • Ecologic added value. • Computational simulation /modeling tools. • Form /function shifting predefined configuration.	• Open. • Open (e.g. Social, entertainment, etc.). • Computational simulation /modeling tools. • Form /function shifting predefined configuration.

E-1.4) bioactive vs. parametric /generative (according to tools, methods)

Design theory	***Bioactive***	***Parametric /generative***
Similarities	Tools: • ***Bioactive design*** is model based and directed by its objective in simulating and modeling microbiological systems' behavior that is stochastic and chaotic. This include a wide spectrum of computational design tools that all employs and applies dynamic mathematical models as exhibited in this chapter. These systems by default are parametric (either visual programming or textual); In order to support the stochastic nature of the behavior studies. • ***Parametric and generative design*** applies wide spectrum of mathematical rules not necessarily stochastic.	
Differences	**Methodology:** bottom–up feedback loops.	**Methodology:** parametric design supports both top down and bottom up methodologies; it also supports feedback loops as a tool for specific applications not as a default methodology for design generation. Generative design mainly supports bottom-up methodology and supports chaos and uncertainty in terms of form generation and behavioral design.

[6] Conclusion

From the previous comparative study, it is obvious that bioactive design proposed in this research is a specified branch of biodigital design and is the accumulative effect of many intersections between design trends and theories as: bio inspired, bio mimicry, environmental, green, responsive, interactive, parametric, and generative. This intersection is varied between methodology, methods, tools, objectives, scope, integration and inspiration.

CHAPTER 6

Methodology of BioActive Design

Embedding Microorganisms in Interior Design Elements to Achieve Space Ecology-Self Sufficient system of Microbial Electricity

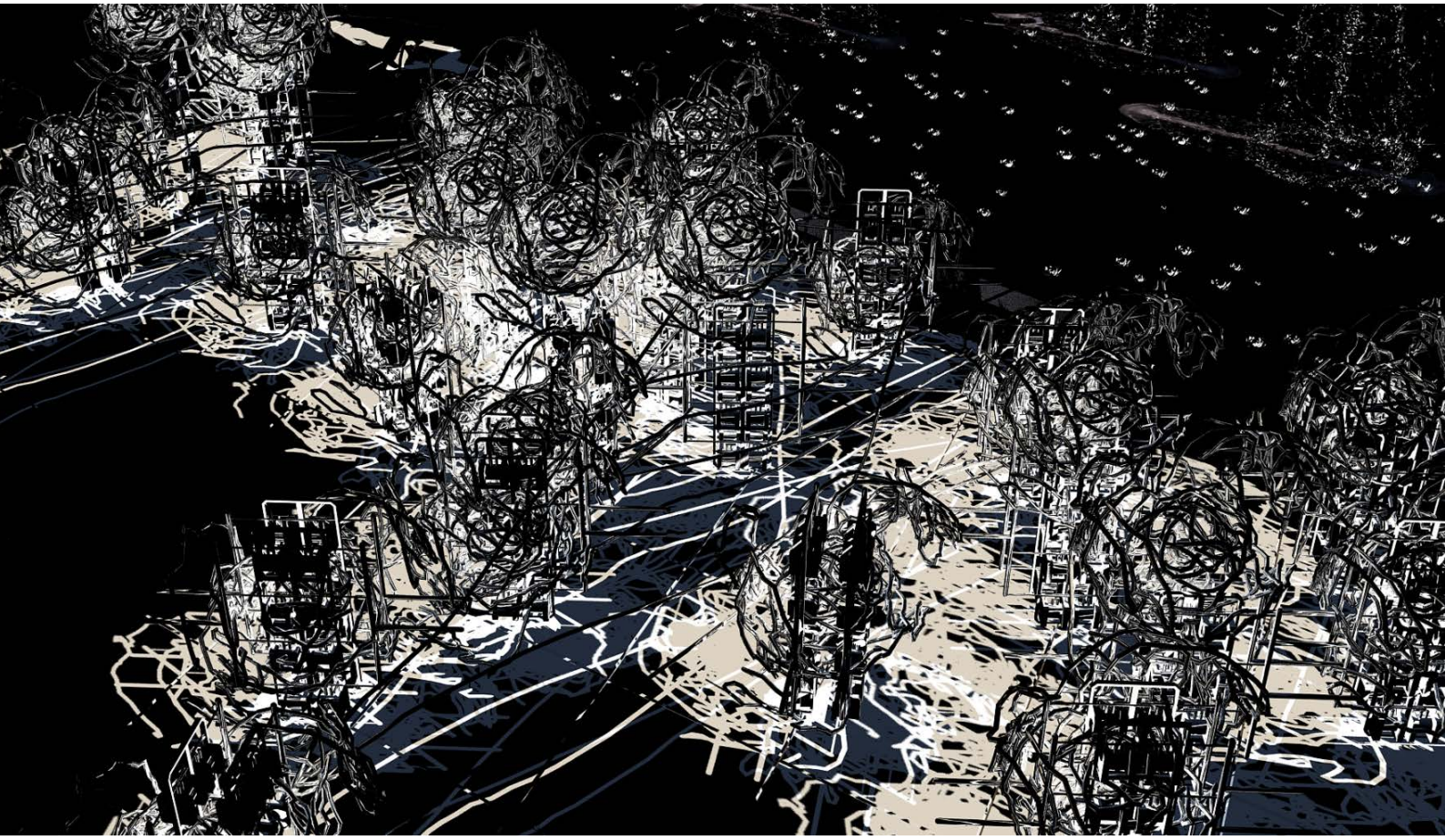

Image. [134]. Lighting intensity simulation of the emitted light from the self-sufficient system for bioelectricity production based on Microbial fuel cell employing *Aspergillus sydowii* NYKA 510 as the biocatalyst. By author.

Introduction

This chapter sums up the **Design methods, procedures** and **criteria** involved in embedding microorganisms in architectural design; from **redefining design process** in terms of **bioactive design** moving to the interior design criteria of the self-sufficient hybrid systems and devices of employing microorganisms in achieving space ecology in different aspects.

[1] Bioactive design as a complex system

In the concept of complex systems, emergence is central and essential, with inherent intelligence as an emergent property of the complex systems; which is a feature that is not reducible to the parts of the system in isolation. As the intelligence observed inside a single cell emerges from interactions among thousands of non-intelligent macromolecules. Similarly, the intelligent behavior of a microbial society is not simply the sum of the behavior of intelligent cells; rather, it is a property that emerges from the interactions amongst many of them. [397]•. As an emergent property, the ***bioactive complex system*** satisfies ***physical monism; Synchronic determinism; Systemic organizational property***. [398]•.

Synchronic determinism *restricts the way systemic properties and the system's microstructure are related to each other, and states that there can be no difference in systemic properties without changes in the structure of the system or in the properties of the components*. Features of intelligence are exhibited exactly by the changes in the system such as chemical reactions between molecules, electrical current between components of a system. *While the inverse statement is invalid as a change in a system's microstructure or properties does not necessarily yield a change in its behavior or properties.*

Strong emergence also satisfies an additional criterion of irreducibility. [399]•.The system components' properties are state dependent, the greater their state dependency is, the greater the degree of irreducibility of the system, implying stronger emergence. [400]•. It is worth mentioning that in this definition of strong emergence, the deduction base does not include systemic knowledge, such as the state of the system.

A description of how components interact with and affect each other within a complex system such as a bioactive system can be represented as ***a network, such as: metabolic networks, signal transduction networks, gene expression networks, microbial ecological networks, etc***. One can generate and model these networks using various approaches. As a result, simulating the dynamic behavior of the network thus, reconstruct its emergent properties in silico. *On the other hand, that is practically challenging because* there is a large degree of uncertainty involved in measuring or even acquiring all system parameters, or the extreme complexity of the system makes it difficult to understand or even know the mechanisms of all system processes.

• **[397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.**
• **[398] A. Stephan, Varieties of Emergentism, *Evol. Cogn,* 5 49–59, 1999.**
• **[399] A. Stephan, The dual role of 'emergence' in the philosophy of mind and in cognitive science, *Synthese,* 151, 485–498, 2006.**
• **[400] F. C. A. Kolodkin, et al., Emergence of the silicon human and network targeting drugs, *Eur. J. Pharm. Sci,* 46, 190–197, 2012.**

[2] Defining design process in terms of bioactive design

The bioactive design theory proposed in the current work includes both special methods and tools to achieve the ultimate coupling of function, form, and behavior in terms of continuous and future propagation, evolution and morphogenesis in real time that defines it as a complex system. [401]•.

The Recipe for designing Complexity in bioactive empirical terms includes criteria of design factors that make a system complex, as:

- The system contains large numbers of similar computational elements.
- Simple rules govern the interactions between elements.
- There is a significant degree of nonlinearity in the element interactions.
- There is adaptation (sensitivity to history) on the part of the elements.
- There is sensitivity to an external environment. [401]•.

The proposed design code presented in the current work, is achieving complexity through the integration of function /form /time and space relation. This design theory is manipulated by two parallel yet intersected categories, the ***complex systems intelligence of devices***, and the ***complex systems intelligence of hydride***s. The complex systems' intelligence of devices begins usually with top-down planning of variables, interactions, boundaries, etc. on the other hand, complex systems' behavior of hybrids always arises from bottom-up improvisation. [402]•. However, the complex systems intelligence of devices would still adopt bottom up methodology including various scales of complexity. Multi-intelligence (MI) is the main characteristic of hybrid beings and systems comprising diverse natural and artificial intelligences.

The multi intelligence characteristic of bioactive design includes ***self-awareness, robust adaptation, and problem solving***; this multi intelligence is considered in terms of whole systems of causal mechanisms and causal contexts encompassing full range of variables that can contribute to intended and unintended consequences in both bioactive hybrids and devices. [403]•. Accordingly, the two main categories of bioactive design are:

[2.1] Bioactive design method for Complex System's intelligence of devices: This design method could include top-down or bottom up methodology; it utilizes an active organism that perform specific function that have a certain value in design ecology. This category couples between form, function, time and space, with some limitations in propagation and morphogenesis in space. This spatial propagation restriction is due to challenging mechanistic extension, automation management type, singularity and global behavior coherence between system agents and the agents with the system, decision making, feedback loops, adaptation and system networking's update.

[2.2] Bioactive design method for Complex System's intelligence of hybrids: This category mainly adopt bottom–up methodology, as the result is a new hybrid that is considered living bioactive, as a mixture between an active organism or any of its parts and a designed host, either material or device. These systems possess the inherent natural intelligence that include

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• **[401] R. Loosemore, Complex Systems, Artificial Intelligence and Theoretical Psychology, Proceedings of the 2007 conference on Advances in Artificial General Intelligence: Concepts, Architectures and Algorithms: Proceedings of the AGI Workshop, 159–173, 2007.**

• **Ibid.**

• **[402] S. Johnson, Emergence: The Connected Lives of Ants, Brains, Cities, and Software, Scribner: New York, NY, USA, 2001.**

• **[403] S. Fox, Beyond AI: Multi-Intelligence (MI) Combining Natural and Artificial Intelligences in Hybrid Beings and Systems, Technologies5, 38, 2017.**

problem-solving capabilities involving self-awareness and robust adaptation. [397•; 404•; 405•], as in microbial communities. [403]•. This type achieves the optimum coupling code of function/form/time and space; as it exhibits morphogenesis and emergence through the ability of reforming by the growth process, various successive functioning coupled with reforming of growth, and propagation in space. Despite the fact that this method will enable maximum freedom and control of the built environment, it raises an ethical issue about biodiversity and ecologic conservation. The process of hybridizing bioactive features included mainly in DNA manipulation or molecular biotechnology raises many related questions about;

- ***The interactions between the bioactive agent and the host in itself. How this affects the hybrid physical and chemical features?***
- ***The potential of evolution of this hybrid as an added creature to the ecologic system, and how it will maintain its functional margins without producing hazards?***
- ***This hybrid ability to reproduce and to decay (biodegradability compatibility with function)?***
- ***The interaction with different environments and conditions?***
- ***Most importantly, the limits of transferred biological intelligence (from the bioactive agent) and handling it by the system?***
- ***The hybrid's ability of developing multi –intelligence?***
- ***The hybrid's ability of extracting new functions imposed by multiple –intelligence ability?***

These questions are key guide to designers/researchers willing to develop bioactive hybrid designs that perform certain ecologic functions, in addition to these questions; the author here proposes ethical design criteria for the bioactive hybrid design.

[3] Ethical Design Criteria for Bioactive Design

Bioactive hybrid design validation: Such bioactive hybrids should be subjugated for long experimentation that monitors its functionality under different environments, conditions and even extremes, this monitoring aim is to test functional ability extents and detects hybrid's reaction in the most extreme conditions.

Directed bioactive intelligence: Although biological intelligence is inter-reciprocal, and by no means is restricted, the absolute integration of such intelligence in functional devices or materials is a menacing issue, as such, hybrids' behavior could develop to be uncontrollable or unpredictable, that could hinder its functionality or even worse (mal-functioning, poisonous effect, etc.). Thus, designers and researchers should specify certain functions and insert typically biological elements responsible for that function (specific genes, enzymes, proteins, etc.).

Bioactive hybrid's life cycle planning: Designers and researchers should work collaboratively to design the life cycle of the bioactive hybrid in regard to ecologic system conservation in main three phases:

• **[397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.**
• **[404] L. Chittka, et al., What is comparable in comparative cognition? Philos. Trans. R. Soc, 367, 2677–2685, 2012.**
• **[405] M. Wilson, Six Views of Embodied Cognition, Psychon. Bull. Rev, 9, 625–636, 2002.**
• **[403] S. Fox, Beyond AI: Multi-Intelligence (MI) Combining Natural and Artificial Intelligences in Hybrid Beings and Systems, Technologies5, 38, 2017.**

- The first phase is **Emergence**: That include the standard function of the hybrid and the initial growth phases in space, time and morphogenesis as well. In this phase, designers and researchers guarantee optimum conditions for the growth of the hybrid, concerning safety measures.
- The second phase is **Evolution:** This critical phase implies a significant transformation in form, structure and functional capacities and qualities mainly. Resulting in continuous growth and propagation in time and space, as the scale of performance would grow or decline. In this phase designers should consider growth margins and scenarios of form / function transformation respecting time frames and resources management (including base material and supporting resources (medium)), this would be conducted with the help of parametric simulation that utilizes mathematical modeling (cellular automata/ agent based, partial differential equations, etc. as mentioned in chapter 5) extracted from similar biological systems.

The third phase is **Decay or degradation:** In this phase designers and researchers guarantee that the designed bioactive hybrid joins the ecologic system cycle**,** this includes two possible scenarios: the first is **Finite decaying**; in this scenario all the hybrid parts should be biodegradable or recyclable, not to impose pollutants in any aspect on ecologic system. The second scenario is the **Infinite evolution**; in this scenario the hybrid possesses an ultimate endless ability of fast and diverse adaptation physiologically in form and function. Yet this scenario is not achievable in the near future, as it demands high level of biological intelligence insertion in the hybrid (intelligence in terms of consistency and evolution, as well as highly flexible and compatible formal qualities of the host.

[4] Bioactive Interior design criteria

According to the previous categorization of Bioactive Design to intelligence of devices, and intelligence of hybrids, developing design process and criteria is categorized accordingly. The Bioactive Design criteria will focus on the design quadratic code of function /form/time and space in terms of process as well as safety measures.

Interior Design Criteria of Bioactive -Complex System's Intelligence (hybrids/ devices)

[4.1] Function

Designing function in Bioactive design exploits organic intelligence and potentials mainly to harmonize with the natural eco-system. The notion of function plays an important role in systems' biology. [397]•. Distinguishing between mechanistic explanations and biological design explanations. Mechanistic explanations categorize a system into a number of functional components; they describe how these components are arranged, how their activities are organized in time, and relate these features to some phenotype. [406]•. Mechanistic models are mathematical models related to the activities of cellular reaction networks involving transport, metabolism, signal transduction, or gene expression. However, mechanisms suffice to explain how the features work.

• **[397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.**
• **[406] F. C. Boogerd, et al., Emergence and its place in nature: a case study of biochemical networks, *Synthese,* 145, 131–164, 2005.**

Understanding *why* certain mechanisms exist (rather than other, alternative organizations) requires biological design explanations. [407•; 408•].

Mahner and Bunge (2001) considered the "cognitive" functions of decision-making, robust adaptation, association, anticipation, self-awareness and problem solving as the main set of complex intelligence functions. Microbial like intelligence targeted in this context of bioactive complex systems, in principle can be mechanistically explained if the properties and behaviors of the parts and their relationship within the system are fully known, and, thus, can also be reconstructed in mathematical models of the underlying mechanism. [406]•.

This sort of complex systems (bioactive hybrids and devices) are semi-open systems. It selectively interact with its environments by way of mass and energy exchange, where the decrease of free energy in the environment is coupled with the increase of the order of the bioactive system itself (decreasing its own entropy), or with the maintenance of the bioactive system against the activity of the many processes that tend to disintegrate it. [409]•. In other words, there is always a flow of mass and energy through the system, causing the emergence of a certain function.

The bioactive functional design criteria include:

[4.1.1] Achieving Ecology and sustainability (hybrids and devices): This is the pivotal criteria in bioactive design and is the main reason towards using bioactive agents specifically instead of other sustainable systems i.e. renewable energy systems of wind or solar energy. as achieving ecological design by using bioactive agents have further specifications and more benefits.

"Ecological design" or "eco-design" provides a framework for uniting conventional perspectives on design and management with environmental ones, by incorporating the consideration of ecological concerns at relevant spatial and temporal scales. [410]•. Moving towards an ***integration of the needs of humans, other species, and natural ecosystems.*** In this context, bioactive design of both hybrids and devices emphasize on the integration of alive bioactive agents as part of the built environment. Maintaining all their natural environmental conditions and their bio abilities as well (functions), this is a wider and more invasive application in design than biomimicry, which "studies nature's models and imitates or takes inspiration from these processes to solve human problems". In Biomimicry many of the technologies and ideas, such as alternative building materials, renewable energy sources, and conservation and recycling of materials have been adopted. *However,* ***bioactive design moves further from just imitating natural laws to bringing the very natural and bioactive agents into action by themselves. In order to achieve ultimate integration of natural laws that would be inherited and activated by default and in a spontaneous manner, obviating the need of designing each single function to a separate complex system or face the consequences of problematic relations and synchronization between all these reciprocal systems and functions****. Moreover, bioactive design utilizes natural potential of bioactive agents to perform variable functions within an ecologic performance, (for example, exoelectrogeneses, bioluminescence, etc.). Those functions are inherited physiological processes that the bioactive agent normally conduct for living. Thus, bioactive design achieves double benefit; by proposing natural ecologic solutions for required functions in the built*

• **[407] A. G. Wouters, Viability explanation, *Biol. Philos,* 10, 435–457, 1995.**
• **[408] A. G. Wouters, Design explanation: determining the constraints on what can be aliv, *Erkenntnis* 67, 65–80, 2007.**
• **[406] F. C. Boogerd, et al., Emergence and its place in nature: a case study of biochemical networks, *Synthese,* 145, 131–164, 2005.**
• **[409] H. V. Westerhoff, K. Van Dam, *Thermodynamics and Control of Biological Free Energy Transduction,* Amsterdam: Elsevier Science Ltd, 1987.**
• **[410] F. Shu-Yang, et al., Principles and practice of ecological design, Environmental Reviews, 12, 2, 2004.**

environment, and maintain and enhance the presence and richness of the biodiversity of the ecosystem.

Principles of ecological design; the author emphasize on the related principles to the ecologic criteria of bioactive design, as follows;

- Meeting the inherent human needs.
- Sustaining integrity of the structure and function of both natural, enhanced, engineered managed ecosystems within the bioactive hybrid or device and in the whole built environment.
- Embedding the inherent properties of nature in built environment functional systems.
- Greater reliance on renewable resources, recycling, and efficient use of materials and energy.
- Conserving natural ecosystems and indigenous biodiversity at viable levels.
- Increasing environmental literacy to build social support for sustainable development, resource conservation, and protection of the natural world, and dealing with bioactive systems. [410]•.

Thus, eco-design framework of a bioactive design seeks to provide a framework for environmentally appropriate systems of design and management by incorporating ecological values, at relevant spatial and temporal scales. While maintaining standards of quality of services and reducing overall resource consumption, waste generation, and ecological damage through efficiencies of use, and recycling.

These principles are applied to the design process and operation of bioactive design in the built environment (interior, architecture and urban) as follows;

- **Maintain ecological integrity**: Ecosystems are life systems; that are environments that support biodiversity and natural communities i.e. biofilms, while also providing critical support for the human initiative. ***As such, a purpose of eco-design generally and bioactive design specifically is to integrate human activities and required functioning systems with the structure and dynamics of natural flows and cycles of materials, organisms, and energy***. This begins with development of an understanding of the ecological context of particular design case, and developing solutions consistent with these circumstances. To achieve this, the designer must have a clear idea of both;

  (a) The activity (required function) being contemplated with its requirements and operative conditions.

  (b) The limitations of natural embedded bioactive systems (either hybrids or devices) to support the design enterprise.

The designer needs a detailed understanding of local ecosystems and environments, including climate, topography, resources, i.e. (soil and water, flows of energy and materials, biotic communities, and critical habitat). This information can be used to define the carrying capacity of the study region for the proposed activity, allowing the bioactive eco design to be accommodated within the identified ecological limits.

- **Embedding natural ecosystems:** Natural ecosystems are characterized by complex patterns and dynamics of biodiversity, materials, and energy, occurring at various spatial and temporal scales. ***These patterns reflect the long- and short-term influences of biological evolution*** (including speciation and extinction), disturbance and successional regimes, environmental

• **[410] F. Shu-Yang, et al., Principles and practice of ecological design, Environmental Reviews, 12, 2, 2004.**

change (i.e., in climate), species introductions, and anthropogenic influences associated with pollution and other stressors. Biodiversity plays a critical role in ecosystems, in part by forming a globally integrated network of healthy functioning of symbiotic relationships, resource availability, nutrient and biomass cycling and retention, and the intensity of environmental stressors. ***A central goal of bioactive design is to embed these natural ecological qualities in design when planning for anthropogenic activities, so the resulting effects will be totally "natural" and evaluative.***

Embedding of the structure and function of natural ecosystems can be expressed in various scales. For example, design towards an integrated web of ecological activities; accommodate the natural regime of ecological stressors and disturbances, thereby maintaining a high degree of support for environmental services and indigenous biodiversity.

- **Biocompatibility:** Biocompatibility is an essential characteristic principle of bioactive eco design, as it ensures the following
    1. (Bio/ chemical–compatibility) between bioactive agents (organisms or any of its specific parts) and the host components and structure inside the bioactive hybrid or device.
    2. Compatibility between the host components (chemically, biologically, physically) along different operation phases.
    3. Compatibility between the bioactive hybrid or device along its life cycle (emergence –evolution –decay-loop) and the surrounding environment.
    4. Support bio diversity in the surrounding environment through compatibility with different species.
- **Biodegradability:** Biodegradability in this context focuses on the ability of bioactive system to be digested by nature and join the eco system natural life cycle after the end of its own life cycle, not to impose wastes on the eco system; this criterion includes both bioactive agents and hosts. It is worth mentioning that bioactive agents should not produce any toxins or pollutants in any phase of the operational phases of the bioactive hybrid or device, and to have the ability to be further exploited in the bioactive system closed loop or to be employed in other useful recycling applications.
- **Protect natural habitat:** To sustain species and natural ecosystems that are incompatible with the proposed project in general.
- **Increase sustainability consciousness**: Environmental protection is a broad societal responsibility. Bioactive design usage is proposed not only for specialists use, but also for wide spectrum of non-specialized users, it entails deep cooperation among designers, government, businesses, and citizens.
- **Biophilic Design (human sustainability):** Biophilic Design builds on ''Biophilia,'' a term presented by the biologist E. O. Wilson, (1984), who described it as ''***the innate tendency to focus on life and lifelike processes***'' and later expanded to suggest ''***the innately emotional affiliation of human beings to other living organisms***'' by Kellert and Wilson, (1993). 'Biophilic design' is both an ideology and a method for conceptualizing individual bioactive design features as well as the larger holistic bio based systems. [411]•.

• **[411] S. K. Kellert, et al., Biophilic design: The theory, science and practice of bringing buildings to life, Hoboken, NJ: John Wiley & Sons, 2008.**

Biophilic design emerges from "the increasing recognition that the human mind and body evolved in natural biodiverse world that is critical to people's health, productivity, emotional, intellectual and spiritual well-being. [411]•.

Biophilic Architecture is a part of an emerging concept in architecture, that work intensively with human health, ecology and sustainability precepts, such an integrate part of architectural formation which must be in optimal proportion with other buildings material. [412]•.

[4.1.2] Function growth and morphogenesis (ultimate application in bioactive hybrids, limited functional shifting in bioactive devices): The function growth and morphogenesis means the ability of the system to adjust transition in its outer and inner phenotypes (function, form, orientation and propagation in space) according to evolution of its internal genotypes. The change in the system's genotypes evolve from the continuous responsiveness to its outer environment conditions and inner abilities, in other words it's the natural intelligence found in all living creatures with different rates defined according to the system's ability of fast responsiveness, maneuver and organization of its surrounding resources, and inner abilities in sync.

Achieving this level of synchronization and sophisticated intelligence could be via *genetic manipulation in organisms inserted in built environment*, and as long as microorganisms are, fast, resistant and controllable in scale; they are perfect material for such hybridization.

Functional morphogenesis depend on two main aspects; reading inner and outer environment and acting responsively according to imposed conditions with suitable functional morphogenesis. ***In case of bioactive hybrids***, this functional morphogenesis can be applied to maximum extents depending on ***genetic manipulation and bioactive material engineering*** that add a spontaneous inherited biological ability of functional management and shifting according to environmental changing conditions. ***This is not the case in bioactive devices, which is limited in functional shifting in terms of formal and spatial compatibility to the new imposed function, limiting the responsiveness abilities of the bioactive device to predefined designed and limited functional shifting***.

Tim, functional customization, scale, spatial recognition, spatial propagation and spatial orientation are the main attributes of bioactive design functional morphogenesis criteria.

[4.1.2.1] Time (hybrids and devices equally): Designing function transition in time in bioactive design implies an accurate study of the embedded bioactive agent (gene, whole cell, tissue, etc.), and its required functional aspects. This study focuses on:

- ***Latency period of performance:*** Biochemical, physiochemical, and electrochemical reactions that occur on different scales according to the inserted bioactive agent (for instance; growth period).
- ***Optimum Efficiency:*** This includes the study of timing to maximum performance achieved by the bioactive agent in nature (for example; enzymes production, bioluminescence activity, etc.), and the study of optimum efficiency of the new bioactive hybrid. This is to ensure in time margins that none of the hybrid living or nonliving components would hinder the natural

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• Ibid

• [412] A. Almusaed, Biophilic architecture (Towards a new potential of healthy architecture), Conference: Rethinking Sustainable Construction, 12thRinker International Conference, USA, 2006.

potentials of the bioactive agent, and in some cases that these hybrid parts boosts these natural potentials of the bioactive agent.

- ***Responsiveness synchronization:*** The consumed time of functional adjustment to the new imposed function, in other words it is the functional shifting latency time.

[4.1.2.2] Function customization (hybrids full featured functional customization, devices limited predefined customization): This aspect is highly related to ***time***. Functional customization means the ability to perform different functions along the hybrid life span. These different functions emerge from continuously changing surrounding environmental conditions and needs. *Thus,* ***latency period*** *is a defining aspect for the efficiency of this* ***functional transition****. Latency period here includes the time consumed by the bioactive hybrid to analyze the new surrounding environmental conditions, analyze the required function that would fit that change, the latency period to organize its inner genotypes and the time consumed to translate these genotypes into the resulting behavior*. In other words, it is natural evolution wise with faster timescale.

Although this is the most accurate defining characteristic of the bioactive design, it is almost not achieved yet in design disciplines, due to the variation and complexity in unpredicted functions that are imposed by continuously changing conditions in surrounding environment and in usage requirements as well. It is worth mentioning here that variation in function doesn't mean multifunction, but it literally means different functions, for example; making the bioactive hybrid shifting from generating bioelectricity to performing bioluminescence activity or sensing toxic compounds in the surrounding environment. ***This level of flexibility and smooth transition between varied and different functions requires a high level of biological complexity working on the genetic scale, to control the complex network of feedback loops with multiple stimuli, and requires a sufficient management of components, resources and materials***.

This could be achieved by mimicking ***Biological networks*** that constitute a type of ***information and communication technology (ICT)***: They receive information from the outside and inside of cells, integrate and interpret this information, and then activate a response. Biological networks enable molecules within cells, and even cells themselves, to communicate with each other and their environment. [397]•.

One type of biological networks that is essential for functional customization design in bioactive hybrids are ***Macromolecular networks*** in microbes, which confer intelligent characteristics, such as ***memory, anticipation, adaptation and reflection and network organization*** that reflects the type of intelligence required for the environments in which they were selected. [397]•. The genome-wide data production and analysis, in microbes, provide an extensive guide of microbial intelligence and propose how the insights derived from quantitatively characterizing ***bio-molecular networks enable synthetic biologists to create intelligent molecular networks for bioactive hybrids***, possibly generating new forms of intelligence.[397]•.

- ***Another type of biological networks is Neural networks*** that also serve as potent intelligence networks in bioactive agents, they are mainly classified into two groups:

• **[397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.**
• **[397] Ibid.**
• **[397] Ibid.**

(i) The ***feedforward neural networks*** (FFNNs) where data is propagated from input to output using combinatorial machines, e.g., radial basis function (RBF), multilayer perceptron (MLP), self-organizing map (SOM);
(ii) The ***recurrent neural networks*** (RNNs).

- **FFNNs**: Several important feedforward loop motifs have been identified in both ***neuronal connectivity networks*** and ***transcriptional gene regulation networks***. [413]•, despite these networks operating on different spatial and temporal scales. This similarity in motifs reflect a fundamental similarity in the evolved designs of both types of networks. The main motif to be applied in bioactive design is ***to reject transient input fluctuations/noises and activate output only if the input is persistent, a so-called persistence detector***. [414]•. This is in order to avoid bioactive hybrid mal-functioning caused by over sensitivity to environment. ***In addition, a multi-input feedforward structure serves as a so-called coincidence detector: the output is activated only if stimuli from two or more different inputs occur within a certain period of time***. [415]•. This motif is utilized for specific functional customization that requires more than one environmental condition shifting to occur in the bioactive hybrid response.
- **RNNs:** Have immediate biological application (i.e., self-organizing dynamic systems) and can describe complex non-linear dynamics, including both feedforward and feedback structures. ***This allows the network to reflect the input presented to it, but also its own internal activity at any given time.***

Designing functional transition in bioactive design also requires that the bioactive hybrid or device is able to ***learn and to remember*** various previous responses. Learning and memory are two important, counter posed features of intelligence. The former assimilates new information, requiring flexibility in the network to produce complex dynamics; the latter retains old information, requiring stability in the network with sufficient storing capacity. Tradeoffs between the two can be modeled and observed using ***neural networks***.

RNNs may be a more appropriate choice for describing memory-like structures. In addition, feedback structures can increase network stability and exhibit the paradoxical property of near-perfect adaptation, where many properties of the system remain constant even when the system is subject to an environmental challenge or strong change in other network properties. [416]•.

Bioactive design functional transition needs also ***decision-making*** intelligence, to act and respond sufficiently and on time according to the imposed environmental challenges or internal requirements. *In the microbial world, decisions are made by monitoring the current state of the system, by processing this information and by taking action with the ability to take into account several factors such as recent history, the likely future conditions and the cost and benefit of making a particular decision.* At the population level, ***microbes are also capable of getting around their risks, by having individuals of an isogenic population in different states even when experiencing the same environmental conditions,*** and they are also able to make ***collective decisions that cause the entire***

• **[413] R. Milo, et al., Network motifs: simple building blocks of complex networks, *Science,* 298, 824–827, 2002.**
• **[414] U. Alon, Network motifs: theory and experimental approaches, *Nat. Rev. Genet,* 8, 450–461, 2007.**
• **[415] N. Kashtan, et al., Topological generalizations of network motifs, *Phys. Rev. E Stat. Nonlin. Soft Matter Phys,* 2004.**
• **[416] F. He, et al., (Im)Perfect robustness and adaptation of metabolic networks subject to metabolic and gene-expression regulation: marrying control engineering with metabolic control analysis, *BMC Syst. Biol,* 7, 131, 2013.**

population to respond in a particular way. *Microbes are able to make decisions based on different criteria of information and to perform the decision-making using different mechanisms, utilizing different types of* ***molecular networks.*** [397]•.

This decision is made through the action of a complex hierarchical regulatory network, simultaneously involving ***gene expression, signal transduction, metabolic regulation and transport.*** [417]•. Although the network components may vary, the networks involved and the parameters controlling their interactions allow the *microbes to monitor their environment, process the information and react, effectively making a decision in an intelligent manner by taking into account such factors as the cost–benefit ratio and population survival strategies*.

Another important feature of bioactive design intelligence is ***the robust adaptation*** to changes in environments. It describes an organism's response to an external perturbation by returning state variables to their original values before perturbation. [397]•. Such robust adaptions include ***homeostasis***, as well as ***adaptive tracking of nutrient sources*** and ***evasion of harmful compounds***.

Almost all adaptation mechanisms involve feedback or feedforward regulation structures. These can be relevant for signaling, gene regulatory and metabolic networks. Relatively there are two types of robust adaptation intelligence in bioactive agents:

1. ***Long-term adaptations*** often involve changes in genetic expression, such as *gene mutations, transcription/translation activities or rewiring of gene regulatory networks*. Examples include adaptation to temperature.
2. ***Short-term adaptation*** typically involves regulation mediated by
   (i) Protein–protein interactions and covalent modifications in signal transduction pathways.
   (ii) Direct substrate–product effects in metabolic networks.

Such perfect adaption behaviors of the bioactive hybrid specifically are thought to be introduced through a time integral on the ***"controlled variable"*** in the network, which corresponds to a specific control system structure, i.e., an integral feedback control. [418]•. In non-robust proportional regulations, the appearance of a specific signal or environmental condition can be a direct indicator/predictor of a particular response. [416]•. The feedforward regulatory mechanism, then, is introduced to respond directly to the signal rather than to the disturbance.

Different regulation mechanisms in bioactive agent living cells often occur at multiple levels simultaneously with a hierarchical structure. In this case within research scope, in ***microbial metabolic network, the regulation of a reaction rate can be achieved by the modulation of***

(i) Enzyme activity through a substrate or product effect, i.e., metabolic regulation,
(ii) Enzyme covalent modification via signal transduction pathway,
(iii) Enzyme concentration via gene expression, gene-expression regulation.

Such multi-level regulation corresponds to different control loops in a control system, which may ensure the robustness versus perturbations at various frequencies in the bioactive hybrid.

• **[397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.**
• **[417] W. C. van Heeswijk, et al., Nitrogen assimilation in *Escherichia coli*: putting molecular data into a systems perspective, *Microbiol. Mol. Biol. Rev,* 77, 628–695, 2013.**
• **[397] Ibid.**
• **[418] M. E. Csete, J. C. Doyle, Reverse engineering of biological complexity, *Science,* 295, 1664–1669, 2002.**
• **[416] F. He, et al., (Im)Perfect robustness and adaptation of metabolic networks subject to metabolic and gene-expression regulation: marrying control engineering with metabolic control analysis, *BMC Syst. Biol,* 2013.**

When interpreting metabolic and gene-expression regulation separately as specific "***control system structures***," the metabolic regulation is recently identified as more of a ***proportional control action*** with limited range, [419]• and the gene expression regulation as more of an ***integral control*** action with potentially a wider range, but acting more slowly. [416•; 420•; 421•].

Associative learning is another feature of bioactive hybrids. It allows one to model how two or more features co-vary and respond accordingly. This type of learning specifies how several features in the environment, or within cells, change together. ***It implies that the learner has a mechanism to encode mutual information.*** Associative learning in bioactive hybrids occurs when environmental variables are physically coupled, or somehow co-vary non-randomly. For example, the increase in the level of light intensity, signals associated changes in the environment, such as increase in temperature, etc. **Bioactive hybrids weight these physical associations to better adjust their physiology in specific environments** [422]•, to employ easily measured proxies as indications for other phenomena and, in some cases, even use the cues themselves to prepare or anticipate subsequent alterations to the environment.

It is important to note, that time-scale for associative learning in bioactive agents (genetic regulations) is about evolutionary processes and most likely involves genetic changes. [423•; 424•; 425•]. Typically, associative learning in microbial populations involves some sort of social communication (such as quorum sensing). This capacity for associative learning among microbes is the reason why they could be reverse engineered. Since microbes do not respond to stimuli independently, but rather their internal networks direct common responses to diverse but related environmental signals, regulatory networks in microbes can be reconstructed by measuring their response across a broad range of conditions.

For instance, Gene regulatory networks, can be inferred in three simple steps:

(i) Perturb cells across a broad range of relevant conditions;

(ii) Measure their transcriptional response in each environment;

(iii) Cluster similar gene expression patterns observed reproducibly across environments.

Mining for genetic similarities among genes sharing a particular expression pattern, in turn helps link these transcriptional modules to some of the molecular mechanisms responsible for regulating them.

Self-awareness is another crucial aspect of bioactive design functional customization for the bioactive agent in both bioactive hybrids and bioactive devices. ***Self-awareness can be described as the ability to recognize oneself as an individual separate from the environment and other individuals (singularity and global behavior indicator)***. One essential strategy of it is ***quorum sensing***. Quorum sensing provides the entire microbial network with the ability to recognize and

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• **[419] H. Samad, et al., Calcium homeostasis and parturient hypocalcemia: an integral feedback perspective, *J. Theor. Biol,* 214, 17–29, 2002.**

• **[416] F. He, et al., (Im)Perfect robustness and adaptation of metabolic networks subject to metabolic and gene-expression regulation: marrying control engineering with metabolic control analysis, *BMC Syst. Biol,* 7, 131, 2013.**

• **[420] D. Kahn, H. V. Westerhoff, Control theory of regulatory cascades, *J. Theor. Biol,*153, 255–285, 1991.**

• **[421] D. A. Fell, *Understanding the Control of Metabolism*, London: Portland Press, 1997.**

• **[422] R. Bonneau, et al., A predictive model for transcriptional control of physiology in a free-living cell, *Cell,* 131, 1354–1365, 2007.**

• **[423] M. Sorek, et al., Stochasticity, bistability and the wisdom of crowds: a model for associative learning in genetic regulatory networks, *PLoS Comput. Biol,* 2013.**

• **[424] S. McGregor, et al., Evolution of associative learning in chemical networks, *PLoS Comput. Biol,* 2012.**

• **[425] J. Carrera, et al., Computational design of genomic transcriptional networks with adaptation to varying environments, *Proc. Natl. Acad. Sci, U.S.A,* 2012.**

adjust itself collectively once a specific population threshold is exceeded. This is specific for all individuals of a certain organism and even strain.

[4.1.2.3] Scale: Scale is pivotal in controlling function in bioactive design, this aspect focuses on the physical ability to grow and maintain functional potentials of the bioactive hybrid.

- ***Reaction scale***: This is highly determinant by the scale of reaction inside the hybrid; on the genetic, molecular, and cellular level. This also includes the bioactive agent's density ratio to the hosting agent (material /system). It studies the bioactive hybrid physical ability to grow in dimensions (spatial propagation and orientation), and how this affect the bioactive agents' density ratio to the host.
- ***Bioactive agents' multiplication (bioactive hybrid and device)***: this affect ecological balance in terms of the bioactive agents' population and diversity included in a semi open continuously growing system. It also deals with the growth in media or supplementary resources needed to support the growth of the bioactive agent, guarantee adequate functional relations between the systems' components without hindering the output function; in other words it is not just growth in volume, it is growth in form according to function imposed by new environmental conditions as a response. This aspect is pivotal in controlling the ***functional customization***, designing this aspect should be conducted through extensive experimentation on scaling up the hybrid's function /functions as computational simulation alone wouldn't be a sufficient solution.
- ***Material properties:*** It is the role of the researcher/ designer to determine how the growth in the bioactive hybrid scale will be achieved. This is highly relevant to hybrid's materials and their physical and chemical properties, their ability to shape shift and the mechanism of propagation in space. This aspect is related to spatial propagation and will be further explained.
- ***Inverse restoration:*** Designs the system's ability to restore its previous scale (volume), this is highly connected with ***functional customization,*** assuming that environmental conditions will impose shrinkage in the system's scale and vice versa, and provided that growth in scale means multiplication in bioactive agents and propagation in volumetric properties of materials, components and resources. The decrease in scale would impose the need to define specifically the needed population of the bioactive agents, specific amount of media (resources, concentrations), and specific bulk of materials of the host. As hard as it sounds controlling the growth in scale is already challenging aspect that needs extensive research; yet achieving the ***inverse restoration*** is more challenging; especially in controlling microelements (media components concentration that affects the required biological reaction and resultant function).

Inverse restoration also deals with rearranging and managing resources of excess agents (bioactive, materials, resources, etc.). As it is necessary to design how these excess agents will be recycled, flushed out of the system, will be employed in other supporting functions or will be recycled through the system to be restored and redistributed when needed. This sort of resources management

could be achieved by increasing the bioactive agent dominance in the design hybrid in order to exploit natural intelligence.

[4.1.2.4] Spatial recognition (bioactive hybrids): Spatial recognition is a determinant aspect of the ability of growth in scale and propagation in space. ***Spatial recognition is the ability of the bioactive hybrid to read its surrounding space in 360 degrees in the three dimensions or more***. It is a sort of embedded navigating intelligence system in the bioactive hybrid that enables it from roaming the space effectively. This navigator system have specific functions. These include; ***reading and locating the current spatial configuration of the bioactive hybrid, reading the vectored growth space (the space planned for the hybrid growth in its direction) and locate possible obstacles or insufficient locations for growth (mal-conditioned)***. ***The last would be very complicated task if coupled with time (scanned according to future prediction of spatial propagation).***

- ***Dimensional cognition***: Dimensional cognition is an embedded intelligence that could be a natural consequence of the complex intelligence of embedded bioactive agent (genes) in the hybrid, or could be a separate navigating system that is based on embedding specific bioactive agents for spatial recognition specifically (e.g. fungal hyphae intelligence). It also could employ engineered devices that mimic natural microbial intelligence in navigating surrounding environment, these engineered system would be either biological engineered devices or artificial intelligence devices for scanning and analysis.
- ***Environmental Conditions cognition:*** Focuses on analyzing environmental conditions in the surrounding space, this simulator works dependently on the previous spatial dimensional scanner, as to analyze environmental conditions region by region in the target growth vector identified by the spatial scanner. The data obtained by this simulator controls spatial propagation decision-making as it either would approve the next spatial spot for propagation if suitable in conditions, or cancels the propagation decision in this spot to begin new simulation and analysis in the successive spatial region scanned by dimensional cognition scanner.
- ***Synchronized spatial navigation and recognition:*** Navigation is the ability to read space and move accordingly to suitable and sufficient spatial spots for colonization (hybrid growth), and recognition is the ability to describe the current spatial configuration of the hybrid, each element that have been scanned in the space, and to re-locate and direct the hybrid towards favored spots for colonization. The two functions are dependent on each other and ***must*** happen at the same time, recognition will direct navigation and navigation supplies recognition with information in real time. ***This could be achieved by feedback and feedforward loops that are based on mathematical models applying the specific rules of morphogenesis*** *(cellular automata, agent based) of the specific bioactive agent that was used in the hybrid to achieve ultimate coupling between functional customization and spatial configuration.*

[4.1.2.5] Spatial propagation and orientation (bioactive hybrid): Spatial propagation is the result of previous factors all together, the most defining aspect of it; is the mechanism of propagation the ability to move in all spatial direction in a natural wise. This includes:

- ***Motion mechanism***: Movement in the scale of micro, molecular and sub cellular level could be defined according to the resulting effect of growth, which is directed, by certain biological, biochemical and physiological processes inside the bioactive agent. This motion mechanism could be categorized according to resultant effect as:

  -Macro: Which affects the whole bioactive agent by growth in itself or colonization environment (organism, organism-environment).

  -Micro: Which affects certain tasks achieved by this movement inside the bioactive agent's building blocks (cells).

- ***Functional movement mechanisms***: According to this research scope, the author presents only functional movement mechanisms that are possible to be applied in the motion of the bioactive hybrid easily. These mechanisms are related to fugal growth and morphogenesis in summarized points, as motion mechanisms in microorganisms and their application in design is a very wide and nascent field of research that is out of scope of this research.

  -Micro movement: Many microorganisms cells are able to move through liquids or over moist surfaces by using a variety of motility mechanisms ***(swimming, swarming, gliding, twitching, floating) and mostly use complex sensory devices to control their movements***. [426•]. In order to make the decision of movement, the cell monitors the environment by means of multiple receptors in the cell membrane. The information of the ligand binding to the receptor, and the processing of this information inside the cell, is achieved by means of a signaling pathway. The level of the downstream protein of the signaling pathway, determines which movements the cell undertakes: ***when this protein is bound to the flagellar motor it rotates counter-clockwise, resulting in a straight swimming movement***; and ***in the case of absence of such protein the unbound flagellar motor rotates clockwise, resulting in a tumbling motion***. Using this mechanism, organisms make a ***biased-random walk,*** with the length of the periods of straight swimming dependent on the signal, resulting in movement toward or away from different stimuli. [426•; 427•].

  - Macro movement: Hyphal growth motion mechanism

The typical scales for fungal movements vary over many orders of magnitude in time and length, but they are mainly based on ***hydraulics and mechanics.*** [428]•. Hyphal growth is the essential mechanism of movement in bioactive hybrids. As hyphae grow by ***polarized exocytosis*** at the apex, which allows the organism to overcome long distances and invade many substrates, including host tissues. ***Establishment of a growth site and the subsequent maintenance of the growth axis (***polarity establishment and polarity maintenance) are the two strategies controlling this hyphal propagation, two types of polarity establishment events are commonly observed in a typical hypha: ***spore polarization and branch formation.*** It has been firmly established that spatially coupled exocytosis and endocytosis are essential for the stabilization of polarity axes. [429]•.

In both cases, specification of a polarity axis occurs in a cell that is otherwise displaying isotropic or a polar growth (i.e., germinating spores or an existing hyphal compartment). Stabilization of the

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• **[426] K. F. Jarrell, M. J. McBride, The surprisingly diverse ways that prokaryotes move, *Nat. Rev. Microbiol,* 6, 2008.**
• **[426] Ibid**
• **[427] R. B. Bourret, A. M. Stock, Molecular information processing lessons from bacterial chemotaxis, J Biol Chem, 277, 12, 9625-8, 2002.**
• **[428] J. M. Skotheim, L. Mahadevan, Physical Limits and Design Principles for Plant and Fungal Movements, Science, Vol. 308, Issue 5726, pp. 1308-1310, 2005.**
• **[429] S. Upadhyay, B. D. Shaw, The role of actin, fimbrin and endocytosis in growth of hyphae in *Aspergillus nidulans*, Mol Microbiol, 68, 690–705, 2008.**

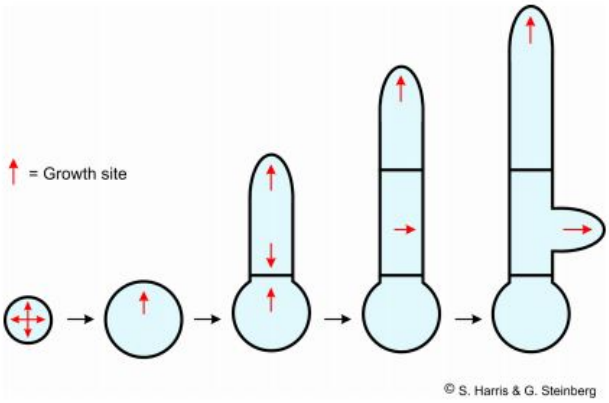
resulting axis requires the recruitment of the morphogenetic machinery to the specified site. As a result, cell surface expansion and cell wall deposition are subsequently confined to a discrete cortical site, which ultimately leads to the formation of a new hypha.

The transport of growth supplies along with polarity establishment and maintenance, initiate hyphal tip growth. ***Those supplies include membranes and proteins, delivered by motors along the cytoskeleton to the hyphal apex***. The regulation of these processes over time and space presumably accounts for much of the variation in hyphal morphology and growth patterns observed in the filamentous fungi. [430]•. Different fungal strains normally utilizes an internal program to specify the position of localized cell surface expansion and cell wall deposition. This internal program utilizes a set of cortical landmark proteins (the Bud proteins) to specify new polarity axes [431]•, whereas others relies on microtubule-based delivery of a set of marker proteins to position new growth sites. [432]•. Microtubules are biopolymers that support long-distance transport of organelles and vesicles in fungal cells. [433]•. They elongate at their plus end. Motility along microtubules is mediated by molecular motors, which use the polarity of microtubules to transport their cargo.

The ability to re-orient tip growth in response to environmental cues is critical for colony ramification, the penetration of diverse host tissues and the formation of mating structures. [434]•. Mutations that affect the processes of hyphal reorientation generate normal-shaped, growing hyphae that have either abnormal winding trajectories or attenuated tropic responses. Hyphal tip orientation and tip extension are distinct regulatory mechanisms that operate in parallel during filamentous growth, thus allowing fungi to organize their reproduction in relation to gradients of effectors in their environments. [434•; 435•; 397•].

However, some fungal strains are able to override the program in response to external signals. [435]•. Accordingly, the signaling pathways that mediate growth and stress responses in filamentous fungi likely interface with the mechanisms that temporally and spatially regulate hyphal morphogenesis. ***Filamentous fungi exhibit tropic responses to chemical, mechanical, electrical, and other environmental stimuli***. The dynamic nature of polarity maintenance provides an explanation for how the direction of hyphal extension can be rapidly reoriented in response to those external signals. Additional processes that contribute to the complexity of hyphal morphology include septation and the formation of septal pores. [436]•.

• [430] S. D. Harris, Hyphal morphogenesis: an evolutionary perspective, Fungal Biol, 115, 475–484, 2011.
• [431] J. Chant, Cell polarity in yeast, Annu Rev Cell Dev Biol, 15, 365–391, 1999.
• [432] F. Chang, S. G. Martin, Shaping fission yeast with microtubules, Cold Spring Harb Perspect Biol, 2009.
• [433] G. Steinberg, Hyphal growth: a tale of motors, lipids, and the Spitzenkörper, Eukaryot Cell, 6, 351–360, 2007.
• [434] C. Brand, N. A Gow, Mechanisms of hypha orientation of fungi, Current opinion in microbiology, 12, 4, 350-7, 2009.
• Ibid.
• [435] F. Chang, M. Peter, Yeasts make their mark, Nat Cell Biol, 5, 294–299, 2003.
• [397] H. V. Westerhoff, et al., Macromolecular networks and intelligence in microorganisms, Front Microbiol, 2014.
• Op.cit.
• [436] G. J. Celio, et al., Assembling the fungal tree of life: constructing the structural and biochemical database, Mycologia, 98, 850–859, 2006.



[437]•

Figure. [78]. Growth patterns in fungal hyphae growth occurs in an isotropic fashion during spore germination. Specification of a polarity axis ultimately results in the formation of a hypha that continues to grow at the tip. While tip growth is maintained. The specification of additional polarity axes enables the formation of septa and lateral branches. Whereas septum formation is transient, branching results in the formation of a secondary hypha that also continues to grow at the tip, red arrows designate polarity axes.

Among the enzymes delivered are cell wall synthases that are exocytosed for local synthesis of the extracellular cell wall. Exocytosis is opposed by endocytic uptake of soluble and membrane-bound material into the cell. The first intracellular compartment in the endocytic pathway is the early endosomes, which emerge to perform essential additional functions as spatial organizers of the hyphal cell. Individual compartments within septated hyphae can communicate with each other via septal pores, which allow passage of cytoplasm or organelles to help differentiation within the mycelium. [437]•.

Actually, most hyphae propagate in a spatially heterogeneous environment that is characterized by a patchy distribution of nutrients. Accordingly, two possible strategies could plausibly be invoked to account for hyphal extension under variable conditions. First, hyphal growth could be a ***"brute force"*** mechanism by which a hypha harnesses the morphogenetic machinery (i.e., the cytoskeleton and vesicle trafficking machinery), and ***turgor pressure*** to invest ahead with colonization of the substrate. Extension rates and the frequency of branching may only be minimally adjusted to account for a changing environment. Alternatively, hyphae may ***fine-tune extension rates and morphogenesis*** to reflect the local environment at the tip and at budding sites of branch formation. [437]•. *However, the latter strategy would apparently rely more on the ability of hyphae to adjust the timing and location of polarization events, as well as to modulate the degree of polarity maintenance, in response to local environmental conditions.*

A unique feature of filamentous fungi that enables the formation of mycelia is the ability to simultaneously **sustain multiple axes of hyphal polarity** that is essential in terms of application in the bioactive hybrid to adjust the synthesis of new growth vector according to environmental and spatial analysis. These axes result in the formation of branches, either lateral branches that emerge from subapical compartments or apical branches that form by splitting of the hyphal tip. [16]•.

• **[437] G. Steinberg, et al., Cell Biology of Hyphal Growth, Microbiology Spectrum, 2017.**
• **Ibid.**
• **Ibid.**
• **[16] S.D. Harris, Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems, Mycologia, 6, 100, 823–32, 2008.**

As in some fungi whose spores are capable of producing multiple hyphae, it is important to ***ensure that the second polarity axis is opposite to the first***. This type of bipolar spore polarization pattern is often observed and can be perturbed by disruption of the vesicle trafficking machinery, microtubules, and marking system. [438•; 439•].

The Delivery of Cell Wall-Building Enzymes

Hyphal growth occurs at the apices. The accumulation of a monomeric unit of chitin, preferentially at hyphal tips, indicates that apical growth is correlated to a polarized mechanism of cell wall assembly. [440]•. This assembly of cell wall at the apex include the participation of synthetic as well as lytic (plasticizing, softening) activities. [441]•. By contrast, an alternative model not involving lytic activity proposed a plastic cell wall at the apex that would become rigid at the sub apex, where remodeling glycosyltransferases would cross-link the cell wall material and rigidify it. [442•; 443•; 444•]. **For application in bioactive hybrid, the first model is more likely to be employed by means of embedding natural intelligence of delivery of cell wall building enzymes to build new extensions of the growing morphogenetic hybrid.**

Septum Formation

As exhibited previously, fungal cells divide via the process of septation, whereby the localized synthesis of a cross-wall divides an existing cell into two distinct cells. Septa divide the hypha into compartments. These septa contain pores that allow intra- and inter-hyphal translocation of metabolites, proteins, and RNA. [445]•.

The process of septum formation in fungal hyphae requires the presence of intact actin filaments, which assemble into a ring. [446]•. This contractile acting ring (CAR) initially appears at the incipient septation site as a tangle of actin filaments that is associated with myosin (one of three major classes of molecular motor proteins), [447]• and tropomyosin. This structure subsequently consolidates into a thin "proto-CAR" that circumscribes the hypha. Constriction of the CAR coincides with ingrowth of the plasma membrane and the deposition of the new cell wall material that will become the septum. The CAR remains positioned at the advancing edge of the invaginating plasma membrane, which is also the active site of cell wall deposition that is mediated by the delivery of exocytic vesicles. The CAR does not fully close, thereby retaining cytoplasmic continuity between adjacent hyphal cells through the resulting septal pore. The formation of CARs at septation sites is subject to both spatial and temporal regulation. [448]•. **In a similar fashion, applying these septation in bioactive hybrid includes the ability to pass essential nutrients and to control their flow according to the need.**

• **[438] S. D. Harris, Morphogenesis is coordinated with nuclear division in germinating *Aspergillus nidulans* conidiospores, Microbiology, 145, 2747–2756, 1999.**

• **[439] S. Konzack, et al., The role of the kinesin motor KipA in microtubule organization and polarized growth of *Aspergillus nidulans*, Mol Biol Cell, 16, 497–506, 2005.**

• **[440] S. Bartnicki-Garcia, E. Lippman, Fungal morphogenesis: cell wall construction in Mucor rouxii, Science, 165, 302–304, 1969.**

• **[441] S. Bartnicki-Garcia, Fundamental aspects of hyphal morphogenesis, In Ashworth JM, Smith E (ed), Microbial differentiation. Cambridge University Press, Cambridge, United Kingdom, 1973.**

• **[442] J. G. H. Wessels, A steady-state model for apical wall growth in fungi, Acta Bot Neerl, 37, 3–16, 1988.**

• **[443] T. M. Bourett, R.J. Howard, Ultrastructural immunolocalization of actin in a fungus, Protoplasma, 163, 199–202, 1991.**

• **[444] S. Bartnicki-Garcia, et al., Computer simulation of fungal morphogenesis and the mathematical basis for hyphal tip growth, Protoplasma, 153, 46–57, 1989.**

• **[445] D. H. Jennings, Translocation of solutes in fungi, Biol Rev Camb Philos Soc, 62, 215–243, 1987.**

• **[446] D. L. Delgado-Álvarez, et al., Septum development in *Neurospora crassa*: the septal ctomyosin tangle, PLoS One, 2014.**

• **[447] https://proteopedia.org/wiki/index.php/Myosin.**

• **[448] C. Fiddy, A. P. Trinci, Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans*. J Gen Microbiol, 97, 169–184, 1976.**

This could be achieved by coupling to a sensory system that tests the concentration of main medium substrates for the bioactive hybrid's growth, as well as building a feedback network to control the closure or openings of these septation.

Mechanisms of cytoplasmic flow

Cytoplasmic streaming depends on the architecture of the mycelium and is the result of turgor pressure gradients, diffusion, and/or turbulence due to movement of organelles along the cytoskeleton. Hyphal fusion plays an important role in long-distance translocation of nutrients. [449•; 445]•. However, motor proteins move organelles in hyphal cells. [450•; 451•]. Such motor-driven motility of organelles was shown to mix the cytoplasm and increase diffusion within the cytoplasm [452]•, which could support the exchange between adjacent cells. [453]•.

Selective transport across closed septa

Fungi have the ability to close their septa, which limits exchange between hyphal compartments. Septal pores mediate communication between these compartments. [454]•. ***However, it is essential for the hyphal cell to keep this exchange under tight control and thus be able to close the septal pore upon stress or cell injury or during particular developmental stages and differentiation***. To this end, fungi have developed various ways to plug the septal pore. [455]•. The process of pore closure is based on ultrastructural basis, which show closure by organelles, small crystalline bodies, or electron-dense proteinaceous material. [455•; 456•; 457•].

Hype ramification

Vegetative hyphae exhibit positive aero-tropism and negative auto-tropism to enable hyphae in a colony to ramify into evenly dispersed mycelial networks that maximally exploit nutrients in the substratum. **Hyphae navigate around impenetrable objects**. [458]•. ***The mechanism underpinning this negative auto tropism has been hypothesized to be mediated*** by;

(i) ***Aero tropism*** towards oxygen, (away from oxygen-depleted zones around metabolically active hyphae) or,

(ii) ***Negative chemotropism*** (away from staling products).

Hyphae can also fuse with one another, tip-to-tip or tip-to-hyphal side.

• **[449] A. Simonin, et al., Physiological significance of network organization in fungi, Eukaryot Cell, 11, 1345–1352, 2012.**
• **[445] D. H. Jennings, Translocation of solutes in fungi, Biol Rev Camb Philos Soc, 62, 215–243, 1987.**
• **[450] X. Xiang, M. Plamann, Cytoskeleton and motor proteins in filamentous fungi, Curr Opin Microbiol, 6, 628–633, 2003.**
• **[451] M. J. Egan, et al., Microtubule based transport in filamentous fungi, Curr Opin Microbiol, 15, 637–645, 2012.**
• **[452] C. Lin, et al., Active diffusion and microtubule based transport oppose myosin forces to position organelles in cells, Nat Commun, 7, 11814, 2016.**
• **[453] A. Abadeh, R. R. Lew, Mass flow and velocity profiles in Neurospora hyphae: partial plug flow dominates intra-hyphal transport, Microbiology, 159, 2386–2394, 2013.**
• **[454] R. R. Lew, Mass flow and pressure-driven hyphal extension in Neurospora crassa, Microbiology, 151, 2685–2692, 2005.**
• **[455] P. Markham, Occlusions of septal pores in filamentous fungi, Mycol Res, 98, 1089–1106, 1994.**
• **[455] Ibid.**
• **[456] R. T. Moore, J. H. McAlear, Fine structure of mycota. 7. Observations on septa of ascomycetes and basidiomycetes, Am J Bot, 49, 86–94, 1962.**
• **[457] A. F. van Peer, et al., Cytoplasmic continuity revisited: closure of septa of the filamentous fungus *Schizophyllum* commune in response to environmental conditions, PLoS One, 2009.**
• **[458] N. A. Gow, et al., Fungal morphogenesis and hostinvasion, Curr Opin Microbiol, 5, 366-371, 2002.**

- **Spatial propagation of growth in bioactive design) hybrid reproduction /device propagation)**.

Spatial propagation and orientation in bioactive hybrid differ from the spatial propagation in bioactive device. Bioactive hybrid carries the original active natural characteristics of growth, which emerges from the hybrid's response to growth in user requirements, sensing the surrounding environmental conditions and analyzing inner capabilities coupled with responding to all these factors by searching resources (nutrients), organizing genotypes and consequently phenotypes and reproducing the hybrid cells (bioactive agents + host particles). This reproduction method follows typically the reproduction regulations of the natural bioactive agent cells (either bacteria, fungi) that is embedded in the particles of the host, thus reproduction method is more automatic and coherent process in bioactive hybrid, which is not exactly the case in bioactive devices.

Bioactive devices do not reproduce in a biological manner, except for only bioactive agents employed inside the bioreactors. Otherwise the bioreactor hardware and components cannot reproduce itself, cannot grow utilizing fresh media or nutrients, and do not of course search for nutrients the same way as bioactive hybrids do. Bioactive devices spatial propagation depends on artificial interference determined by user's definition not by nature's default and achieved by different means of artificial automation (i.e. artificial intelligence, or installation of new bioactive reactors by the designer or user).

Bioactive devices have environmental sensing abilities only for its current spatial limits, in other words, it senses its zone not the growing space. This implies that the spatial propagation may not be achieved naturally, but by the imposed growing requirements of the user and embedded only by the designer decision as added clusters for different ecologic functions.

[4.2] Form

[4.2.1] Time: (Frames), phases, transition ***(related to scale & mass customization: controller).***

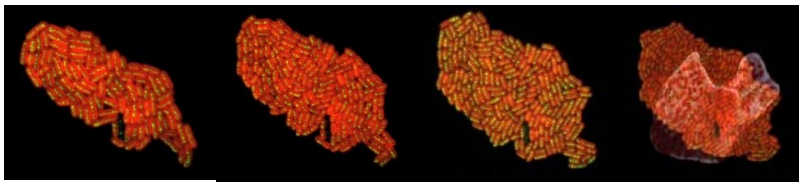
Time controls form conception in Bioactive design hybrids and devices; as discussed previously, bioactive design changes over a wide time variants; each of these variants is translated into consequent phases that compose the overall transition from a configuration to another in hybrids (physical/spatial/functional configurations) and in devices (planar/functional configurations). To design the formal and spatial maneuver in the overall interior or architectural design that hosts the bioactive hybrid or device; designer must utilize computational animation of the design model. This model is based on mathematical modeling (agent based, cellular automata) and conducted with the help of parametric modeling software that supports animation based on simulation of changing parameters (conditions) that control the transition between different phases in the emergence, evolution and decay of the bioactive hybrid or device.

Conducting model's simulation allows the designer to capture every key frame in transition process on the least reaction scale of the bioactive hybrid or device (mainly derived from the bioactive agent in the hybrid or device). These key frames will enable formal and aesthetical evaluation and spatial maneuver in designing the interior space.

An example of cellular proliferation simulation is represented in Mushtari wearable design project conducted by Mediated Matter Lab–MIT (Neri Oxman); Bacterial cellular proliferation time laps. The project is a 3D printed wearable with internal fluid channels that is designed to use synthetic biology to convert sunlight into useful products for the wearer. Through a symbiotic relationship

between two organisms: a photosynthetic microbe such as microalgae or cyanobacteria and compatible microbes such as baker's yeast and E. coli that make useful materials. The photosynthetic microbe converts sunlight to sucrose, which is then consumed by compatible microbes and converted into materials such as pigments, drugs, food, fuel and scents. [459]•. It was designed using ***generative growth algorithms*** to mimic biological growth by generating recursive forms over many iterations of time growth achieved by the algorithm. Initial geometry and parameters defined by the algorithm inform the overall geometry, *the local mesh geometry as well as variations in material property by altering the relative strength of relaxation, attraction and repulsion between mesh vertices.*

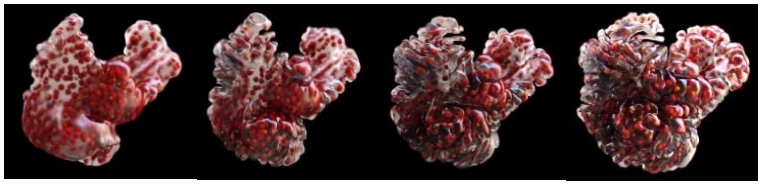
Phase (1) cellular proliferation



[459]•.

Image. [135]. Cellular proliferation simulation time lapse, Mushtari wearable design by Mediated Matter Lab–MIT (Neri Oxman).

Phase (2) from cells to tissue



[459]•.

Image. [136]. Cells development and differentiation to tissue, simulation time lapse, Mushtari wearable design by Mediated Matter Lab–MIT (Neri Oxman).

The initial geometry and parameters created a single long channel that grew over numerous iterations into a wearable with 58 meters of inner channels varying in diameter from 1 mm to 2.5 cm. Transparency was graded regionally within the design to create areas where photosynthetic microbes could receive light and produce sucrose. The design was 3D printed using a color multi-material 3D Printer. [459]•.

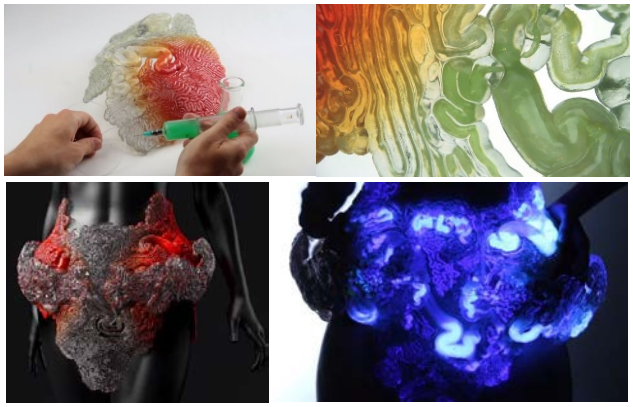


Image. [137]. ***3D printing and inoculation of the wearable device*** **[459]•. Mushtari wearable design by Mediated Matter Lab–MIT (Neri Oxman).**

• **[459] https://www.media.mit.edu/projects/living-mushtari/overview.**
• **Ibid.**
• **Ibid**
• **Ibid**
• **Ibid**

[4.2.1.1] Cellular simulation parametric software

Multi-cellular systems are inherently of multi-scale nature. The research in systems biology simulation dedicated to time study is directed towards whole cells or populations of cells to complement sequence analysis simulation, gene expression profiles, research on signal transduction pathways and the analysis of biochemical systems. The steadily increasing computational power of modern processors today permits the simulation of up to several millions of cells by agent-based models provided an efficient implementation especially in three dimensions.[460]•

Modern cell based parametric simulation soft wares enable Cell–cell and cell–matrix interaction that are mimicked by the experimentally validated models that summarizes deformation, compression and adhesion forces, and displays hysteresis if cells move close to or away from one another. For instance, model cells are parameterized by the Young modulus (defined by the relation(10.03) σl = Eel Thus E is the ratio of longitudinal stress, σl, to the longitudinal strain el) [461]•, the Poisson ratio (defined as the ratio of the change in the width per unit width of a material, to the change in its length per unit length, as a result of strain [462]•), and the density of membrane receptors responsible for cell–cell and cell–substrate adhesion and by its intrinsic radius. Proliferating cells double volume and deform into dumbbells after a given number of time steps before splitting into two daughter cells.

[4.2.2] Scale (fractal dimension) (L-systems / Cellular automata): Scale is a main attribute in controlling form design of bioactive hybrids specifically and devices as well. Scale in bioactive design depends on formal cluster proliferation in fractal wise. Thus, designing any bioactive hybrid or device must be directed towards clustering its formal properties to enable repetition in different configurations of this cluster. This criterion is strongly dependable and related to ***Mass customization***: as mass customization and manipulation specify design methods that control form clustering and proliferation.

Bioactive scale design includes the **Fractal dimension controller;** a term that is previously mentioned to describe the measuring unite of fungi hyphal growth. Here it represents a measuring tool to measure the bioactive design ability to proliferate in spatial dimensions around 360 ^{0} degrees (not just X, Y, Z). Consequently, this controller depends on various spatial relations between clusters (juxtaposition, interpolation, cross sectioning, repulsion, etc.). These spatial relations are further specified in mass customization.

[4.2.3] ***Mass customization (planar, spatial)***

[4.2.3.1] Bio-receptive vs. bioreactor: (enclosure/properties)

Formal limitations in bioactive reactors (devices)

Limitation in mass customization in bioactive devices stems from the fact that they depend on insulation, enclosure and separation between the bioactive agent, the host, and the environment taking the form of bioreactors. These bioreactors ensure the maintenance of certain growth conditions for different organisms that vary in their capacity of persistency in open environment as well as their opportunity to pathogenicity. This is achieved by maximum enclosure and isolation from outer conditions, and to maintain certain temperature, pH, aeration status (aerobic or anaerobic) and certain concentration of media substrates. *Although bioreactors achieve maximum safety in dealing with*

• **[460] S. Hoehme, D. Drasdo, A cell-based simulation software for multi-cellular systems, Systems biology Advance, 2010.**
• **[461] https://www.sciencedirect.com/topics/materials-science/youngs-modulus.**
• **[462] https://www.sciencedirect.com/topics/materials-science/poisson-ratio.**

microorganisms it hinders formal potentials in mass customization and spatial propagation aspects. In addition to the need for automation design or user interference in the system in case of expanding the system or adjust it according to emerging new needs or sudden environmental conditions. Bioreactors also require specific design for each function, which is sometimes complicated when it comes to supplies, fixtures, and circulation design.

The formal limitations of a bioreactor are as follow:

- ***Optimum enclosure*** in bioreactor design including tight control over inlets of growth medium and outlets of the exhausted medium to ensure the circulation efficiency.
- ***Orientation limits,*** as bioreactors need to be adjusted horizontally in a stable configuration as mechanical forces affect physio and biochemical reactions that occur on sub cellular scale.
- ***Spatial propagation limitations,*** the bioreactor sensitivity to mechanical forces implies the deficiency in orientation variations, thus limiting spatial orientation iterations to the least that could achieve stability of the bioreactor. Moreover, spatial propagation and orientation in bioreactors depend almost entirely on the shape of the device that defines the proliferation (fractal grid) and the axes of proliferation as well. As the bioreactor design affects the physiochemical processes that controls the various ecologic functions that it perform, varying the bioreactor shape is limited by many functional constraints resulting in few formal design iterations.

Formal potentials in bioactive hybrids (bio-receptive hosts + bio integrated hosts)

On the other hand, the concept of bio-receptive hosts varies in range from engineered materials that augment specific characteristics and abilities in the bio-agent, for example to be nonpathogenic, that the bioactive agent can grow in open air, and can resist changing environmental conditions (i.e. temperature). To bio-receptive, surfaces that only form a passive host to bio agent. These bio-receptive surfaces are hard textured surfaces of biocompatible materials that do not react or biodegrade by the bio-agent and enables it from colonizing its surface by attaching the bio-agent to its niches. Bio-receptive surfaces limit the functional application that exploits natural potentials of different useful organisms to those who are the simplest and do not include sophisticated physiological processes that produce toxic chemicals or impose any hazards. Bio-receptive surfaces are also limited in functional customization and shifting, unlike ***bio-integrated hosts or bio engineered materials (bio printed materials)***. The bio-integrated hosts are bio designed and engineered materials (usually by genetic manipulation and insertion). These materials are designed according to specific ecological functions that is integrated with the type and function of the bio-agent species, thus achieving the most compatibility and integration of the bioactive hybrid enabling naturally inherited smoothness in functional and formal transitions with maximum freedom and infinite iterations while maintaining the function and safety criteria.

[4.2.3.2] Planar manipulation (bioactive devices)

Patterning the functional clusters: This controls the method of utilizing bioactive clusters to build a global form. This is strongly related to scale.

In designing bioactive devices, each cluster is either a ***differentiated pixel*** as a part in the global form (pixels completing a global form), or a ***uniform pixel*** (fractal) that is repeated in the global form.

either way the repetition or tessellation method is the pivotal attributes in controlling the resulting form, tessellation method depends mainly on the following:

- ***Pixel's shape (form) (dimension /orientation/relation):*** Pixel's outer shape, edges, total dimension, and orientation affects the global form and its visual conception. Pixel's edges define its joinery method and defines the formal transition between multiple pixels in accordance to scale and population (count), as this edge line will build with other edges a visually defining line in the final form. Besides pixel's form define its ability of repetition or integration with other pixels thus pixel's form relates the first step of design to the final global design and must be considered integrally along the design process.
- ***Tessellation layers:*** This aspect focuses on the consequent organization of tessellation groups or layers. This aspect controls the tessellation axis, as both aspects control the global form in space, and affects its orientation, its perception, stability and continuity in space. Designer can define tessellation layers integrally with tessellation direction of growth (propagation) axes.
- ***Tessellation axis (rail, orientation):*** Tessellation axes is the orientation of the repeated pixels or clusters along a specific path in space. This path is a vector that have a point of start and a direction but should have an open or growing end enabling adding more clusters in future. This open-ended path should maintain direction and orientation in building successive rows of joined pixels; this could be a challenging aspect in doubly curved, twisted, tangled and kinked paths. Maintaining direction in future added clusters would face functional path alteration (functional demand implies a different path than the predefined designed one). Thus, tessellation axes must contain flexibility to be redefined in space in more than one rail (at least 3 different rails) to enable reorientation in case of sudden changes in environmental conditions or user demands. This flexibility in tessellation axes is achieved according to tessellation cluster dimensions, shape and joinery system.
- ***Tessellation joinery:*** Tessellation joinery system controls a lot of formal and functional attributes as well;
    a) ***Tessellated tiles joinery flexibility according to rail (axes):*** The tiles or clusters should be designed to fit to at least three different configurations of joinery systems in spatial orientation according to the predefined simulated rail iterations that resulted from functional (environmental +user demands) simulation.
    b) ***Tessellated tiles joinery flexibility according to layers:*** Joinery system design should be flexible also according to added layers (in future according to demand or environmental need). Thus, support addition or subtraction of new layers that compose the global form.
    c) ***Tessellated clusters functional flexibility in bioactive system expansion or subtraction:*** This aspect focuses on functional joinery of system infrastructure, suppliers and duct. This is the most important aspect that guarantee the sufficient functioning of the system and achieving flexibility in this aspect

implies the usage of prefabricated flexible parts that can be easily installed in space by user.

[4.2.3.3] Spatial manipulation, propagation and orientation: **(mass customization) (bioactive hybrids and bioactive devices) related to *time and scale*.**

- ***Patterned/ pixeled customized mass:*** Customized mass design in this aspect focuses on adjusting formal design in pixeled fashion. How to pixelate the global formal mass into differentiated tiles, and how to differentiate tiles design in shape, dimension, joinery, layers, axes to achieve the required design form without hindering function or visual continuity of the form. This design process is the opposite of the tessellated planner manipulation as the first result in uniform tiles designed previously. The pivotal difference between the two design processes is that the first (planner manipulation) focuses on the cluster uniform design and how it would be tessellated regardless of the final form (previously simulated to ensure functional and configurable sufficiency). The second (mass customization) focuses on the final form (mass), and how it could be pixeled or tiled with the functional cluster. This difference emerge from the defining nature of bioactive hybrid that carries the vital bioactive proliferation manner that implies diversity in clusters unlike the bioactive device that is limited by the host device (bioreactor) specifications and properties.
- ***Fluid mass (bioactive hybrids only): Axial growth –proliferation method –merging and emergence / morphogenesis:*** Fluid mass is achieved by ultimate bioactive hybrids that behave in natural cellular growth, proliferation and morphogenesis manner. Thus, the resulting mass is the bioactive hybrid through its life cycle in different phases. This bioactive hybrid mass is divers and unconstrained in spatial configuration except by the bioactive hybrid's phenotypes and genotypes. Although the growth mechanism and form mechanics is entirely dependent on the bioactive agent behavior in growth (bacteria, fungi, etc.); the author proposes some criteria to rule formal composition of the resulting mass and volume.
    - ***a) Proliferation axes (axial growth):*** axial growth defines the growth vectors in space; it may be one or more. This is important in designing spatial propagation and orientation in functional and formal aspects as well. Mathematical models that analyze cellular behavior in growth (of the bioactive agent) could simulate this.

An example is provided by The Wanderers project of wearable design by MIT Mediated Matter Lab; the project employs cellular proliferation simulation to propose various growth iterations to be utilized in fashion design. Each wearable is designed for a specific extreme environment where it transforms elements that are found in the atmosphere to one of the classical elements supporting life: oxygen for breathing, photons for seeing, biomass for eating, biofuels for moving, and calcium for building. Some photosynthesize converting daylight into energy, others bio-mineralize to strengthen and augment human bone, and some fluoresce to light the way in darkness. Design research the intersection of multi-material 3D printing and Synthetic Biology. [463]•.

• **[463] https://www.media.mit.edu/projects/wanderers/overview**

Proliferation axes iterations:

- ***Polar /lateral***

Image. [138]. Time lapse of polar/ lateral proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463][•].

- ***Axial –orbital***

Image. [139]. Time lapse of axial- orbital proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463].

- ***Longitudinal –lateral***

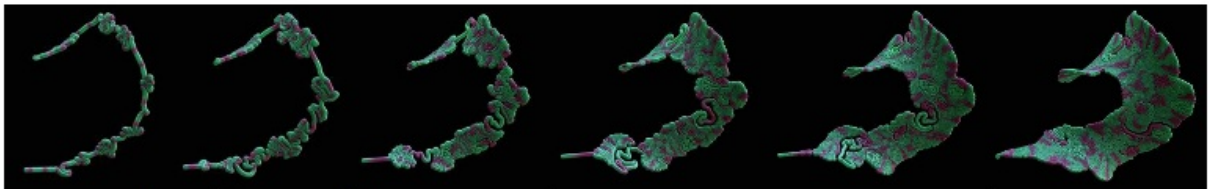


Image. [140]. Time lapse of longitudinal- lateral proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463].

- ***Polar –orbital***

Image. [141]. Time lapse of polar-orbital proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463].

- ***Aerial –longitudinal –orbital***

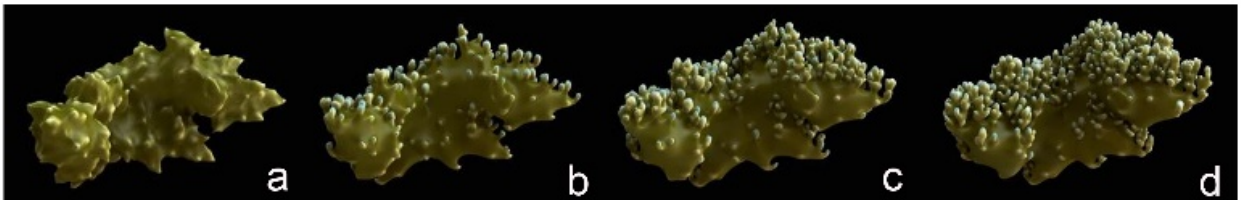


Image. [142]. Time lapse of aerial-longitudinal-orbital proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463].

[•] **Ibid.**

- ***Collateral –sectional***

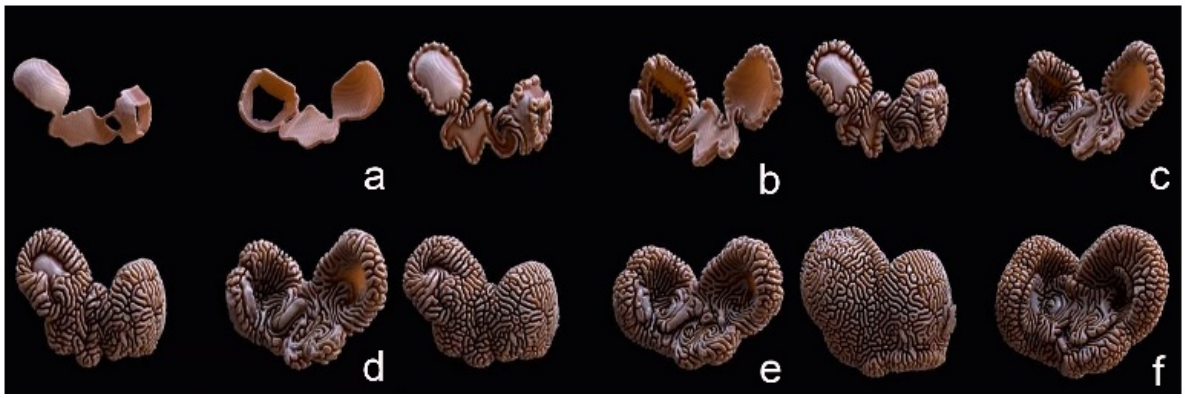
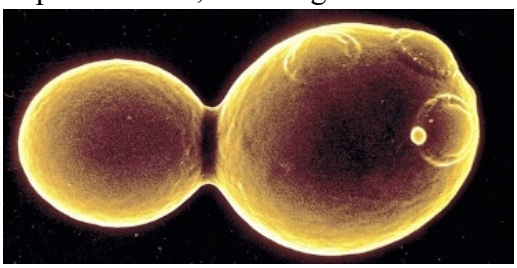
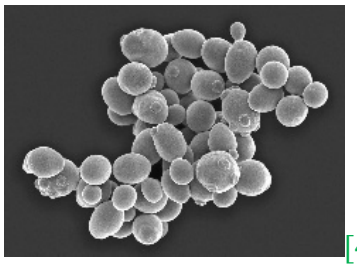


Image. [143]. Time lapse of collateral-sectional proliferation axes of cellular growth, Wanderers project of wearable design by MIT Mediated Matter Lab. [463].

b) ***Proliferation method (formal): budding, branching:*** Proliferation methods are defined by the bioactive agent method of cellular proliferation; thus, it varies according to different species. However, there are common methods that is employed by most of living species and could be utilized in proliferation of bioactive design hybrids, as previously mentioned, budding and branching. As explained previously branching is the method utilized by fungi to form mycelium as a growth and functional spatial propagation method employed by bioactive hybrids. [464]•.

Branching as a formal mass customized attribute affects spatial propagation method and focuses on different patterns resulting from branching according to adjusting to environmental conditions and inner demands strategies as previously explained.

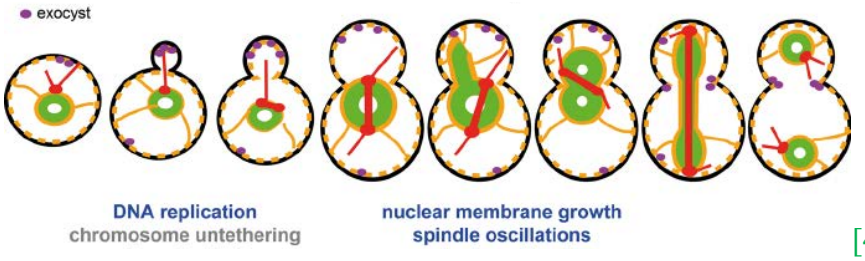
Budding is a type of asexual reproduction in which a new organism develops from an outgrowth or bud due to cell division at one particular site. The small bulb like projection coming out from the parent cell is called a bud. The new organism remains attached as it grows, separating from the parent organism only when it is mature, leaving behind scar tissue. Since the reproduction is asexual, the newly created organism is a clone and is genetically identical to the parent organism. [465]•. ***Budding forms control the final mass texture, as the resulting from depends on the initial parent cell shape, form and volume***, (for example, when considering variation in bacterial cells shape and applying budding method in proliferation, the designer could obtain infinite iterations.)

[466•; 467]•

Image. [144; 145]. Budding Yeast-yeast budding cells.

• **[464] D. J Moore, et al., Branching in Fungal Hyphae and Fungal Tissues: Growing Mycelia in a Desktop Computer, Branching Morphogenesis, 75-90, 2004.**
• **[465] https://en.wikipedia.org/wiki/Budding#cite_note-smyth-1**
• **[466] http://biology-pictures.blogspot.com/2011/10/budding-yeast-picture.html**
• **[467] https://www.britannica.com/science/yeast-fungus/media/652395/203798**



[468]•

Figure. [79]. Model of events during nuclear migration/spindle positioning in budding yeast. Early in the cell cycle, the nucleus is moved close to the bud neck. After entry into mitosis, replication starts and the nuclear membrane expands. Dynein-dependent spindle oscillations pull part of the nuclear envelope into the bud, as it continues to proliferate. Eventually, dynein pulls the spindle and the DNA mass into the bud neck, creating a dumbbell nucleus.

In conclusion mass customization methods (budding, branching) in bioactive hybrids, coupled with proliferation axes and initial cluster form can produce infinite iterations in final mass forms and textures.

c) ***Morphogenesis (formal):*** Morphogenesis in bioactive hybrids occurs synchronously with cellular proliferation, the new cells emerge into various tissues. The defining aspect that differentiate the resultant tissue formal type depends on a group of parameters:

- *The parent cell form and texture.*
- *The proliferation (growth) axis or axes.*
- *The spatial relations between the cellular generations (proliferation method).*
- *The emergence rule that control cellular proliferation:* This aspect controls the step following proliferation ***according to the ratio between available spatial margins for proliferation and the cellular population (scale) needed*** *(according to environmental conditions and user needs). The emergence rule is also controlled by time (proliferation time) the faster it takes the larger population it produces. Thus controlling this aspect is complicated and demands* a highly synchronized and tight design to control all these related parameters.
- ***Emergence (transformation type):*** This includes the physical forces involved in reforming the new (resultant) tissue; such as fusion, overlapping, contiguity and juxtaposition of cells. These scenarios of morphogenesis could be simulated by mathematical models with the help of computational design tools that control the large processed data involved in these models. It's worth mentioning that employing such methods while varying the previously mentioned parameters can produce infinite number of iterations for the bioactive hybrid design which implies that the real-life bioactive hybrid would develop to various different forms and tissues according to computational calculation based on the environmental simulations and the users' changing demands.

[4.3] Fabrication methods

Achieving maximum coupling between functional and formal customization in time and space in sync and according to changing demands and environmental conditions need very specific qualities in both bioactive hybrids and devices. This flexibility and morphogenesis in real time could be achieved by employing bioactive material bioengineering (bioprinting) or self-building architecture.

• **[468] D. Liakopoulos, An auxiliary, membrane-based mechanism for nuclear migration in budding yeast-*Marisa Kirchenbauer*, Molecular biology of the cell, 24, 9, 2013. https://www.researchgate.net/figure/Model-showing-events-during-nuclear-migration-spindle-positioning-in-budding-yeast-Early_fig7_235750939.**

To fabricate the bio-engineered or bio printed materials in pilot scale, 3D bioprinters are scale up and developed to sort of robotic fabrication by KUKA-**Robots, the fabrication process programming with Kuka algorithmic software make it easier to build different free and complex formed bioactive designs extracted from biological behavioral patterns. One example is the** KUKA|prc plug in which is a set of Grasshopper components that provide procedural robot control for KUKA robots (PRC). These components are straightforward to use and to program the robots using them. [469]•.

Robotic modeling -Motion Types: KUKA provides several motion types. These are Point-to-Point, Linear, Circular, or Spline. [469]•.

Robotic fabrication by KUKA Extrusion printing arms: Robotic 6-Axis 3D Printing, developed by Lei Yu (ASW, LCD, Tsinghua University), Shanghai. 2014. For the robotic extrusion that is based on robotic 6-axis 3D printing, which is a highly integrated installation combining robotics, 3D printing technique and interactive interface.[470]•

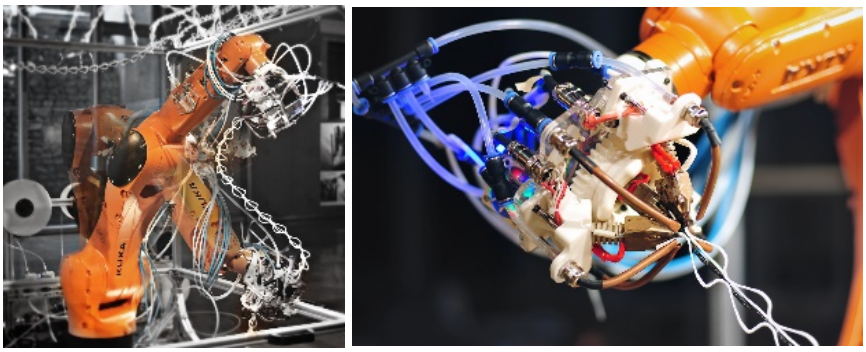


Image. [146; 147]. Robot printing tool detail inspired from spiders web generation process.

Self-building architecture could mainly be employed in bioactive hybrids that perform full featured morphogenesis based on complicated feedback and neural networks determining the growth specifications. It is also possible to employ this strategy in bioactive devices for building the propagation of the system. In this scope, ***self-building architecture is defined by the author as the architecture that can build itself as it has embedded robotic parts (systems) that can regenerate itself (such as the early model of Von Neumann's self-replicating cellular automata).*** These robotic architectural machines are designed according to their specific case of application, for example, part of these robots could utilize 3D printing for various host materials or utilize winding and weaving robots. The use of these robotic parts in the bioactive hybrid is a combination between the hybrid agents' natural intelligence and the artificial intelligence that is based on their behavior.

An example of such self-replicating system is exhibited in **FIBERBOTS** project, which is the design of a multi-agent, fiber composite digital fabrication system. The FIBERBOTS developed by the Mediated Matter group at the MIT Media Lab, is a digital fabrication platform fusing cooperative robotic manufacturing with abilities to generate sophisticated material architectures. The platform can enable design and digital fabrication of large-scale structures with high spatial resolution leveraging

• **[469] https://mkmra2.blogspot.com/2019/01/robot-programming-with-kukaprc-v3.html**
• **Ibid.**
• **[470] https://www.behance.net/gallery/22536831/ROBOTIC-EXTRUSION(6-Axis-KUKAABS-3D-Printing)**

mobile fabrication nodes, or robotic "agents" designed to adjust the material make-up of the structure being constructed in real time as informed by their environment. [471]•.

Image. [148]. The FIBERBOTS project.

The project is composed of a swarm of robots designed to wind fiberglass filament around themselves to create ***high-strength tubular structures***. These structures can be built in parallel and interwoven to rapidly create architectural structures. ***The robots are mobile, using sensor feedback to control the length and curvature of each individual tube according to paths determined by a custom, environmentally informed, flocking-based design protocol***. This gives designers the ability to control high-level design parameters that govern the shape of the resulting structure without needing to manually provide commands for each robot. The 16 robots, including the design system to control them, were developed and deployed to autonomously create a 4.5m-tall structure. [471]•.

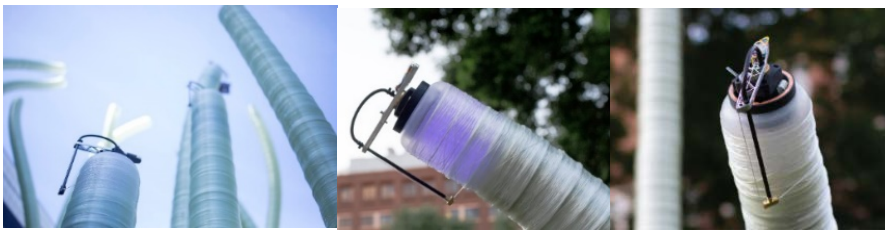


Image. [149]. The FIBERBOTS robot winding head detail.

This method is based on ***swarm sensing and actuation, systems become responsive and adaptive to environmental conditions in real time.*** This FIBERBOTS project develops fabrication units capable of being highly communicative while simultaneously depositing tailorable, multifunctional materials. [472]•.

• [471] M. Kayser, et al., Design of a multi-agent, fiber-composite digital fabrication system, Sci. Robot, 2018. https://link.springer.com/chapter/10.1007/978-3-319-92294-2_22.

• Ibid

• [472] https://www.media.mit.edu/projects/fiberbots/overview

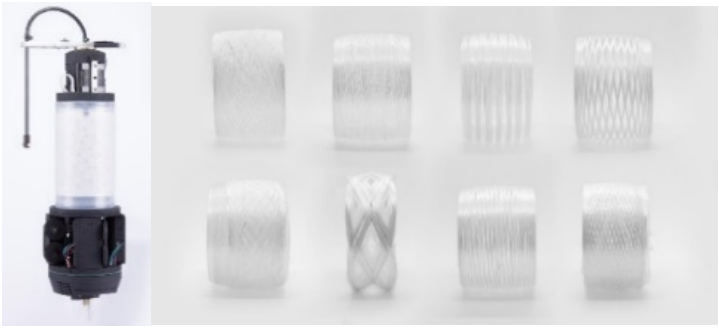


Image. [150]. The FIBERBOT project robotic parts for the winding process in real time building achieving maximum responsiveness to environmental conditions and liberating formal features accordingly.

Bioactive material syntheses (bio-engineering / bio-printing)

Three-dimensional bioprinting is the utilization of 3D printing techniques to combine cells, growth factors, and biomaterials were originally to fabricate biomedical parts that maximally imitate natural tissue characteristics. [473]•. Generally, 3D bioprinting utilizes the layer-by-layer method to deposit materials known as bioinks to create tissue-like structures that are later used in medical and tissue engineering fields. Bioprinting covers a broad range of biomaterials. [474]•. However, emerging innovations span from bioprinting of cells or extracellular matrix deposited into a 3D gel layer by layer to produce the desired tissue or organ. In addition, 3D bioprinting has begun to incorporate the printing of scaffolds. [475]• that were normally produced to regenerate joints and ligaments. [476]•.

Pre-bioprinting: *Pre*-bioprinting is the process of creating a model that the printer will later create and choosing the materials that will be used. One of the first steps is to obtain a sample (biopsy) of the organism. Common technologies used for bioprinting of usually full human organs are ***computed tomography (CT)*** and ***magnetic resonance imaging (MRI)***. ***To print with a layer-by-layer approach, tomographic reconstruction is done on the images. The 2D images are then sent to the printer to be made. Once the image is created, certain cells are isolated and multiplied***. [477]•. ***These cells are then mixed with a special liquefied material that provides oxygen and other nutrients to keep them alive. In some processes, the cells are encapsulated in cellular spheroids 500µm in diameter. This aggregation of cells does not require a scaffold, and are required for placing in the tubular-like tissue fusion for processes such as extrusion***. [478]•.

In the second step, the liquid mixture of cells, matrix, and nutrients known as bioinks are placed in a printer cartridge and deposited using the scans. [479]•. ***When a bioprinted pre-tissue is transferred to an incubator, this cell-based pre-tissue matures into a tissue***. [475]•.

Normally, 3D bioprinting for fabricating biological constructs typically involves dispensing cells onto a biocompatible scaffold using a successive layer-by-layer approach to generate tissue-like three-

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• **[473] D. Singh, D. Thomas, Advances in medical polymer technology towards the panacea of complex 3D tissue and organ manufacture, American Journal of Surgery, 2018.**

• **[474] T. J. Hinton, et al., Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels, *Science Advances*, 1, 9, 2015.**

• **[475] D. J. Thomas, Could 3D bioprinted tissues offer future hope for microtia treatment?, *International Journal of Surgery*, 32, 43–44, 2016.**

• **[476] Y. Nakashima, et al., Bone and Joint Diseases in Present and Future. Fukuoka Igaku Zasshi = Hukuoka Acta Medica, 108, 1, 1–7, 2017.**

• **[477] A. Shafiee, A. Atala, Printing Technologies for Medical Applications, *Trends in Molecular Medicine*, 22, 3, 254–265, 2016.**

• **[478] C. K. Chua, W. Y. Yeong, *Bioprinting: Principles and Applications*, Singapore: World Scientific Publishing Co, 296, 2016.**

• **[479] M. Cooper-White, How 3D Printing Could End The Deadly Shortage Of Donor Organs, *Huffpost Science*, TheHuffingtonPost.com, Inc. 2016.**

• **[475] D. J. Thomas, Could 3D bioprinted tissues offer future hope for microtia treatment?, *International Journal of Surgery*, 2016.**

dimensional structures. [480]•. *Given that, every tissue is naturally composed of different cell types, many technologies for printing these cells vary in their ability to ensure stability and viability of the cells during the manufacturing process. Some of the methods that are used for 3D bioprinting of cells are* ***photolithography, magnetic bioprinting, stereo lithography, and direct cell extrusion***. [478]•.

Some of the primary benefits of 3D printing lie in its capability of mass-producing scaffold structures, as well as the high degree of anatomical precision in scaffold products. This allows for the creation of constructs that more effectively resemble the microstructure of a natural organ or tissue structure. [481]•.

- ***Bioprinting approaches:*** 3D bioprinting is based on three main approaches: Biomimicry, autonomous self-assembly and mini-tissue building blocks. [482]•.

a) Biomimicry: The main goal of this approach is to create fabricated structures that are identical to the natural structure that are found in the tissues and organs. Biomimicry requires duplication of the shape, framework, and the microenvironment of the organs and tissues. [483]•. ***The application of biomimicry in bioprinting involves creating both identical cellular and extracellular parts of organs. For this approach to be successful, the tissues must be replicated on a micro scale. Therefore, it is necessary to understand the microenvironment, the nature of the biological forces in this microenvironment, the precise organization of functional and supporting cell types, solubility factors, and the composition of extracellular matrix***. [482]•.

b) Autonomous self-assembly (future research field combining animal cells in design): This approach relies originally on the physical process of embryonic organ development as a model to replicate the tissues of interest. [483]•. ***When cells are in their early development, they create their own extracellular matrix building block, the proper cell signaling, and independent arrangement and patterning to provide the required biological functions and micro-architecture***. [482]•. Autonomous self-assembly demands specific information about the developmental techniques of the tissues and organs. [483]•. ***A scaffold-free model that uses self-assembling spheroids subjects to fusion and cell arrangement to resemble evolving tissues. Autonomous self-assembly depends on the cell as the fundamental driver of histogenesis, guiding the building blocks, structural and functional properties of these tissues. It demands a deeper understanding of how tissues mechanisms develop as well as the microenvironment surrounded to create the bioprinted tissues***. [482]•.

c) ***Mini-tissue:*** The third approach of bioprinting is a combination of both the biomimicry and self-assembly approaches, which is called mini tissues. [484]• Organs and tissues are built

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• **[480] K. Harmon, A sweet solution for replacing organs, *Scientific American*, 308, 4, 54–55, 2013.**
• **[478] C. K. Chua, W. Y. Yeong, *Bioprinting: Principles and Applications,* Singapore: World Scientific Publishing Co, 2016.**
• **[481] L. A. Hockaday, et al., Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds, *Biofabrication*, 4, 3, 2012.**
• **[482] S. Murphy, A. Atala, 3D bioprinting of tissues and organs, *Nature Biotechnology*, 32, 773–85, 2014.**
• **[483] J. Yoo, A. Atala, Bioprinting: 3D printing comes to life, *Manufacturing Engineering*, 2015.**
• **Op.cit.**
• **[483] J. Yoo, A. Atala, Bioprinting: 3D printing comes to life.**
• **Op.cit.**
• **[483] J. Yoo, A. Atala, Bioprinting: 3D printing comes to life.**
• **Op.cit**
• **[484] D. Thomas, D. Singh, Novel techniques of engineering 3D vasculature tissue for surgical procedures, *The American Journal of Surgery, 2018.***

from very small functional components. Mini-tissue approach takes these small pieces, manufacture and arrange them into larger framework. [483]•.

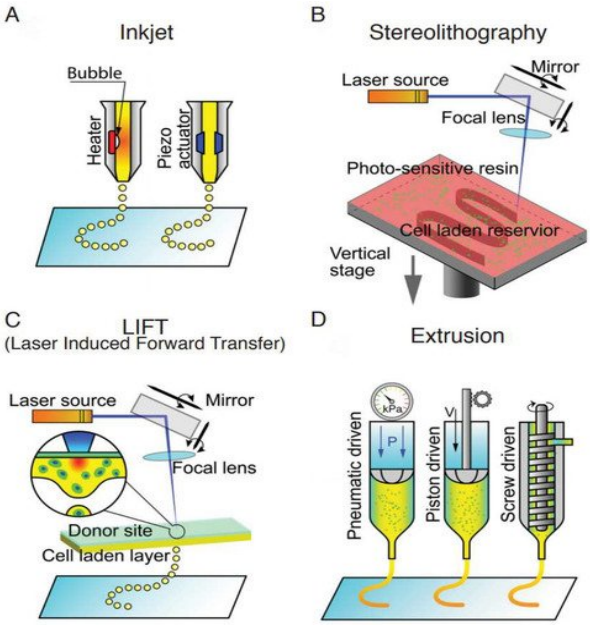

Figure. [80]. Schematic of various 3D printing methodologies. (A) Inkjet. A heater or piezo actuator deposits droplets. (B) Stereolithography. Layer by layer photopolymerization of a liquid resin by laser. (C) Laser induced forward transfer. Droplets of the material induced by a laser source. (D) Extrusion. Material exiting a nozzle that is pneumatic, piston, or screw driven. [485]•.

Bioprinters have three major components. ***These are the hardware used, the type of bio-ink, and the material it is printed on (biomaterials)***. [477]•. ***Bio-ink is a material made from living cells that behaves much like a liquid, allowing printing living tissue in desired shape***. To make bio-ink, a slurry of cells is created that can be loaded into a cartridge and inserted into a specially designed printer, along with another cartridge containing a gel known as bio-paper.[486]•. In bioprinting, there are three major types of printers. ***These are inkjet, laser-assisted, and extrusion printers***. Inkjet printers are mainly used in bioprinting for fast and large-scale products. One type of inkjet printer, called ***drop-on-demand inkjet printer***, prints materials in exact amounts, minimizing cost and waste. [487]•.

Printers that utilize lasers provide high-resolution printing; however, these printers are often expensive. Extrusion printers print cells layer-by-layer, just like 3D printing to create 3D constructs. [488]•. ***3D printing allows for the layer-by-layer construction of a particular tissue structure to form a cell scaffold. This can be followed by the process of cell seeding, in which cells of interest are pipetted directly onto the scaffold structure***. ***Additionally, the process of integrating cells into the***

• **[483] J. Yoo, A. Atala, Bioprinting: 3D printing comes to life, *Manufacturing Engineering*, 2015.**
• **[485] T. jiang, et al., Extrusion bioprinting of soft materials: An emerging technique for biological model fabrication, *Appl. Phys. Rev*, 2019**
• **[477] A. Shafiee, A. Atala, Printing Technologies for Medical Applications, *Trends in Molecular Medicine*, 22, 3, 254–265, 2016.**
• **[486] J. J. Manappallil, Basic Dental Materials, JP Medical Ltd, 2015.**
• **[487] 3D Printing Technology At The Service Of Health", *healthyeve*, 2016.**
• **[488] Z. Ali, et al., Development and analysis of a 3D printed hydrogel soft actuator, *Sensors and Actuators A: Physical*, 2017.**

printable material itself, instead of performing seeding afterwards, has been explored. [482•; 489]•. In addition to just cells, extrusion printers may also use hydrogels infused with cells. [477]•.

Drop-based bioprinting (Inkjet): Drop-based bioprinting creates cellular constructs using individual droplets of a designated material, which has oftentimes been combined with a cell line. ***Upon contact with the substrate surface, each droplet begins to polymerize, forming a larger structure as individual droplets begin to coalesce. Polymerization is activated by the presence of special ions on the substrate, which diffuse into the liquefied bioink and allow for the formation of a solid gel***. Drop-based bioprinting is commonly used due to its efficient speed, though this aspect makes it less suitable for more complicated organism structures. [490]•.

Extrusion bioprinting: Extrusion bioprinting involves the constant deposition of a particular printing material and cell line from an extruder, a type of mobile print head. This tends to be a more controlled and gentler process for material or cell deposition, and allows for greater cell densities to be used in the construction of 3D tissue or organ structures. However, such benefits are set back by the slower printing speeds entailed by this technique. Extrusion bioprinting is often coupled with UV light, which photo-polymerizes the printed material to form a more stable, integrated construct. [491]•.

Printing materials: Materials for 3D printing usually consist of ***alginate or fibrin polymers*** that have been integrated with cellular adhesion molecules, which support the physical attachment of cells. Such polymers are specifically designed to maintain structural stability and be receptive to cellular integration. The term "bioink" has been used as a broad classification of materials that are compatible with 3D bioprinting. [492]•.

Printing materials must fit a broad spectrum of criteria, one of the foremost being ***Biocompatible***. The resulting scaffolds formed by 3D printed materials should be physically and chemically appropriate for cell proliferation. ***Biodegradability*** is another important factor, and insures that the artificially formed structure can be broken down upon successful embedding, to be replaced by a completely natural cellular structure. Due to the nature of 3D printing, materials used must be customizable and adaptable, being suited to wide array of cell types and structural conformations. [493]•.

Hydrogel alginates are one of the most commonly used materials in tissue printing research, as they are highly customizable, and can be fine-tuned to simulate certain mechanical and biological properties of natural tissue. The ability of hydrogels to be tailored to specific needs allows them to be used as an adaptable scaffold material, which are suitable for a variety of tissue structures and physiological conditions. [491]•. ***In the case of bioactive hybrids, it is useful to employ alginate as it has stability and slow degradation, which makes it persistent to environmental conditions until the full maturation of the bioactive hybrid***. It is worth mentioning that the slow degradation of

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• [482] S. Murphy, A. Atala, 3D bioprinting of tissues and organs, *Nature Biotechnology*, 32, 773–85, 2014.
• [489] Z. Ali, et al., 3D printed hydrogel soft actuators, *Region 10 Conference (TENCON), 2016*.
• [477] A. Shafiee, A. Atala, Printing Technologies for Medical Applications, *Trends in Molecular Medicine*, 22, 3, 254–265, 2016.
• [490] F. A. Auger, et al., The Pivotal Role of Vascularization in Tissue Engineering, Annual Review of Biomedical Engineering, 15, 177–200, 2013.
• [491] P. Bajaj, et al., 3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine, *Annual Review of Biomedical Engineering*, 16, 247–76, 2014.
• [492] M. Kesti, et al., A versatile bioink for three-dimensional printing of cellular scaffolds based on thermally and photo-triggered tandem gelation, *Acta Biomaterialia*, 11, 162–72, 2015.
• [493] A. D. Augst, et al., Alginate Hydrogels as Biomaterials, *Macromolecular Bioscience*, 6, 8, 623–33, 2006.
• [491] P. Bajaj, et al., 3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine, *Annual Review of Biomedical Engineering*, 16, 247–76, 2014.

alginate is considered as a draw back in other applications that demand the artificial gel scaffolding to be broken down and replaced with the implanted cells' own extracellular matrix. [494]•. Alginate hydrogel that is suitable for extrusion printing is also often less structurally and mechanically sound; however, this issue can be mediated by the incorporation of other biopolymers, such as nano cellulose, to provide greater stability. The properties of the alginate or mixed-polymer bioink are tunable and can be altered for different applications and types of tissues. [494]•.

[4.4] Built environment evolution

Furniture /Interior /Architecture /urban growth

The aim of bioactive design is to redefine ecologic architecture to be a continuously evolving and active part of natural environment and ecosystem. This extends beyond interior design to include architecture and urban design, the aim is to emerge a mega city that is based on biological communities resulting from a symbioses relation between human, built environment and the ecosystem.

The continuous evolving open system of bioactive design allows integration of natural capabilities of organisms with human needs for various functional practices, thus this bioactive system is liable to rapid and accurate growth according to demand and as a response for environmental changing conditions.

- ***Accurate aggregation by "cluster flexibility***": Bioactive design based on cellular proliferation bases that enable the aggregation of clusters as elements of design to form the interior space, architectural mass and the whole urban context. This clusterization ensures flexibility, reproducibility, sources management and a very accurate exploitation of its functionality (cluster's specific function) to be added precisely on demand and exactly where it is needed.
- ***Independent installation system:*** Clusterization achieved by bioactive design cellular bases also enables the cluster independency in installation processes from the mega infra structure of the architecture or urban context, as each cluster is fully equipped and functional in its own having the ability to proliferate according to demand and environmental conditions. (In bioactive hybrids case through environmental responsiveness and evolution (organizing genotypes that are controlling phenotypes of the bioactive hybrid to enable growth and proliferation or by providing feedback where the proliferation is needed in case of bioactive devices). This installation independency corresponds to achieving this research goal and proves the hypothesis that embedding microorganisms in design is more accurate and advanced clean renewable energy source.

[5] NYKA 510 Bioactive Light Garden.

- **Design concept & objectives:** the design is a self-sufficient power system for bioelectricity generation for domestic use achieving ecology of space by employing Microbial fuel cells that exploits natural electro geneses of certain fungal strain ***Aspergillus sydowii*** NYKA 510.

This design aims to:

- **Achieve coupling between form and function as well on the bases of cellular behavior complying with the bioactive interior design criteria reached by the author.**

• **[494] E. Axpe, M.L. Oyen, Applications of Alginate-Based Bioinks in 3D Bioprinting, *International Journal of Molecular Sciences*, 17, 12, 2016.**

• **Ibid**

- **Apply cellular automata and agent based mathematical simulation and modeling methods in extracting behavioral intelligence, exhibited by fungal hyphae in searching nutrients with specific concentrations for growth and chemotaxis.**

As exhibited in chapter 4 the author experimented on Air Cathode Membrane less Single Chamber Microbial Fuel Cell fed by the filamentous fungi strain *Aspergillus sydowii* NYKA510 and optimized media. From the statistical results obtained, a system of 8 cells connected in series configuration produced 1.5 Watt after 7 days of incubation (***optimum efficiency-time criteria***) and sustained this performance for 4 days.

The author exploited these results in maximizing the gained electrical power by doubling the 8 cells system to a 16 cells system, with each group of 8 cells connected in series separately (to avoid power loses due to current reversal). The 16 cells system produces ***3 Watt***. This design was clustered in a compact system with all required circulation suppliers and ducts encapsulated in it, identifying inputs of fresh supplies and output of exhausted media.

The bioactive cluster was then embedded in two designs iterations employing a cellular form that emerged from cellular automaton simulation of fungal hyphae chemotaxis.

Design Methodology

- **Mathematical model**

The author employed parametric simulation software (Rhino 3D +Grasshopper –Biods plugin) to simulate the cellular behavior of *Aspergillus sydowii* NYKA 510 in the ***oxidation-reduction reaction in air cathode membrane less single chamber microbial fuel cell to generate bioelectricity***. ***The oxidation-reduction reaction occurs when the fugal cells oxidize the carbon source in the media, resulting in fungal growth and formation of hypha inside the MFC and generating electrical current as explained in chapters 3, 4. Thus simulating the bioelectrical behavior is achieved by simulating the process of searching for and reacting with carbon source in the growth media***. This intelligent behavior direct the hyphal formation in search for nutrients to regions of optimum concentration.

In this research and according to experimental procedures conducted and statistical results exhibited in chapter 4, simulating the biological processes involved in oxidation-reduction reaction requires defining the main controlling constraint, which is the Carbon source, the banana peel at concentration of 15.1 g/l.

As exhibited previously in chapter 5, the filamentous fungal hyphae exhibit a ***biased random walk. By definition Biased random walks*** result in the derivation of a ***master equation in which the rate of change of the value of a system variable at a given point is related via transition probability rates (movement probability rates), to the values of this variable at a number of neighboring points.***

This is explained by the following hyphal intelligence strategies:

As exhibited previously of fungal mycelia property of distributed intelligence. The rules that controls the colony are local but lead to patterns on a large-scale. This is represented by ***cellular automat to describe the spatial dynamics of physical, chemical or biological processes occurring in the fungal hyphae***. [267]•.

• **[267] J. M. Halley, et al., Competition, succession and pattern in fungal communities: towards a cellular automaton model, OIKOS, 70, 435-442, 1994.**

- ***Translocation:*** As mentioned previously, the two different translocation mechanisms responsible for nutrient reallocation in fungal strains are simple ***diffusion and the active movement of intracellular metabolites from regions of local excess to regions of local scarcity***. Only newly formed hyphae use active translocation, while older, established hyphae use diffusion as the major means of internal nutrient reallocation. [265]•. As mentioned previously these translocation mechanisms align with the two distinct growth phases; **exploration and exploitation**. ***The exploration phase is adopted in low-nutrient conditions and features fast moving hyphal tips, the exploitation phase is adopted in high-nutrient conditions and features slower moving hyphal tips, resulting in a dense mycelial network***. Changes in the active translocation process *alone* account for the switch between exploration and exploitation phases. [265]•.
- **Chemotaxis:** Microorganisms moves towards higher concentrations of nutrients-chemo attractants and away from toxins chemo repellents. Chemotaxis by cells offer a mechanism for aggregation, either to a pacemaker or to a self-organized common center. If the cells secrete a chemoattractant, then a random fluctuation will cause local chemoattractant concentration to increase, drawing in more cells and again increasing chemoattractant concentration in a positive feedback loop. Eventually the cells will all move into one or more compact clusters (depending on the range of diffusion of the chemoattractant and the response and sensitivity of the cells). [261]•. ***Each cell alternates between periods of running and periods of tumbling. The resulting motion is a random walk that samples the local environment. By contrast, when exposed to a gradient of chemoattractant these cells bias their random walk by tumbling less frequently when moving in the good direction and more frequently when moving in the bad direction***. Once a cell finds itself again in a uniform environment, it returns to the original tumbling frequency, even if the homogeneous level of attractant (or repellent) is significantly different from before-the sensory mechanism adapts to the new nominal environment (on a time-scale of minutes). [261•; 267•].

Forming mathematical equation

Master biased random walker equation

- ***Motion type****:* fungal cells exhibit initial free 3D spatial searching inside the liquid medium space (the MFC volume) for the carbon source molecules, then these cells begin to sense the chemo traction caused by carbon source molecules optimum concentration, this biased vectored motion defines two spatial domains:

- ***Polar domain:*** *(Around 360 ^{0}surrounding the fungal cell), defines the initial free search in the liquid medium.*

• **[265] G. P. Boswell, et al., The Development of Fungal Networks in Complex Environments, Society for Mathematical Biology, Bulletin of Mathematical Biology, 69, 605–634, 2007.**

• **[265]**

• **[261] M. S. ALBER, et al., On Cellular Automaton Approaches To Modeling Biological Cells, https://www2.seas.gwu.edu/~simhaweb/iisc/Alber.pdf.**

• **[261]**

• **[267]**

- ***Vectored domain****: Under the chemo-physical attraction of the optimum concentration of carbon, source molecules that starts to bias the cells motion of searching behavior to more directed and vectored.*

- ***Constraint*** (chemoattractant): Banana peel concentration as the carbon source in growth media, statistical result = 15.1 g/l

  This constraint (slider) determines:

  1. Direction: vector of motion of carbon source molecules inside the liquid media space.
  2. This direction will lead the fungal cells (agents/ walkers) motion (attraction chemical) towards these molecules in order to perform the oxidation reaction.
  3. Carbon source molecules specific position in space.

- ***Parameters***

  a) Agents /walkers: These are the population of fungal cells inside the media. This parameter could be adjusted according to two objectives:

- Study the full population of the inoculum in medium; to obtain the whole colony behavioral pattern; this implies running simulation by varying the spatial position of the carbon source molecule while recording history, in order to animate the searching motion of the whole population in time to capture the over all in time behavioral pattern of the colony. This method will result in a large amount of computational data that won't be easy to handle in further design steps (according to hardware specifications), besides that it gives an approximate analysis of the real behavior, as it drowse the fact that fungal cells diffuse in media which implies the distribution of fungal population in medium space .
- Regional analysis (fungal cells distribution in media); this is a more realistic analysis as it simulates different population numbers randomly complying with the fact that fungal cells are not statically located in one spot in the medium, but are rather in continuous dynamism across different regions of it. This implies that fungal cells first move randomly in different regions of the medium. Thus, the author chose to randomly change the agents count with consideration to computational abilities available in this project (hardware specifications).

  b) ***Chemotaxis (searching) spatial domain***: Diffusion / repulsion reaction to molecule concentration, this parameter controls the fungal cells (agents) motion that result from two contradictory forces;

  - The diffusion (repulsion) behavior that causes the agents to wander freely in the medium space in three dimensions around 360^{0} at each point in space,
  - The attraction force towards carbon source molecules that they will bind with (fungal cells).

This parameter was set to 360^{0} as it is the maximum search domain at each point in space assuming the spherical spatial grid (domain) containing carbon source molecules.

c) ***Random initial molecular sits of the chemical reaction***: This parameter is to propose different random points inside the medium that the oxidation reaction starts at. This parameter is entirely random and its function is to adjust the cellular biased random walks simulation to real design case with specific space coordinates, in other words, at this point, the oxidation-reduction reaction between fungal cells and carbon source is linked to a specific design case.

In this project, the author proposed further parametrization of the code by presenting more iterations. Those iterations are achieved by moving the initial site of reaction (point) along a defined curve (a curve that is implied by the design case and could vary according to different design layouts), this curve is divided to a number of points that could be increased. By running the simulation, the number slider changes which point along the curve is selected, thus the agents head towards it.

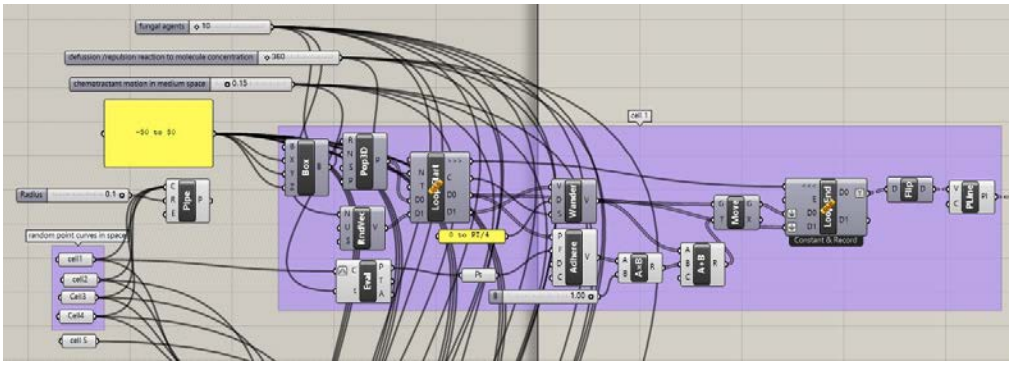

Image. [151]. Biased random walk model equation simulating the chemical reaction of oxidation-reduction based on fungal cells of *Aspergillus sydowii* NYKA 510 oxidation of carbon source, including chemotaxis and reaction active site simulation. By author.

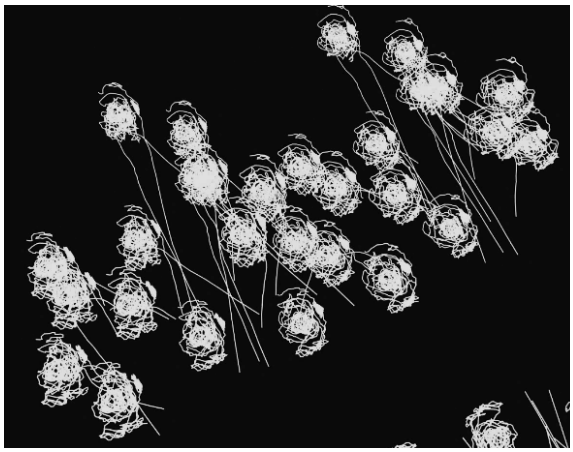

Image. [152]. Biased random walk model of fungal cells chemotaxis behavior and reaction active site initiation. By author.

- ***Clustering the code***: This equation resembles the oxidation reduction reaction that occurs inside the MFC, thus to find the total form of the design in the specified space (design case), the author clustered the equation and repeated it according to two design solutions;
    a) ***Morphogenetic growing bioactive design: (from interior element to architecture and urban design)***;

The first design case grows from the single interior design element to a partition that extends from interior space to semi-open space to outdoor architecture, and would continue its growth to urban.

- ***Interior design element:*** Cell cluster than contains a 16 MFC system for bioelectricity generation embedded inside the design form obtained from the design code (biased random walk). This design element is compact and fully clustered environmentally, functionally and formally, which enables its repetition and distribution interiorly and exteriorly and along various axes in space.

Image. [153]. Formal design derived from biased random walk model designed by the author for the self-sufficient cluster for microbial bioelectricity production appliance as an interior design element acting as a partition and a lighting unite. By author.

b) ***Finite state bioactive design:*** ***this*** case includes design elements that stand-alone and do not emerge into other contexts; including furniture, and lightweight structures.

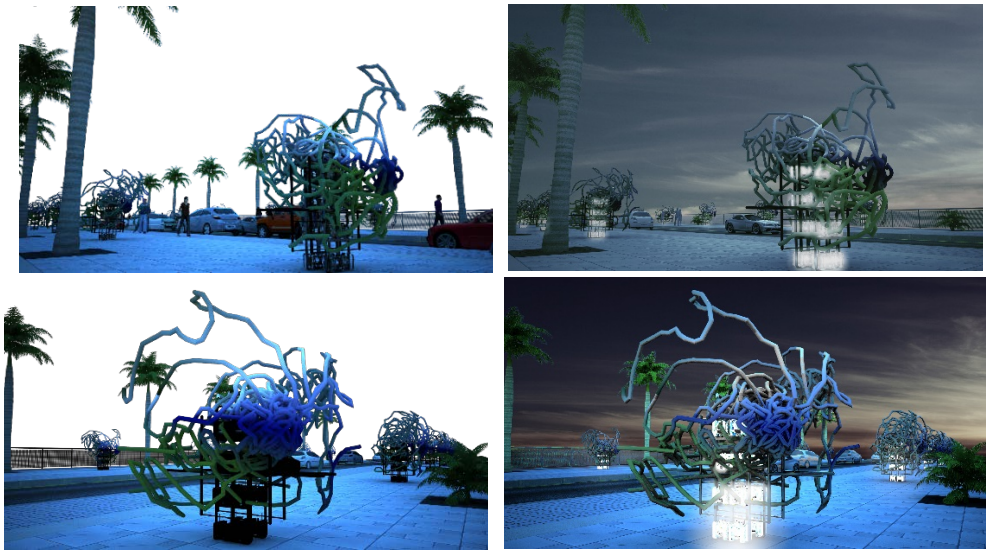

Image. [154]. Self-sufficient cluster for microbial bioelectricity appliance in urbane/ exterior design as a lighting unite. Showing two different formal iterations of the biased random walk model simulating fungal cells in the oxidation-reduction reaction behavioral pattern inside the MFC. The images exhibit two phases of the self-sufficient cluster; power saving mode at day and power usage mode at night. By author.

The second design case: Is an outdoor pavilion that employs the resultant form in design. This pavilion could be inoculated with bioluminescent species to achieve soft lighting when mechanically stimulated.

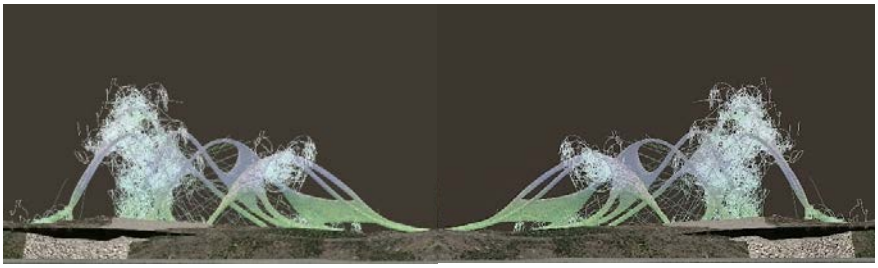

Image. [155]. Elevation and plan of outdoor pavilion based on employing biased random walk model equation of fungal cells chemotaxis, on rale of 5 curves to identify spatial coordinates of oxidation reaction active sites. By author.

Achieving Interior Design Criteria

Function

- ***Electrical properties***: The system depends on the cluster self-sufficient unit that generates 3W for 4 days before recharging the MFCs in the cluster with fresh media and dispersed spores.
- ***Environmental conditions sensory and Power management:*** The self-sufficient cluster is equipped with a power management and sensory systems that performs two integrated ecologic functions:

1. Generating clean bioelectricity for lighting that could be gained from each cluster separately and in-group as well (by connecting two or more clusters in series by a simple predefined connection that is safe for users).
2. Managing the generated electrical power according to the needed lighting intensity (LUX) in interior, semi interior and exterior space as well. This management depends on a sensory system that scan the surrounding space around each cluster and calculates the current lighting intensity, accordingly if these readings are lower than a certain threshold defined by the user, the power management device starts using the stored electrical power. In addition, in case that the lighting intensity value is above threshold the generated electricity is stored in power banks that could be used in need.

- Sensory system: Is composed of lighting sensor that responds to certain thresholds.
- Power management system: That is connected to the sensory system and includes power bank to store electrical power when not needed.

- ***Maintenance & recharging:*** The system is easily recharged every 4 days, each cluster includes an additional sensory for electrical current, when the fungal cells oxidizes all the substrate in media and there is no longer electrical current, the system automatically flushes the exhausted media into a tanker attached to the cluster. After that, the user injects each cluster separately with fresh medium. The author designed the recharging process per each cluster separately complying with processing specification as each cluster is located in a different spatial coordinate, accordingly lighting intensity values varies across various spatial points, resulting in different power consumption at each

cluster, this implies that different clusters along the space will exhibit different power status.

- ***Processing & usability:*** The cluster's design complies with the safety criteria of enclosure and mechanical stability, In addition to the protection provided by the container design that provides safe margins of usability circulation in interior space. The container's design is open to achieve aeration necessary for the air cathode MFC and reachability to MFCs in the cluster in cases of repairing mal functioning or damage or to apply periodical checkup and maintenance.

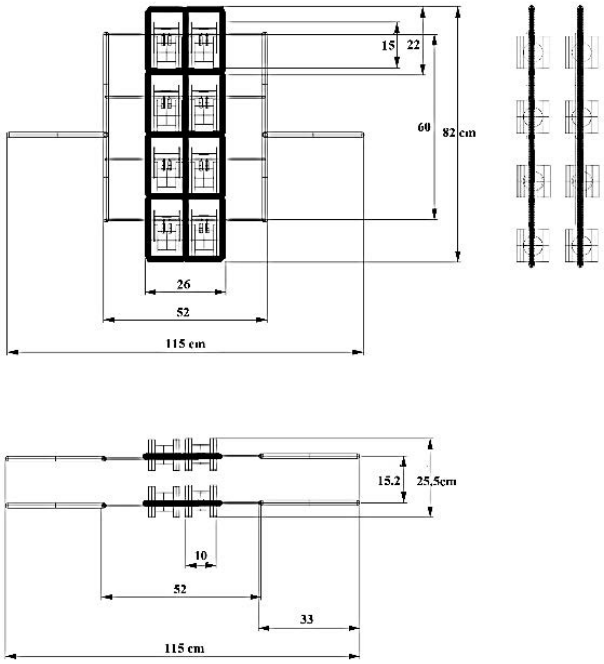

Figure. [81]. Single self-sufficient cluster functional unite of MFCs. By author.

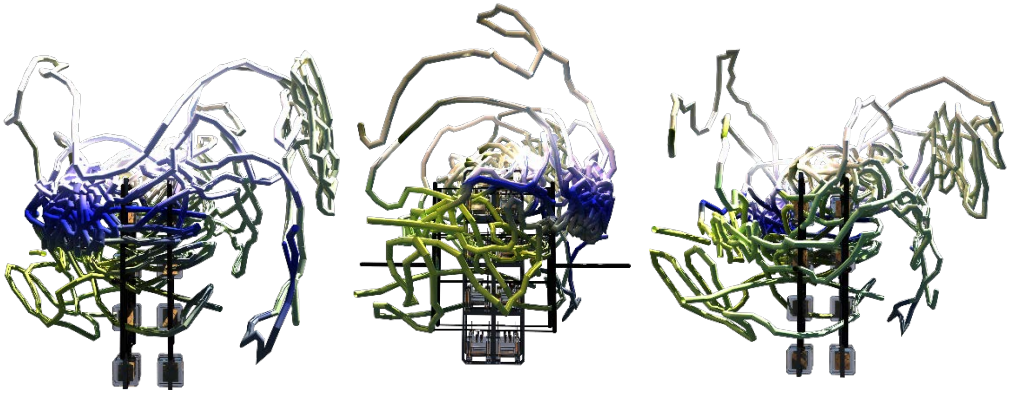

Image. [156]. Self-sufficient cluster for bioelectricity generation of MFCs, side views and elevation, shows varied formal composition around 360 degrees resulting from the biased random walk model of fungal cells. By author.

Image. [157]. Different profile for single unit self–sufficient cluster extracted from the biased random walk simulation model, shows variety and realistic walks tracking of different fungal cells. By author.

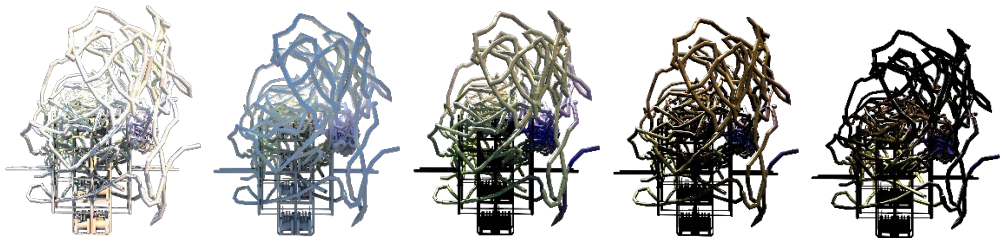

Image. [158]. Chronological simulation time lapse showing fungal culture growth resembled in changing colors and culture density. By author.

- **Achieving Ecology & sustainability**
  - ***Maintain ecological integrity:*** Embedding *Aspergillus sydowii* NYKA 510 as a biocatalyst in bioelectricity production for interior design use. Emphasizing biological integration in built environment as functional agents and exploiting their natural exoelectrogeneses in achieving ecological function of producing clean renewable energy at least costs.
  - ***Embedding natural ecosystems*:** Providing biological agents with their typical growth environment, maintaining growth conditions of temperature, pH, media substrates, aeriation conditions, and mechanical conditions to perform their natural abilities of bio catalysis that is exploited as electrical power sources for lighting gadgets in interior design.
  - ***Biocompatibility and Biodegradability*:** Design materials achieve biocompatibility as MFC cells are made of Plexiglas and do not interact with the bio agent or its enzymatic reactions. Furthermore, the outer design container is made of silicon tubes that is biocompatible either to enable future usage of these tubes as suppling tubes of the fresh media with dispersed spores of bio agent for mega city applications. In case of morphogenesis from interior use to urban use the maintenance and recharging will need to be done automatically according to the large cells numbers and the complex feedback loops and communication networks. The design materials are recyclable and could be reused in the same device and in other application. In addition, the bio agents impose no hazards after

flushing them with the exhausted media that could be further processed for other applications.

- ***Environmental realization and biophilic design*:** This design achieves biophilic design aspects by engaging biological agents' forms and raise environmental awareness by exploiting natural biological agents' biocatalysts abilities in generating electrical power. This proposed relation of symbiosis between human and bio agents is based on integration and mutual benefit. The design system protects biological diversity and provides the agents with their optimum conditions of growth and the agents in turn provide users with clean cheap renewable source of electricity that could be fixed manipulated and handled with minimum knowledge about the system in form of user manual instructions. These information specifies the latency period for electricity production and maximum performance time and duration, it also instruct the users with procedures of recharging or fixing minor damages in the system, it directs the user to understand how to connect two or more cluster for larger electrical current needed safely.

Function growth & morphogenesis (limited functional shifting in bioactive devices)

- **Time**

  - Latency period of performance: According to experimental results from chapter 4, initializing function in each cluster consumes about 2 days (50 h) to generate considerable current but not the maximum efficiency of the MFC. This amount would not be sufficient for electrical gadgets starting from 1 Watt.

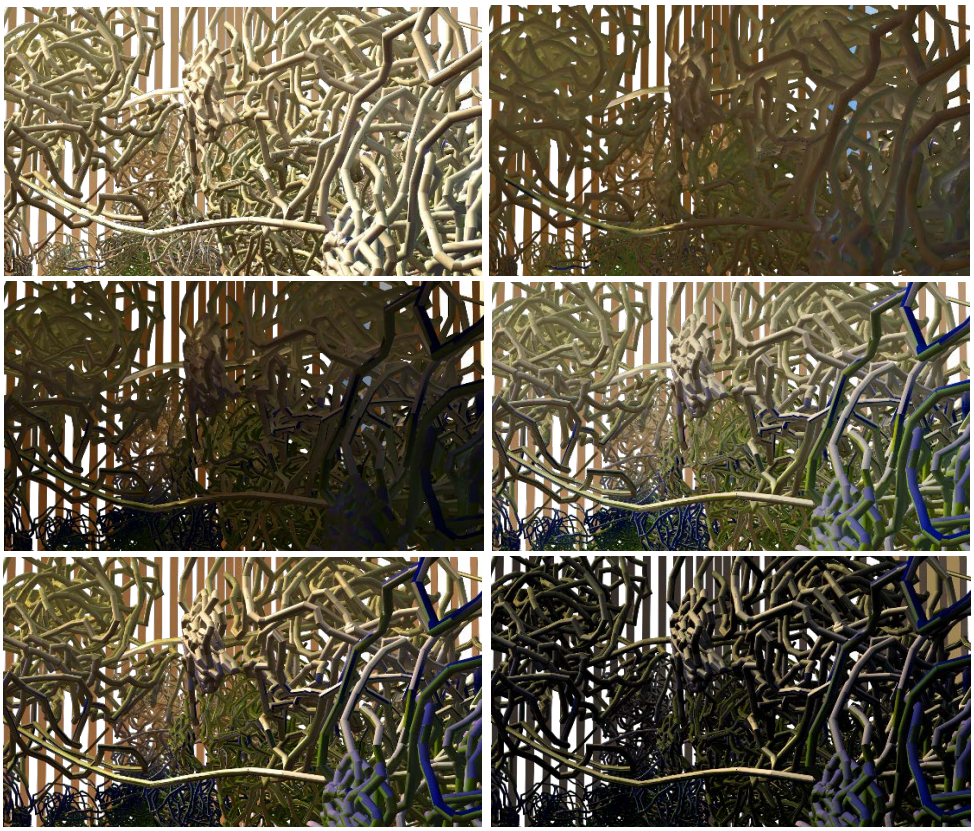

Image. [159]. Detail of interior design installation, Different time frames of growth simulation of the fungal culture of Aspergillus sydowii NYKA 510, injected and cultured in the silicon tubes, showing the growth phases and resulting change in appearance due to blue green color and density of culture. By author.

- Optimum Efficiency: From experimental results obtained, maximum electrical efficiency is obtained after 6 days at the first use of the system. Thus, users should be aware that the system will consume 6 days at first installation before generating electricity sufficient for different gadgets; users also should not interrupt this starting activation process in order not to inhibit or delay the reaction responsible for the bioelectricity generation. After 6 days, the system generates 3 Watt per cluster, which is sufficient for lighting LED bulbs and similar gadgets.

- Responsiveness synchronization: The synchronized response is designed according to the lighting intensity sensory system; the designed response is related to power management, as it determines using or storing the generated electrical power according to defined threshold of lighting intensity in the scanned space. This implies the automated response in real time to switch between the two main moods of power saving or power usage (active performance) without affecting the bio catalysis reaction and the power generation process that continue to produce electrical current, in other words, the response in this case is directed to power management.

- **Function customization (limited predefined customization):** In the case, the self-sufficient system does not alter its function, as it is directed mainly to generate electricity used inside the interior space. However, functional customization aspect would be applied if the system employs a bio agent species that have natural bioluminescence and bio-electrogenesis as well which is found in some bioluminescence bacterial species such as ***Shewanella***. In this case the system would customize the two ecologic functions according to environmental responsiveness defined by the sensory system of the lighting intensity:
    1. In case of sufficient lighting intensity (above threshold): the system switches to biocatalysts for electricity production for power storage or using in other electrical gadgets.
    2. In case of insufficient lighting intensity (below threshold): the system will customize function according to two options, the first is activating bioluminescence when the needed amount of lighting intensity is low (specific Lux), or the second: activating bio catalysis when the needed amount of lighting is more than the estimated intensity produced by the natural bioluminescence.
- ***Biological networks -information and communication technology:*** This point is subject to further research. In case of switching functions between bioluminescent activity and bio catalytic activity, a tight design of feedback network is essential to achieve the responsiveness synchronization needed to activate one reaction and block the other according to environmental conditions. It should be taken into account that activating bio-catalytic activity of the bio-agent will consume a latency period to enable electricity production by the system as explained before, though this is not the case in bioluminescent activity, the defining factor of such

latency period biologically is the reaction time. This should be considered when modeling the biological network feedback loops.

- ***The robust adaptation (Short-term adaptation)***: The system achieves robust adaptation in shifting from power saving mood to active performance mood, depending on an engineered sensory system and microprocessor that compare lighting intensity readings with a predefined threshold and respond accordingly by exploiting the generated electricity or storing it for later use.

- In case of shifting between bio-catalysis activity to generate electricity to bioluminescence or vise –versa, the robust adaptation depends on studying the possibility of achieving both reactions in sync; studying if there is any contradiction between the two reactions. As the bio catalytic activity consumes considerable latency period up to 7 days for microbial species to produce an effective electrical current and this time margins would not achieve robust adaptation if the biocatalysts reaction was interrupted and restarted as the bioelectricity emerges from cumulative effect of the continuous reaction in media.

- **Scale**

  - ***Reaction scale***: According to experimental study this reaction happens on a molecular-cellular level (between fungal cells and carbon source molecules in media). Though the effective scale to achieve the objective of generating considerable amount of bio electricity is the cluster's pixel or unite of 0.4 W ; the single MFC (ACSCMFC) volume of 117.25 ml with specific concentrations of media substrates and the specific amount of inoculum (10 discs (1cm) obtained from a solid pure culture of *A. sydowii* NYKA 510) .

  - Bioactive agents' multiplication: Bioactive agent's multiplication in this case is estimated from the growth process of the fungal inoculum. This growth in culture results in the continuity of the oxidation-reduction reaction until the media is totally exhausted by the fungal cells at these limits the scale parameter turns against the continuity of the reaction and the bioelectricity generation. Thus, the scale factor is restarted each growth course unless the media amount and container volume are scaled up accordingly too which is not the case in this design.

  Spatial recognition. ***Environmental Conditions cognition*:** In this project, spatial recognition is limited to scanning environmental conditions in the surrounding region around each cluster separately. This scanning system measures interior space temperature, exposure to natural lighting and oxygen percentage.

- **Spatial propagation of growth in bioactive design (device propagation)**: Device propagation is achieved in this project depending on clustering and pixilation. Clusters are self-sufficient, confined, controllable and easy for manipulation in space. These clusters could be added or removed according to user's need. Moreover, these clusters could be arranged along different axes in space.

Form

- ***Time: Accumulated frames, phases (related to scale and mass customization):*** Time is presented in this design study by the cumulative effect of biased random walker equation that rules the cellular behavior of the bioactive agents (fungal cells) in their chemotaxis and search for nutrients process. The algorithm was designed in a record history mode to draw the bioactive agent (fungal cell) track through the whole simulation process in a nonstop line (path); this path is the final design form per cell. Another aspect that achieves the formal time criterion is the factual growth phases with its distinguished features of the ***Aspergillus sydowii*** NYKA 510 fungal culture inside the MFCs. The growth from clear media with dispersed spores in light yellow liquid appearance to forming hyphae to turning to light green and bluish green and the distinguished texture of the hyphae to the final dense textured culture. All these changing phases with their formal features contribute to final design form and visual conception as they are contained in transparent glass containers in this design project to engage and integrate the bioactive agent functionally and formally in the design process. As well as building a new aesthetical perspective to users to accept bioactive agents' appearance as a part of the design form.

In designing time frames in this study, the designer was aware of the scale and its contribution to the final formal design ratio. The effect of changing colors and textures was considered as back ground hue in the form of pixels to create a smooth, blended and integrated effect of the bio agent organic appearance. It was also put into consideration the mass customization as these phases exhibit different growth phases features across different clusters (one cluster is in mature phase, other is at the initial phase), thus different clusters will always exhibit different colors and textures of growth according to their growth state.

- Cellular simulation parametric software

The parametric simulation was set to recorded history mode to record the cumulative effect of cellular (fungal cells) search for nutrients in media (the biased random walker behavior); resulting in nonstop splines resembling the history of all walks that the agent achieved in the chemotaxis process.

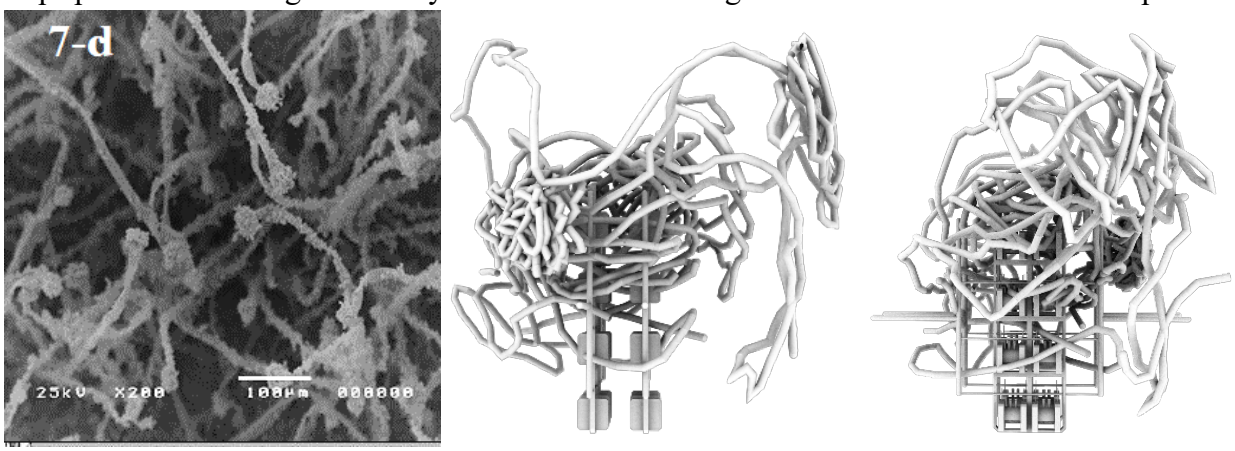

Image. [160]. Formal similarity between (left) the scanning electron microscopy imaging of *Aspergillus sydowii* NYKA 510 culture grew inside microbial fuel cell, (center/ right) and the biased random walk model, simulating oxidation –reduction reaction and chemotaxis of fungal cells oxidizing carbon source inside microbial fuel cell. The formal similarity is shown through the self-sufficient cluster formal design. By author.

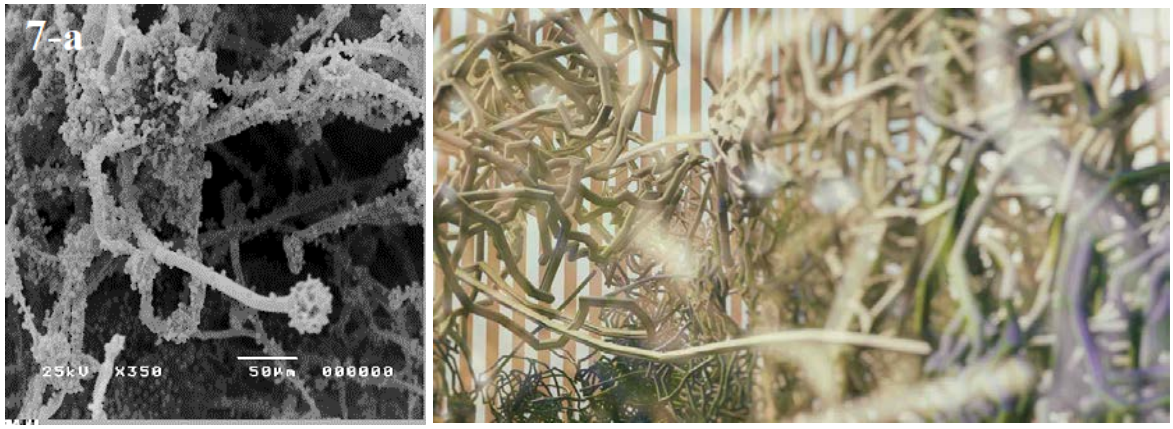

Image. [161]. Similarity between scanning electron microscopy imaging of *Aspergillus sydowii* NYKA 510 and the biased random walk model simulating fungal cell behavior for oxidation-reduction reaction. By author.

- ***Scale (fractal dimension) (Cellular automata)***

The design form of the cluster (the single cell biased random walk) is opened, meshed and allows transparency, these aspects balance its quiet large dimensions (unite dimension) and enables its repetition in space without hindering clarity and visual acceptance.

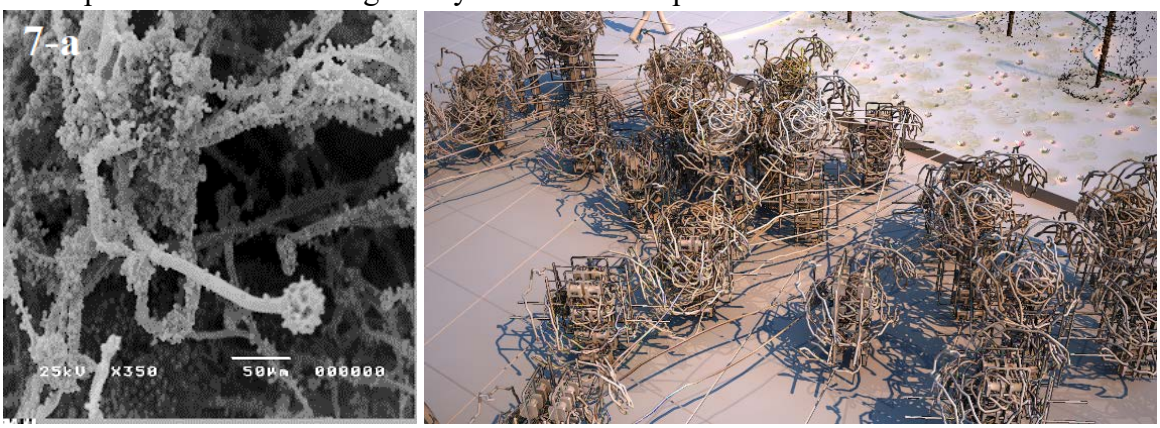

Image. [162]. Form similarity between Aspergillus sydowii NYKA 510 culture grown in MFC and the biased random walk model simulating fungal cells reaction in MFC applied in the self-sufficient system in outdoor. By author.

- ***Mass customization (space)***

The random cellular path that forms the cluster design provides the design with infinite iterations of final mass customizations, and final forms. The intersection and integrity of the opened transparent clusters create various spatial relations between different parts of different clusters emphasized by the different colors and textures of the different growth phases resulting in a new visual conception from different views around the 360º in space and different axes as well.

- ***Formal limitations in bioactive reactors (devices)***

Although the current design proposal liberated formal aspects to the maximum, it is still limited by the formal limitations of functional cluster; these limitations emerge from functional criteria of the MFCs, as horizontal adjustment, stability, the bioreactor design, volume and processing specifications. These aspects limited the functional and mass customization (shifting from function to another accompanied by formal transition and morphogenesis). However, the design maintained the

fluidity and chaos of the design form through using biased random walk in the design process and robotic fabrication in the production process.

- ***Spatial manipulation, propagation and orientation:*** (Related to mass customization and scale).

The spatial manipulation in this project was achieved by locating each formal cluster in space and adjusting its relation with other clusters complying with usability and functional margins, maneuver of recharging and maintenance (mass customization according to function). ***Spatial propagation*** dealt with arranging formal clusters in space and arranging their relations according to visual conception in different views around 360^{O}, this was predefined by the algorithm of biased random walk for multiple cells; it defined to an extent the margins between different clusters and their location in space. ***Spatial orientation*** was limited by the horizontal configuration in the functional cluster; however, the design cluster is free in orientation (rotation) as it is a free fluid form that could be defined in any position.

- Fluid mass

The cluster design achieved both fluidity and pixilation of the mass, the fluid mass conception is achieved in each formal cluster with the chaotic random form resulting from the cellular path of the biased random walk equation on the level of the single cluster and the scale of an element in the interior design. The pixilation is achieved by the ability to repeat these cluster infinite times in space along different axis and in different orientations and relations to each other. This defines the design project as a special case matching both merits of fluid and pixeled design through manipulating scale, mass customization and spatial propagation criteria. The resulting mass is an unpredicted fluid pixeled mass that have uncertain free edges and undetermined solids and voids to exhibit integration in all aspects and create continuity in visual conception.

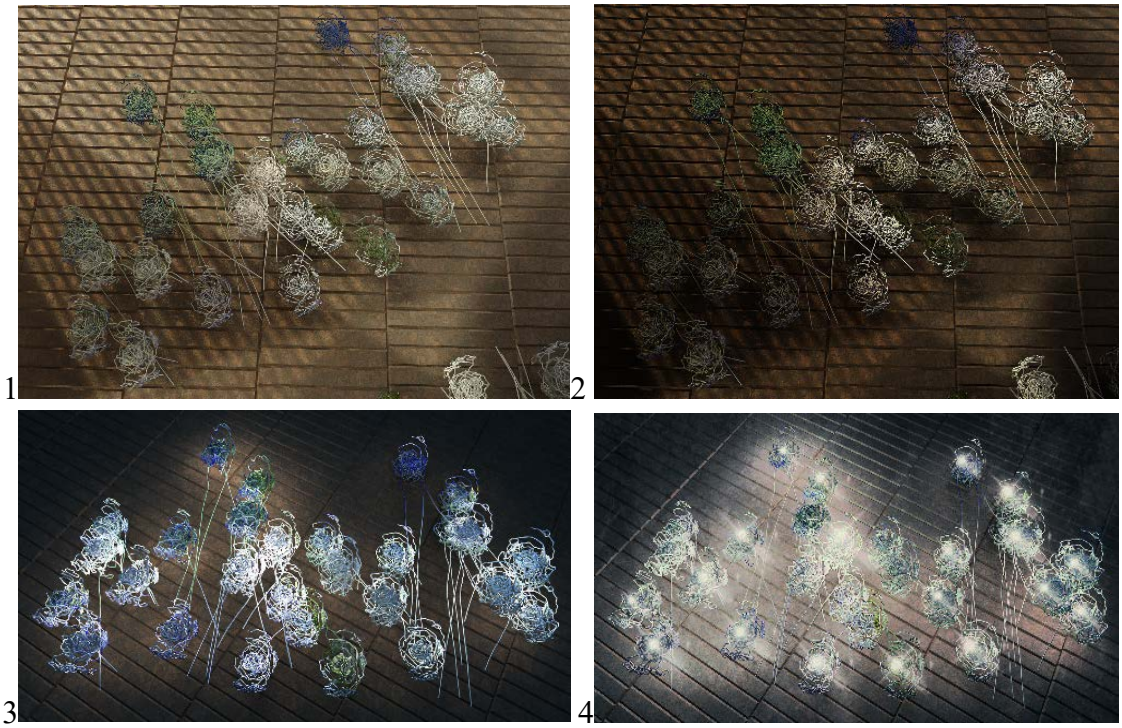

Image. [163]. Plan of distributed self-suffecient clusters showing chronological simulation time lapse of lighting process, from left-up to right-down, day light on power saving mode, sensing light intensity and activating power usage mode. By author.

Image. [164]. Self-sufficient cluster appliance as an interior design element as a partition and lighting unite, showing switching from power saver mode to power usage for bioelectricity consumption exploiting the resulting current from MFCs stack system. By author.

Image. [165]. Outdoor appliance of a system of self-sufficient clusters in landscape design as a source of natural bio electricity, shows the power saving mood in day light preserving electrical current for usage at low light intensity. By author.

Image. [166]. Detail of self-sufficient cluster usage in outdoor design showing integration between the functional unite with MFCs and the formal design. By author.

Image. [167]. Self-sufficient cluster usage in outdoor design showing the appliance of bioluminescent-bio electro genic strain, on the power exploitation mode showing activation of bioluminescence in low light intensity. By author.

Image. [168]. Self-sufficient cluster usage in outdoor design showing the activation of power exploitation mode using electric current harnessed from the MFCs in low light intensities. By author.

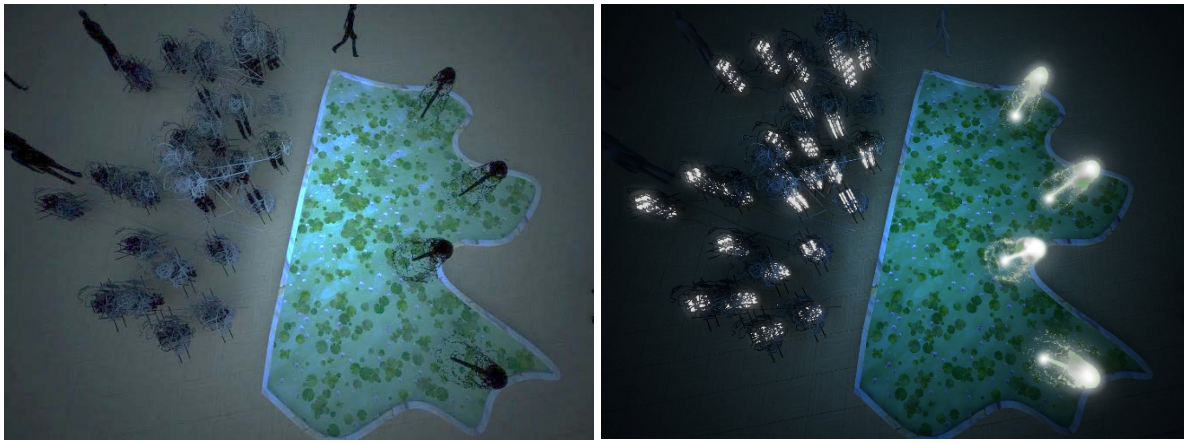

Image. [169]. Plan of Outdoor design of a group of self-sufficient clusters showing switching to activate power exploitation mode to use the harnessed electric current from the MFCs. By author.

Image. [170]. Perspective of outdoor design of a group of self-sufficient clusters showing switching to activate power exploitation mode to use the harnessed electric current from the MFCs. By author.

Image. [171]. Outdoor design of a group of self-sufficient clusters in power exploitation mode using the harnessed electric current from MFCs. By author.

Image. [172]. Detail of self-sufficient clusters lighting at night. By author.

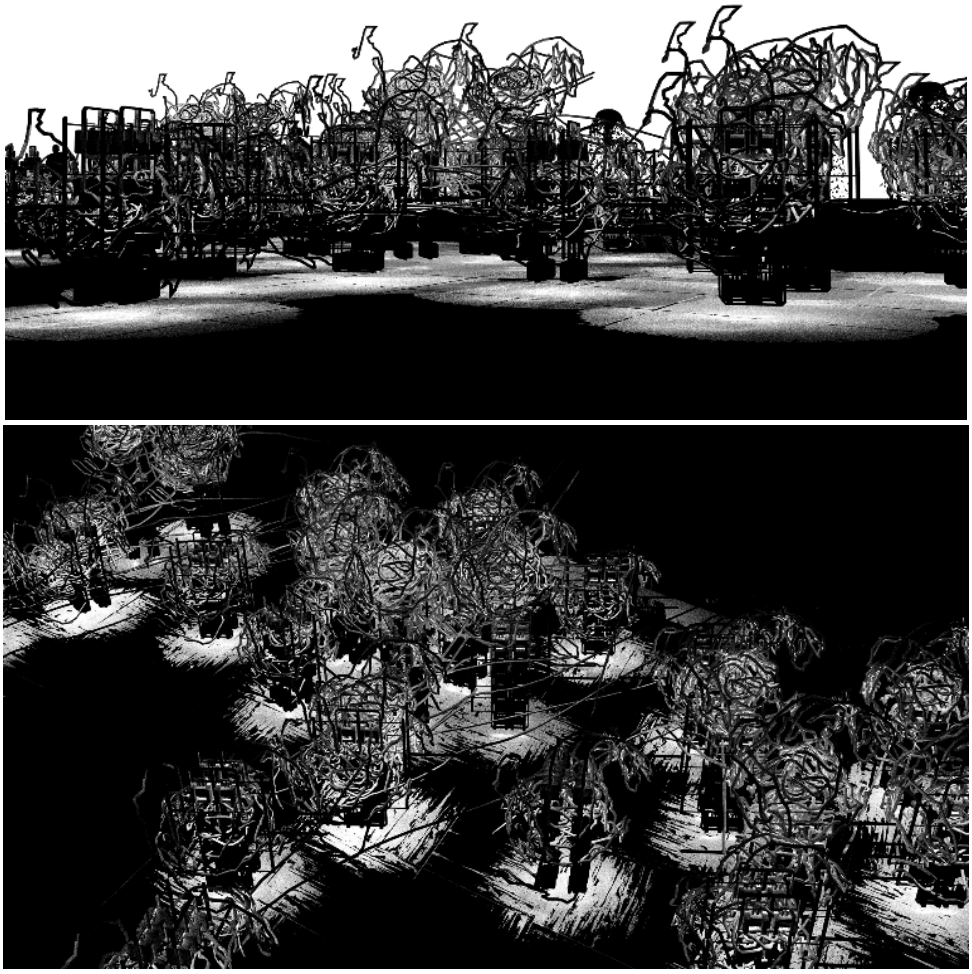

Image. [173]. Simulation of the lighting intensity based on the produced wattage from 16 cell, 3 watt. By author.

a) ***Proliferation axes (axial growth). Aerial –longitudinal –orbital:*** The proliferation axes for the design cluster (functional +formal) is aerial as the cluster is not sit to the ground level in all cases (some clusters are lifted). The longitudinal/ orbital axis is the main path of proliferation of the clusters in the space.

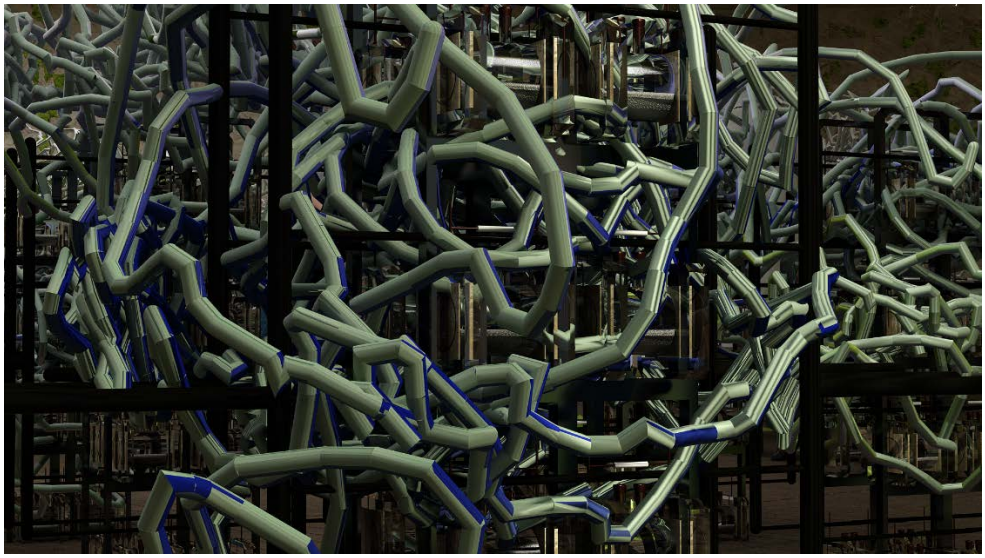

Image. [174]. Interior design detail showing proliferation axes of aerial longitudinal orbital propagation. By author.

b) ***Proliferation method (formal) - branching:*** Branching is the dominant proliferation and growth method of the bioactive agent, the ***Aspergillus sydowii*** NYKA 510 fungal hyphae formation. This proliferation result in electrical current generation and it results in changing formal features inside the functional cluster (MFCs). This branching method is the base of the biased random walk algorithm that generates the cluster form. Thus, the branching proliferation method achieves coupling between form and function as well as spatial propagation through branching along aerial –longitudinal/orbital axis.

Image. [175]. Detail of outdoor cluster proliferation showing integrity in branching proliferation method. By author.

c) ***Morphogenesis (formal):*** This criterion is achieved in the design project through morphogenesis is growth phases of the fungal cells inside the MFCs that resulted in different formal features and the emergence of the whole mass formal design. This design emerges from clusters repetition from the interior design element (single cluster) to (partition design) to growing from interior to semi opened (architecture) and from architecture to urban design.

Fabrication

The design was modeled to be fabricated by KUKA printing arm robot using Rhino3D+Grasshopper –KUKA.prc-2v plugin;

- **Printing material**: Silicon rubber (transparent) - biocompatible material that does not react with bioactive agents.
- **Printing procedures:** Inserting the form finding code (based on the biased random walk of chemotaxis behavior of fungal cells) as the required geometry for printing.
- **Printing code:** The code generated by KUKA.prc-2v sets algorithmic equation that controls real robot arm motion to print the required design.
  - The code is based on specifying the path of the design form ,which defines also the movement path of the robot arm (polyline movement); the main component is KUKA.prc. This component is plugged with the simulation time, the type of movement of the robot arm (to achieve the polyline movement a line movement was adjusted to go throw different planes through the design path),the type of the KUKA robot and the end effector part, which is a spindle tool to generate the hollow tubes form.

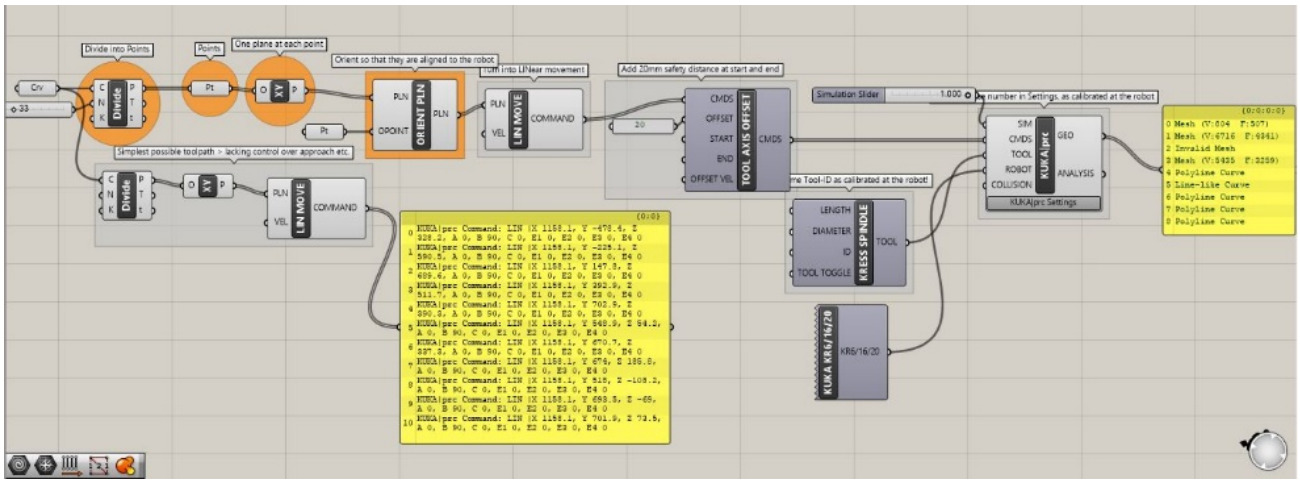

Image. [176]. KUKA.prc-2v robot motion code to print the design form. Developed by author.

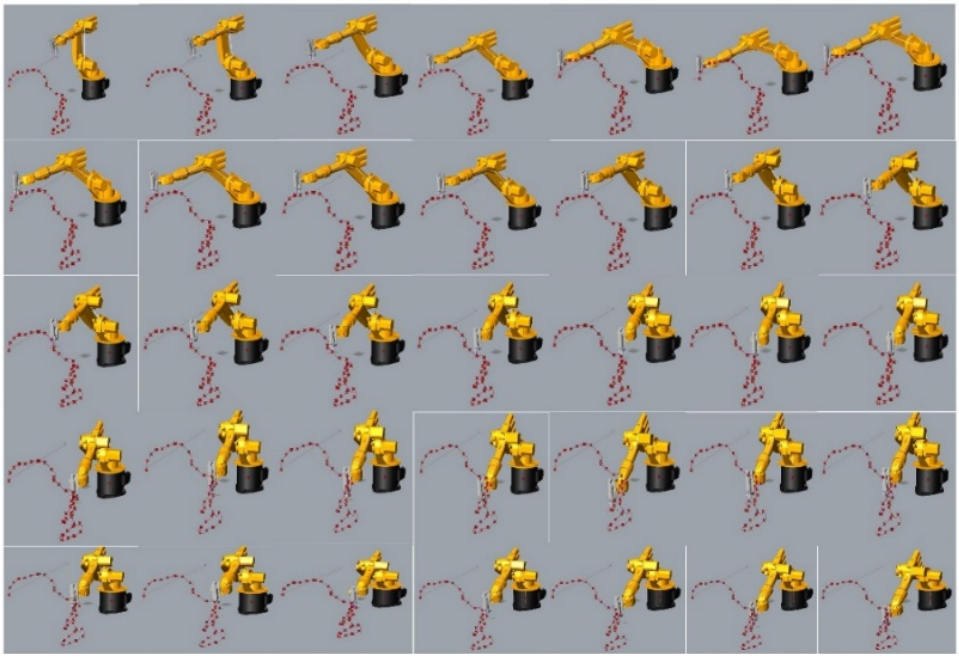

Figure. [82]. Time frames of the robot movement in printing a part of the design form. By author.

Built environment evolution

Achieving this project on urban scale needs more controlled procedures to manage the resulting electrical power of the system and make the maximum use of it, as well as managing the wastes in forms of bioactive agents' wastes, materials or parts of devices.

To control these aspects, the following procedures are required:

- ***Third party installation companies:*** Installation of the power generating self-sufficient clusters will need a specialized organization to manage and control installation points and their distribution and relation to the whole system; it also manages the generated power, as power saving and power usage mode will be exploited on larger scales including regions or districts. The third party will also monitor the efficiency of the whole system and apply periodical maintenance and recharging processes. Third party companies are also responsible for sorting recycling paths of the system's wastes and how to process and reuse it.
- ***Scale-up applications:*** *Scaling* up the system to urban level requires scaling up the self-sufficient cluster (MFCs); this requires a scaled reactor design and demands extensive experimentation on reactions occurring at this grand scale. Scaling up also depends on the ability of materials (host materials) and reactor parts complying with this scale design criteria to maintain the ecologic system, protect and integrate bioactive agents in design.
- **Recharging and maintenance points:** The urban scale applications requires network design defining centers of recharging and maintenance to monitor and organize this process in each district.
- ***Power saving & Redistribution points:*** Managing the electrical power resulting from the system requires main centers to harness the resultant power and manage its redistribution in each district.

General Results and Discussion

[1] This research main results of experimental work conducted by author, were published under the two main experimental phases of this study;

[1.1] Embedding of self-sufficient system of bioelectricity generation employing a bioactive agent (fungal strain) - in architectural design for domestic use. This experimental study was published in;

Y. Abdallah, A. T. Estevez, D. Tantawy, A. Ibrahim, N. Khalil, "Employing laccase producing *Aspergillus sydowii* NYKA 510 for cathodic biocatalyst in self-sufficient lighting microbial fuel cell", *J. Microbiol. Biotechnol.*, Nov 2019.

In this phase: the main results were:

[1.1.1] Isolation and Molecular identification of the most potent fungal strain producing the indicator enzyme Laccase, which is the pivotal element in the oxidation-reduction reaction in generating bioelectricity inside MFC: The procured nucleotide sequence was deposited at the NCBI GenBank. The fungal isolate was thus identified as *Aspergillus sydowii* NYKA 510 with accession number MK060010.

[1.1.2] Optimization of growth conditions of *Aspergillus sydowii* NYKA 510, for medium constituents were optimized to produce the highest possible amount of laccase, which was after 7 days at 31°C and pH 5.2. Banana peel and peptone excelled in inducing laccase production at concentrations of 15.1 and 2.60 g/l, respectively. Addition of copper sulfate elevated enzyme yield to 145%.

[1.1.3] *Aspergillus sydowii* NYKA 510 was employed in a microbial fuel cell (MFC). The best performance was obtained at 2000 Ω achieving 0.76 V, 380 mAm^{-2}, 160 mWm^{-2}, and 0.4 W. the MFC needed initialization time of 4 days for generating steady current, and maintained steady performance of 6 days before the need to be recharged with fresh medium and dispersed spores. The novelty of this research study was achieved in two aspects. The first is the direct use of microbial fuel cell in a design application for bio (electricity) production addressing the problem of non-renewable energy supply for building sector that contributes to total Co_2 emissions by 50% (47% energy supply and 3% buildings sector). The second is the cost effective, available, easy to embed and use, domestic waste treating, self-sufficient cluster design of the self-sufficient unite, addressing the problem of economic poverty and limited resources in developing countries that eliminate the need for large-scale infrastructure intervention, which make this bioelectricity self-sufficient cluster outweighs other abiotic renewable energy resources of solar or wind power. Thus answering research hypothesis positively.

[1.1.4] A self-sufficient lighting unit was implemented by employing a system of 2 sets of 4 MFCs each, connected in series, for electricity generation. The self-sufficient cluster design involved the inner system of the MFCs and the outer container, which gave the cluster its final form. The formal design at this phase only included patterned customized mass depending on bio digital design procedures through utilizing scanning electron microscopy images of *A. sydowii* NYKA 510 in algorithmic form generation equations. Patterned customized mass approach were developed by the author and chosen for application in the design.

[1.1.5] The resulting self-sufficient cluster suffered limitation in mass manipulation in the 3 axes, due to the need of maximum enclosure, mechanic stability, simplicity of design in terms of maintenance reachability and ease of use (initializing and recharging) by non-specialized conventional users. However, the design formal simplicity in this case was favorable due to the need of replication as panels or building blocks (partitions) in the space. In addition, manipulating the biodigital derived pattern did succeed in adding the required design complexity without hindering the main objective of functionality of electricity generation with accessibility, cost effectiveness, and reproducibility (simple manufacturing processes). Which was the main aim of this research solving the domestic and global issue of the need of renewable and ecological source of electricity. (Function 90%, form 70%).

[1.1.6] The self-sufficient cluster still needs further optimization in terms of electrical power management; such as shortening the initialization time by shortening the latency period of the oxidation reduction reaction, lengthen the activity time (more than 6 days before the need to recharge it), to increase system durability. (Further research). However, activity time achieved in this research is suitable for domestic usage. As the main carbon source achieving the maximum laccases production and bioelectricity generation activity was banana peel, this add double benefit of this self-sufficient system as a method of recycling organic waste and generating electricity in the same time.

[1.1.7] The self-sufficient system is subjugated to undergo further research in the electrical power output optimization and management. As proposed in the methodology of embedding microorganisms in interior design elements to achieve space ecology in chapter. 6. The system's power management is a complicated process (in case in networking not separate clusters). This includes managing singularity or global behavior options to control each cluster electrical current output, singular or global responsive behavior to light intensity sensor estimating the surrounding light intensity in the space and controlling singular (only one cluster) or global (group of clusters) response to this. Developing a system of power management like this demand achieving flexibility switching between the singular and global behavior employing Swarmal intelligence embedded in the system control design. (Further research). However, such a complex system was contraindicated in this phase of research with a defined objective of "ease of use", "Clusterization", and "cost effectiveness". for emphasizing on singularity that refute the need for complex systems or infrastructure that demand governmental intervention, and focus more towards a democratic and handy bioactive device that could be adopted in any space easily.

[1.1.8].The self-sufficient cluster's design is subjugated to material development in terms of the bioreactor materials and the outer shell (container) as well. This development is forced towards integrating biocompatible biomaterials that would even facilitate the performance of the Microbial fuel cell (organic materials for solid-state fermentation) and raise the ecologic and economic value of the design as well. (Further research).

[1.1.9]. The self-sufficient cluster is subjugated to scaling up the microbial fuel cell (the bioreactor).

[1.2] achieving coupling between form and function by employing biological imaging tools and mathematical analysis, simulation and modeling tools for extracting mathematical logic of complex biological behaviors. This experimental study was published in;

A. T. Estévez, N. M. Khalil, M. M. Sobhy, Y. K. Abdallah, "Biased Random Walk as a Modeling Tool for Form and Function Integration in Bioactive Design". In Alberto T. Estévez (Ed.), 4th Conference on Biodigital Architecture & Genetics. Barcelona: iBAG, Universitat Internacional de Catalunya. (In printing), 2020.

In this phase: the main results were:

[1.2.1] The design of a cellular automaton- agent based combined mathematical model to simulate the fungal cells behaviors of: 1) Nutrients search in media (diffusion), 2) chemotaxis behavior (motor polarization: motion towards optimum concentration of target molecule / repulsion to higher concentrations of target molecules (carbon source), 3) oxidation-reduction reaction active sites (active site identification in space to start the oxidation-reduction reaction between fungal cells and carbon source in media). This simulation was based on schematic analyses of the oxidation-reduction reaction pathways.

[1.2.2] Dynamic mathematical modeling achieved in this research phase by a developed model combining cellular automata and agent-based modeling in a biased random walk model was recorded in accumulating time frames of fungal cells action in medium space (record history). These continuous time frames is a method to exploit the generated data in the case of static application in design form. Thus, selective accumulation of specific time frames would result in infinite different results from the same equation.

[1.2.3] It was proved in this research the formal similarity between the mathematically modeled fungal cells behavior and the scanning electron microscopy imagery study of different specimens of the fungal cultures used in this research, this proves the accuracy of the biased random walk model developed in this study by the author.

[1.2.4] Microbial behavior emerge from physiological processes to perform specific activities that are essential for their living, growth and morphogenesis, such as: nutrient search and uptake, chemotaxis, bioluminescence, quorum sensing, bioelectro-genesis. These processes are complex interconnected behaviors that usually cannot be traced separated from each other, thus microbial behaviors main characteristics is the unpredictability of the system's global behavior from its agent's behavior, thus following stochastic dynamics.

[1.2.5] The first step in analyzing emergent microbial behaviors, is to understand physio-chemical pathways involved in the studied behavior; e.g. metabolic pathways, signaling transduction pathways, etc. understanding the logic behind these processes eases the task of designing a schematic chart of parameters, interdependences and interrelations. This schematic chart would be translated into mathematical modeling equations through specific software.

[1.2.6] In order to gain full insight and understanding of physio-chemical processes that are performed in microbial behaviors, biological imaging is essential. For achieving maximum benefit and data acquisition out of these tools. The designer should understand the role of scale and state in this process. Scale controls the level of magnification of a specimen, this in turn

control the biological behavior resolution and understanding, the other aspect is state that controls imaging static frames of specific biological behavior, or dynamic imaging that record in real time various physio-chemical pathways that rule different biological/ microbial behavior.

[1.2.7] In order to simulate/ model microbial behaviors, the design process should employ a "case-based" design approach. This approach is essential in tackling the design problem, as to specify the target microbial community and behavior. Accordingly, the scale of reaction of the biological behavior will identify the scale and state of biological imagery study required to identify the behavioral logic to be then modeled by the suitable mathematical modeling tool, e.g. cellular automata, agent based, partial differential equations, etc.

[1.2.8] Simulating microbial behaviors in terms of formal composition and behavioral processes is essential in coupling form and function in bioactive design. Application of microbial formal composition logics resulting from behavioral simulation was the first step in this research phase.

[3] According to the proposed design criteria of bioactive devices and bioactive hybrids by the author, bioactive devices lack the ability of spatial propagation automatically in response to environmental conditions or users' needs. **This eliminate to a great extinct their ability (bioactive devices) to growth and morphogenesis in terms of proliferation, propagation, differentiation in form (hesto-genesis) and function (morphogenesis) in time (evolution) and space**. As these bioactive devices can proliferate and propagate based on the pre-determined or pre-designed method of proliferation and replication in space in form of separated clusters that when joined would achieve some sort of semi-automated spatial propagation.

[4] According to the proposed design criteria of bioactive devices and bioactive hybrids by the author, bioactive devices suffer the limitation of formal manipulation as they demand ultimate enclosure of the bioactive agent from the outer surrounding environment. In order to ensure the optimum growth conditions of bioactive agents and optimum exploiting of the required ecological function that they are embedded in architectural design to achieve. This level of enclosure imposes limitations on formal design of the clusters and the proliferation method ruling the relation between clusters orientation and position in space. It also imposes usage hazards as it may contain liquid bioreactors or exhausted media, which impose more responsibilities on the designer to guarantee not just optimum enclosure but also mechanical stability.

As exhibited through the review of this research (chapter, 1, 2, 3). Bioactive devices have been more handy and available for architects and designers to achieve the embedding of microorganisms in architectural design to achieve ecological values. These manifesting projects all shared the need for a design of a container and the use of bioreactor that is usually not manipulated in its formal design [113•; 114•; 116•]. This obligation of design form a container or a guarding shell point of view imposed not just formal limitations but also formal distortion caused by the fixations and equipment needed for designing the bioactive system in a reproductive fashion so that it could continue functioning with least intervention from user or designer. **Another limitation that faces the employing of bioactive devices is the complex biological pathways of the bioactive agents, causing unpredicted latency periods or in worst cases contamination with other species that may be pathogenic**. However,

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• **[113] https://www.ucl.ac.uk/bartlett/architecture/research/algaezebo/23/2/2018**
• **[114] http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=162-2-2-2018.**
• **[116] http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=148-2-2-2018**

almost all of the exhibited manifesting projects achieves the most possible methods in form manipulation and biological functioning precision [115•; 118•; 45•]. This was mainly due to clever selection of the functioning bioactive agent that was mainly algae or non-pathogenic bacterial strains, ensuring its non-pathogenic nature and achieving multiple benefits of it; being edible, used in cosmetics, performing photosynthesis and so on. **This leaves the designer with only two options; take the safest way regardless from the specific required ecological function needed by employing a safe bioactive agent. Or, the hardest way move from assembly to synthesis, from devices of systems to materials of systems or hybrids.**

[5] As exhibited in this research, almost 70% of the exhibited manifesting projects of designing bio materials did not employ complex behaviors that are naturally inherent by the used bioactive agents; instead, applications were directed towards "single-value" objective. This resulted in non-complex system that lack the ability of morphogenesis in terms of formal functional differentiation in time and space based on environmental conditions, user needs, inner needs, inner resources, genotypes and phenotypes. Thus, these bio-based materials [64•; 67•; 72]•, are not considered bioactive hybrids proposed in the methodology developed by the author in this research.

[6] The key feature of bioactive hybrids or bioactive materials of systems, is to achieve natural automated self-replication and tissue differentiation, these two main features should be achieved based on embedded biological intelligence in materials to proliferate the hybrid cells for multiple purposes; such as locomotion for search of nutrient or escaping hazards, growth and self-healing, and differentiate. Differentiation is the key factor in biological intelligence that was not achieved until now in synthetic biology (biological engineering) disciplines. Cellular differentiation through replication in the growth process based on the intelligent sum of environment, demands, sources, genes and genetic translation (phenotypes), is the most challenging complex system to achieve, not just for the complex physio-chemical pathways involved in this process, but also because its complex dynamic precision. Thus, modeling these features in design is a very nascent field that did not exceed the limits of laboratory experimentation until now.

[7] Modeling self-replication and tissue differentiation in bioactive hybrid materials depends on multidisciplinary research of biological engineering or synthetic biology. The main tool of achieving this is genetic manipulation (recombinant DNA) and biologic robotic materials. (future research). The last combines the complex systems of bioactive devices with bioactive material synthesis. An example of trials of embedding biological intelligence of self-replication for self-healing and locomotion is exhibited in the recent research by S. Kriegman, et al., (2020) of "A scalable pipeline for designing reconfigurable organisms", that Manufactured reconfigurable organisms by aggregation of pluripotent blastula cells harvested from *X. laevis* embryos. The results were layering of cardiac progenitor cells that result in contractile cardiomyocyte tissue, self-locomotion in aqueous environment, and emergent behavior of debris aggregation by

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• http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=20&idproj=163-2-2-2018

• [118] http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=71&idproj=127-2-2-2018

•[45] A. T. Estévez, The Future of Architecture: Bio digital Architecture and Genetics, ESARQ the Architecture School of the Universitat Internacional de Catalunya, Barcelona, Architecture Research, 4(1B), pp.13-20, 2014.

• [64] https://www.treehugger.com/green-architecture/mycotecture-mushroom-bricks-philip-ross.html/23/2/2018.

• [67] https://inhabitat.com/3d-printed-mycelium-chair-sprouts-living-ushrooms/eric_klarenbeek_1.

• [72] https://inhabitat.com/nyc/the-livings-100-organic-hy-fi-towers-win-moma-ps1s-2014-yap-competition/30-1-2018.

individuals within the environment and by groups of reconfigurable organisms. [495]•. Another example of embedding biological intelligence in materials to achieve growth and locomotion is a research study conducted by S. Hamada, et al., (2019) of "Dynamic DNA material with emergent locomotion behavior powered by artificial metabolism". [496]•. In this research a dynamic DNA material with emergent locomotion behavior powered by artificial metabolism was developed for the first time, , an emergent locomotion behavior resembling a slime mold was programmed with this material by using an abstract design model similar to mechanical systems. Dynamic properties, such as autonomous pattern generation and continuous polarized regeneration, enabled locomotion along the designated tracks against a constant flow. An emergent racing behavior of two locomotive bodies was achieved by expanding the program.

[8] It is essential to employ "case-based" design and "multidisciplinary" study when synthesizing a bioactive device, hybrid or bioactive material of systems. A case-based design approach imposes experimentation in various interdisciplinary fields but only the needed in the specific case of design. This approach is also sufficient in terms of addressing ecological problems; as it is aware with the multi scale interconnected nature relations that biological agents is an essential and ever existing element of it. Case-based design approach is also optimum in tackling biological behaviors as it narrows the risk imposed by the multidisciplinary nature of embedding biological agents in design through localization of the problem to specific purpose and specific search areas. It is worth mentioning that this approach does not hinder tracing biological complexity of studied biological behaviors, on contrary it gives a greater ability to analyze these complexities and realize it in terms of realistic translation and application in design process. (As achieved by the author through experimental study of this research in biotechnology, microbiology, bio electro chemistry, electrochemistry, and stochastic dynamics, computation, bio digital design).

[9] In order to move towards bioactive hybrids, or bioactive materials, new digital fabrication tools are needed. These tools should support the biological feature of bioactive designs in terms of supporting continuous growth and synthesizing a method and a base for translating intracellular biological intelligent behaviors to independency. As biological behaviors are singular and global in the same essence and the same time, they support the intelligence mechanism of their morphogenesis and evolution being self-contained in an organism (cellular to tissue). This implies their (biological cultures) ability of independency and activity (active in tracing nutrients, acquiring nutrients, etc.). To this extent, designing a bioactive hybrid or material that should carry these traits should provide to the maximum the survival of these materials by its own, which is not totally achieved until now. Bioprinting is an emergent research discipline focused on printing bioactive materials that can support its self for a certain period exploiting embedded source of nutrition in its own components (in the hydrogel). The current state of bioprinting lacks the durability of active living growth in a long term fashion, as almost all printed bioactive materials need to be embedded in a host to continue living and evolving. Another problematic aspect of printed bioactive materials is the lack of the ability of cellular hestogenesis and tissue differentiation, which means the lack of independent complex biological behavior. (Further research needed).

• [495] S. Kriegman, et al., A scalable pipeline for designing reconfigurable organisms, PNAS, 117, 4, 1853-1859, 2020.
• S. Hamada, et al., Dynamic DNA material with emergent locomotion behavior powered by artificial metabolism, Science Robotics, 2019.

[10] Nascent research areas of robotic materials and their integration in recombinant DNA methods and their interpretation on the bases of trilogy of histology/ physiology/ anatomy system is highly recommended in future research studies, to gain a deeper insight into cellular replication and differentiation.

Conclusions

This study was conducted corresponding to domestic (Egypt) problem of electrical power crisis, concerning the increasing demand in power supply and consumption from 2012 until now, as well as corresponding to universal problem of global warming that was escalating its symptoms and drawbacks aggressively recently. The research area of proposing an adequate sufficient solution was based on analyses of the fact that *(47%) of these total emissions comes from energy supply sector, and (3%) of buildings sectors*. Thus, decarbonizing electricity generation was a key of cost-effective mitigation achieving low-stabilization levels of CO2 as it is more rapidly achievable in electricity generation than other sectors. However, Challenges for integrating RE into energy systems and the associated costs vary by RE technology type, regional circumstances, and the characteristics of the existing background energy system, as it needs large infrastructure interference. Employing bioenergy offers energy supply with large-scale net negative emissions, depending on options with low lifecycle emissions (e.g., sustainable use of biomass residues) and outcomes that are site-specific and rely on efficient integrated biomass-to-bioenergy systems. To this extent this research objective was to achieve self-sufficient systems of bioelectricity generation that achieves the following; safety, electrical power generation efficiency, cost effectiveness, waste treatment, ease of implementation in domestic use, ease of use, reproducibility and availability. As well as designing a general design methodology of embedding and utilizing microorganisms in design elements to achieve ecology. For this, a special research plan that combines quantitative experimental methods with theoretical deduction was designed; this was mandatory introducing a multidisciplinary research application through a design theories aspect. The research study included an extensive review to analyze and categorize previous research in this field, manifesting projects, and state of art, moving towards establishing multidisciplinary scientific bases integrated in the embedding of microbes in design elements for performing ecologic values. The main core of the study was the experimental work conducted utilizing MFC device technology for exploiting natural exoelectrogeneses of microbes, as exhibited potent microbial (fungal strain) was identified and optimized for further implementation in MFC performance for electricity generation. Outcomes were then included in the self-sufficient cluster design that achieved the aforementioned conditions (hypothesis part1 answered). For the second objective of this research, another essential multidisciplinary study based on biological imagery and dynamic mathematical modeling was carried on, in order to achieve coupling between form and function in the self-sufficient bioelectricity generating system. This phase involved a review of scientific bases of mathematical modeling of biological behavior and categorization according to physio-chemical pathways and stochastic dynamics basis. Followed by another experimental phase of designing a cellular automata-agent based combined model of biased random walk that simulate the fungal cells complex behavior in oxidation-reduction reaction responsible for bioelectricity generation inside MFCs. This specific reaction was chosen for the study in order to achieve coherence in coupling form and function.

Results were employed in extensive design study of the design methodology and criteria of embedding microorganisms in interior design elements to achieve space ecology through case-based design approach. (Supported by designs analyses and justification for different applications) (Hypothesis part2 answered). Resulting in two main categories of bioactive devices (applied in this thesis scope and coherent to its objectives), and bioactive hybrids or bioactive materials of system which is a material for further investigation.

Abstract

This research was conducted in corresponding to energy crisis and the global warming effects of utilizing nonrenewable energy sources (fossil fuels). Contributing to high Co_2 emissions. The study proposed bioenergy sources as a sufficient alternative for power supply in building sectors for domestic use as it achieves a multi scale solution for the addressed problem; being ***safe, efficient in electrical power generation, cost effective, waste treating, easy to implement in domestic use, easy to use, reproducible and available.*** In this extent an experimental study on bioelectricity generation employing microbial fuel cell device was conducted based on previous research review, analysis and scientific interdisciplinary study developed for the case based design. Including introduction to interdisciplinary sciences, mainly biotechnology, tissue engineering, bio materials, synthetic biology, bio informatics and biodigital design involved in the embedding and integration of microbes in design for ecological purposes, bioluminescent activity physio-chemical basis and biological synthesis potentials and possible design application. Bioelectricity bio-electro chemical basis and introduction of fuel cells and microbial fuel cells devices technology as a specific bioreactor for exploiting natural exoelectrogeneses of microbes. The experimental study surveyed various microbial species for optimum production of **indicator enzyme (laccase)** that is the main precursor agent in the **oxidation-reduction reaction of bioelectricity generation**; the potent strain was molecularly identified as ***Aspergillus sydowii* NYKA *510*, and further optimized for its growth condition to be employed in a single chamber membrane-less MFC for bioelectricity generation and optimization. The system achieved at 2000 Ω, 0.76 V, 380 mAm^{-2}, 160 mWm^{-2}, and 0.4 W. the MFC needed initialization time of 4 days for generating steady current, and maintained steady performance of 6 days before the need to be recharged with fresh medium and dispersed spores. A self-sufficient lighting unit was implemented by employing a system of 2 sets of 4 MFCs each, connected in series, for electricity generation. The self-sufficient cluster design involved the inner system of the MFCs and the outer container, which gave the cluster its final form. The formal design included patterned customized mass depending on bio digital design procedures through utilizing scanning electron microscopy images of *A. sydowii* NYKA 510 in algorithmic form generation equations. Patterned customized mass approach were developed by the authors and chosen for application in the design. Following this, a multidisciplinary study based on biological imagery and dynamic mathematical modeling was carried on, in order to achieve coupling between form and function in the self-sufficient bioelectricity generating system. This phase involved a review of scientific bases of biological imagery study and mathematical modeling of biological behavior and categorization according to physio-chemical pathways and stochastic dynamics basis; as cellular automata, agent based, partial differential equations, and introduced the basis of complex intelligence systems. Followed by another experimental phase of designing a cellular automata-agent based combined model of biased random walk that simulate the fungal cells complex behavior in oxidation-reduction reaction responsible for bioelectricity generation**

inside MFCs (including nutrients search, chemotaxis, oxidation-reduction reaction active site). This reaction was chosen for the study in order to achieve coherence in coupling form and function. Results were employed in extensive design study of the design methodology and criteria of embedding microorganisms in interior design elements to achieve space ecology through case-based design approach. Resulting in two main categories of bioactive devices (applied in this thesis scope and coherent to its objectives), and bioactive hybrids or bioactive materials of system which is a material for further investigation.

References

- **[a] https://en.wikipedia.org/wiki/Ecological_design/.**
- **[b] https://climate.nasa.gov/causes/11/2019.**
- **[c] IPCC Fifth Assessment Report/ 2014.**
- **[d] Mediterranean Energy Regulations -reporting methodologies -how to collect data and monitor regulated entities -Egypt ERA -www.egyptra.org-milan/10/10/2017.**
- **[e] https://tradingeconomics.com/egypt/electricity-production-kwh-wb-data.html/1/9/2017**
- **[f] E. M. Milner, et al., Microbial fuel cells with highly active aerobic biocathodes, Journal of Power Sources, 8-16, 2016.**

- **[1] M.D. Robertson, C. R. Figueroa, M. Zhang, 2015, Material ecologies for synthetic biology: Bio mineralization and the state space of design, Computer-Aided Design journal, 60, 28-39, 2015.**
- **[2] https://en.wikipedia.org/wiki/Microorganism#cite_note-1-28/1/2018.**
- **[3] H. Schulz, B. Jorgensen, Big bacteria, Annual Review of Microbiology, 1, 55, 105–37, 2001.**
- **[4] J.A. Shapiro, Thinking about bacterial populations as multicellular organisms, Annual Review of Microbiology, 1, 52, 81–104, 1998.**
- **[5] J. Muñoz-Dorado, F. J. Marcos-Torres, E. García-Bravo, A. Moraleda-Muñoz, J. Pérez, Myxobacteria: Moving, Killing, Feeding, and Surviving Together, Frontiers in Microbiology, 7, 781, 2016.**
- **[6] O. Johnsborg, V. Eldholm, L. S. Havarstein, Natural genetic transformation: prevalence, mechanisms and function, Research in Microbiology, 10, 158, 767–78, 2007.**
- **[7] R. Eagon, Pseudomonas Natriegens, a Marine Bacterium with a Generation Time of Less Than 10 Minutes, Journal of Bacteriology, 4, 83, 736–7.1962.**
- **[8] Eukaryota: More on Morphology. (Retrieved 10 October 2006)/30/1/2018.**
- **[9] S. Dyall, M. Brown, P. Johnson, Ancient invasions: from endosymbionts to organelles, Science, 5668, 304, 253–7, 2004.**
- **[10] https://www.biology-online.org/dictionary/Haploid-6-2-2018.**
- **[11] S. In Kimura, S. Shimizu, DNA repair as the primary adaptive function of sex in bacteria and eukaryotes, DNA Repair: New Research. Hauppauge, N.Y. Nova Sci. Publ, 1–49, 2012.**
- **[12] https://theartofmed.wordpress.com/2015/06/03/types-of-pathogenic-microorganisms/29/1/2018.**
- **[13] https://theartofmed.wordpress.com/2015/06/03/types-of-pathogenic-microorganisms/ 8/2/2018.**
- **[14] "Fungus". Oxford Dictionaries. Retrieved 26 February 2011/8/2/2018.**
- **[15] https://www.nature.com/articles/nmicrobiol2017120/8/2/2018.**
- **[16] S.D. Harris, Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems, Mycologia, 6, 100, 823–32. 2008.**
- **[17] J. W. Deacon, Fungal Biology, Wiley, 267–276, 2013.**
- **[18] http://www.medical-labs.net/fungi-hyphae-1522/forms-of-hyphae-septate-and-coenocytic-hyphae.**
- **[19] J. R. Hanson, Chemistry of fungi, 127–141, 2008.**
- **[20] S. T. Moss, The Biology of Marine Fungi, Cambridge, UK: Cambridge University Press, 76, 1986.**
- **[21] M. A. Peñalva, H.N. Arst, Regulation of gene expression by ambient pH in filamentous fungi and yeasts, Microbiology and Molecular Biology Reviews, 3, 66, 426–46, 2002.**
- **[22] J. L. Pereira, E.F. Noronha, R. N. Miller, O.L. Franco, Novel insights in the use of hydrolytic enzymes secreted by fungi with biotechnological potential, Letters in Applied Microbiology, 6, 44, 573–81, 2007.**
- **[23] R. Fischer, N. Zekert, N. Takeshita, Polarized growth in fungi--interplay between the cytoskeleton, positional markers and membrane domains, Molecular Microbiology, 4, 68, 813–26, 2008.**
- **[24] M. J. Christensen, R.J. Bennett, H.A. Ansari, H. Koga, R.D. Johnson, G. T. Bryan, W. R. Simpson, J. P. Koolaard, E. M. Nickless, C. R. Voisey, Epichloë endophytes grow by intercalary hyphal extension in elongating grass leaves, Fungal Genetics and Biology, 2, 45, 84–93, 2008.**
- **[25] N. P. Money, Mushroom stem cells, BioEssays, 10, 24, 949–52, 2002.**
- **[26] M. Willensdorfer, On the evolution of differentiated multicellularity, Evolution; International Journal of Organic Evolution, 2, 63, 306–23, 2009.**

- **[27] K.J. Daniels, T. Srikantha, S.R. Lockhart, C. Pujol, D.R. Soll, Opaque cells signal white cells to form biofilms in Candida albicans, The EMBO Journal, 10, 25, 2240–52, 2006.**
- **[28] G. A. Marzluf, Regulation of nitrogen metabolism and gene expression in fungi, Microbiological Reviews, 3, 45, 437–61, 1981.**
- **[29] J. Heitman, Sexual reproduction and the evolution of microbial pathogens, Current Biology, 17, 16, R711–25, 2006.**
- **[30] D. Redecker, P. Raab, Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers, Mycologia, 6, 98, 885–95, 2006.**
- **[31] R. L. Metzenberg, N. L. Glass, Mating type and mating strategies in Neurospora, BioEssays, 2, 12, 53–9, 1990.**
- **[32] M. B. Linder, G. R. Szilvay, T. Nakari-Setälä, M. E. Penttilä, Hydrophobins: the protein-amphiphiles of filamentous fungi, FEMS Microbiology Reviews, 5, 29, 877–96, 2005.**
- **[33] F. Trail, Fungal cannons: explosive spore discharge in the Ascomycota, FEMS Microbiology Letters, 1, 276, 12–8, 2007.**
- **[34] A. Pringle, S. N. Patek, M. Fischer, J. Stolze, N. P. Money, The captured launch of a ballistospore, Mycologia, 4, 97, 866–71, 2005.**
- **[35] H. J. Brodie, The Bird's Nest Fungi, Toronto, Ontario: University of Toronto Press, 80, 1975.**
- **[36] R. E. Lee, Phycology, Cambridge University Press, 2008.**
- **[37] Z. K. Yang, Y. F. Niu, Y. H. Ma, J. Xue, M. H. Zhang, W. D. Yang, J. S. Liu, S. H. Lu, Y. Guan, H. Y. Li, Molecular and cellular mechanisms of neutral lipid accumulation in diatom following nitrogen deprivation, Biotechnology for biofuels, 1, 6, 67, 2013.**
- **[38] https://www.scitechnol.com/scholarly/pathogenic-microorganisms-journals-articles-ppts-list.php.**
- **[39] Nonpathogenic, Definition, meaning & more, Collins Dictionary, Retrieved 2016-06-30.**
- **[40] A. Zargar, D. N. Quan, David, K. K. Carter, M. Guo, H. O. Sintim, G. F. Payne, W. E. Bentley, Bacterial Secretions of Nonpathogenic Escherichia coli Elicit Inflammatory Pathways: a Closer Investigation of Interkingdom Signaling, mBio, 2, 6, 25–15. 2015.**
- **[41] G. F. Rall, P. J. Esteves, J. Abrantes, S. Bertagnoli, P. Cavadini, D. Gavier-Widén, J. S. Guitton, A. Lavazza, E. Lemaitre, J. Letty, A. M. Lopes, A. S. Neimanis, N. Ruvoën-Clouet, J. Le Pendu, S. Marchandeau, C. Le Gall-Reculé, Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump?, PLOS Pathogens, 11, 11, 2015.**
- **[42] D. Liu, Molecular Approaches to the Identification of Pathogenic and Nonpathogenic Listeriae, Microbiology Insights, 59, 2013.**
- **[43] Z. Naz, INTRODUCTION TO BIOTECHNOLOGY, Research Gate, 2015.**
- **[44] R. Christensen, J. Davis, G.M.P. Ochoa, W. Schmidt, BIOTECHNOLOGY: AN OVERVIEW, P.J. Industry & Services, European investment bank, 2002.**
- **[45] A. T. Estévez, The Future of Architecture: Bio digital Architecture and Genetics, ESARQ the Architecture School of the Universitat Internacional de Catalunya, Barcelona, Architecture Research, 4(1B), 13-20, 2014.**
- **[46] A. Bartoszek, A. Bekierska, J. Bell-lloch, T. De Groot, E. Singer, M. Woźniak, Managing innovations in biotechnology, European Project Semester, 2006. https://dugi-doc.udg.edu/bitstream/handle/10256/4289/1Memoria.pdf?sequence=1&isAllowed=y.**
- **[47] ACGT: Alliance for Cancer Gene Therapy, https://acgtfoundation.org/.**
- **[48] Intellectual Property and the Commons in Synthetic Biology: Strategies to Facilitate an Emerging Tec, W97 binnenwerk-8, Rathenau Constructing Life, IEEE, 2006.**
- **[49] T. Nakano, Molecular Communication, Cambridge. 2013.**
- **[50] Registry of Standard Biological Parts, Retrieved 2014-09-11. http://parts.igem.org/Main_Page.**
- **[51]E. C. Hayden, Synthetic-biology firms shift focus, Nature, 7485, 505, 598, 2014.**
- **[52] A. E. Osbourn, P.E. O'Maille, S. J. Rosser, K. Lindsey, Synthetic biology, New Phytologist, 3, 196, 671–677, 2012.**
- **[53] E. Andrianantoandro, S. Basu, D. K. Karig, R. Weiss, Synthetic biology: new engineering rules for an emerging discipline, Molecular Systems Biology, 2006.**

- **[54] http://www.materialecology.com-http://2012.acadia.org.**
- **[55] M. Hensel, (Synthetic) life architectures: ramifications and potentials of a literal biological paradigm for architectural design, Architecture Studies, 76, 18–25, 2006.**
- **[56] B. M. Cruz, S. Pike, Neoplasmatic design, Architecture Studies, 76, 6–7, 2008.**
- **[57] A. Tathe, M. Ghodke, A. P. nikalje, A Brief Review: Biomaterials and Their Application, International Journal of Pharmacy and Pharmaceutical Sciences, 2,4, 2010.**
- **[58] B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons, Biomaterials Science: An Evolving, Multidisciplinary Endeavor, In book: Biomaterials Science, 2012.**
- **[59] https://en.wikipedia.org/wiki/Bio-based_material-30-1-2018.**
- **[60] M. Vert, Y. Doi, K. H. Hellwich, M. Hess, P. Hodge, P. Kubisa, M. Rinaudo, F. Schué, Terminology for biorelated polymers and applications, Pure and Applied Chemistry, 2, 84, 377–410, 2012.**
- **[61] G. K. Sims, A. M. Cupples, Factors controlling degradation of pesticides in soil, Pesticide Science, 55, 598–601, 1999.**
- **[62] G.K. Sims, The effects of sorption on the bioavailability of pesticides, London: Springer Verlag, 119–137, 1991.**
- **[63] Measuring Biodegradability, The University of Waikato, June 19, 2008.**
- **[64] https://www.treehugger.com/green-architecture/mycotecture-mushroom-bricks-philip-ross.html/23/2/2018.**
- **[65] https://inhabitat.com/phillip-ross-molds-fast-growing-fungi-into-mushroom-building-bricks-that-are-stronger-than-concrete/30-1-2018.**
- **[66] https://www.dezeen.com/2013/10/20/mycelium-chair-by-eric-klarenbeek-is-3d-printed-with-living-fungus/30-1-2018.**
- **[67] https://inhabitat.com/3d-printed-mycelium-chair-sprouts-living-ushrooms/eric_klarenbeek_1.**
- **[68] J. B. Cleveland, The potential application of Natural Fiber Reinforced Bio-polymer (NFRbP Composites in Architecture), Faculty of The School of Architecture University of Southern California, Master of Building Science. 2008.**
- **[69] F. C. Campbell, Introduction to Composite Materials, Structural Composite Materials, Material Information Society, 2010. www.asminternational.org.**
- **[70] E. M. González, E. Keller, M. Joachim, Ecovative Design-Growing Architecture Through Mycelium and Agricultural Waste, AAR, Columbia University, GSAPP.2010.**
- **[71] L. Drzal, R. Inc, Sustainable Bio-based Structural Composite materials for Housing and Infrastructure Applications: Opportunities and Challenges.**
- **[72] https://inhabitat.com/nyc/the-livings-100-organic-hy-fi-towers-win-moma-ps1s-2014-yap-competition/30-1-2018.**
- **[73] https://inhabitat.com/nyc/hy-fi-tower-made-of-self-assembling-mushroom-root-bricks-rises-at-moma-ps1.**
- **[74] P. Watnick, R. Kolter, Biofilm, city of microbes, Journal of Bacteriology, 10, 182, 2675–2679, 2000.**
- **[75] C. D. Nadell, J. B. Xavier, K. R. Foster, The sociobiology of biofilms, FEMS Microbiology Reviews, 1, 33, 206–224, 2009.**
- **[76] D. López, H. Vlamakis, R. Kolter, Biofilms, Cold Spring Harbor Perspectives in Biology, 7, 2, 2010.**
- **[77] L. Hall-Stoodley, J. W. Costerton, P. Stoodley, Bacterial biofilms: from the natural environment to infectious diseases, Nature Reviews Microbiology, 2, 2, 95–108, 2004.**
- **[78] M. D. Robertson, A. K. Paz, M. Zhang, I. K. Gal, Architects of nature: growing buildings with bacterial biofilms, Microbial Biotechnology, 5, 10, 1157–1163, 2017.**
- **[79] G. A. R. Kolter, 1998, Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis, Molecular Microbiology, 3, 28, 449–461, 1998.**
- **[80] G. A. O'Toole, R. Kolter, Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development, Molecular Microbiology, 2, 30, 295–304, 1998.**
- **[81] E. Karatan, P. Watnick, Signals, regulatory networks, and materials that build and break bacterial biofilms, Microbiology and Molecular Biology Reviews, 2, 73, 310–47, 2009.**

- **[82] D. An, M. R. Parsek, The promise and peril of transcriptional profiling in biofilm communities, Current Opinion in Microbiology, 3, 10, 292, 2007.**
- **[83] C. Case, B. Funke, G. Tortora, Microbiology an Introduction, (10th eds) Creative Pultrusions, Pultex Global Design Manual, 2000.**
- **[84] V. A. Martins dos Santos, M. M. Yakimov, K. N. Timmis , P. N. Golyshin, Genomic Insights into Oil Biodegradation in Marine Systems, In book: Microbial Biodegradation: Genomics and Molecular Biology, Caister Academic Press, UK, 2008.**
- **[85] E. In Díaz, Microbial Biodegradation: Genomics and Molecular Biology, Horizon Scientific Press, 2008.**
- **[86] S. L. Chua, V. B. Wang, Z. Cai, K. Sivakumar, S. Kjelleberg, B. Cao, S. C. Loo, L. Yang, A stable synergistic microbial consortium for simultaneous azo dye removal and bioelectricity generation, Bioresource Technology, 155, 71–76, 2014.**
- **[87] R. Y. Zhang, S. Bellenberg, W. Sand, T. R. Neu, M. Vera, The Biofilm Lifestyle of Acidophilic Metal/Sulfur-Oxidizing Microorganisms, In: Biotechnology of Extremophiles: Advances and Challenges, Rampelotto Pabulo H (Ed.), Springer International Publishing, Cham, Switzerland, 177-213, 2016.**
- **[88] T. Abee, A. T. Kovács, O. P. Kuipers, S. Van Der Veen, Biofilm formation and dispersal in Gram-positive bacteria, Current Opinion in Biotechnology, 2, 22, 172–9. 2011.**
- **[89] F. Rossi, R. De Philippis, Role of Cyanobacterial Exopolysaccharides in Phototrophic Biofilms and in Complex Microbial Mats, Meeks JC, Haselkorn R, eds, Life, 2, 5, 1218-1238, 2015.**
- **[90] T. Danhorn, C. Fuqua, Biofilm formation by plant-associated bacteria, Annual Review of Microbiology, 61, 401–22, 2007.**
- **[91] L. M. Joubert, G. M. Wolfaardt, A. Botha, Microbial Exopolymers Link Predator and Prey in a Model Yeast Biofilm System, Microbial Ecology, 2, 52, 187–197, 2006.**
- **[92] A. Orell, S. Schopf, L. Randau, M. Vera, Biofilm Lifestyle of Thermophile and Acidophile Archaea, In: Witzany G (ed). Biocommunication of Archaea, Springer, Switzerland, 133-146, 2017.**
- **[93] C. Van Colen, G. J. C. Underwood, J. Serôdio, D. M. Paterson, Ecology of intertidal microbial biofilms: Mechanisms, patterns and future research needs, Journal of Sea Research, 92, 2 – 5, 2014.**
- **[94] M. Burmølle, A. Kjøller, S. Sørenses, Microbial Biofilms: Current Research and Applications, Caister Academic Press, 2012.**
- **[95] G. Lear, G. D. Lewis, Microbial Biofilms: Current Research and Applications, Caister Academic Press, 2012.**
- **[96] G. Lear, G. D. Lewis, Microbial Biofilms: Current Research and Applications, Horizon Scientific Press, pp. 61–71.**
- **[97] M. D. Robertson, C. R. Figueroa, M. Zhang, Radical Vernacular: Bacterial Architecture on Mars, Journal of the British Interplanetary Society, 67, 213-217, 2014.**
- **[98] http://www.ncl.ac.uk/press.office/press.release/item/cracks-in-your-concrete-you-need-bacillafilla#.UY5Lib9Z-8 [11.02.2014].31-1-2018.**
- **[99] S. S.Fisher, J. Galinat, S. Bang, Microbiological precipitation of CaCO3, Soil Biol., 31, 1563–1571, 1999.**
- **[100] E. B. Jacob, Bacterial Complexity: More is Different on All Levels, In Systems Biology: The Challenge of Complexity, ed. S. Nakanishi, R. Kageyama, D. Watanabe, Springer, Tokyo, 25–35, 2009.**
- **[101] E. B. Jacob, From snowflake formation to growth of bacterial colonies II: Cooperative formation of complex colonial patterns, Contemp. Phys., 38, 205-241, 1997.**
- **[102] R. M. Brown, Jr. Microbial Cellulose: A New Resource for Wood, Paper, Textiles, Food and Specialty Products, Department of Botany, The University of Texas at Austin, Austin, Texas, 78713-764, http://www.botany.utexas.edu/facstaff/facpages/mbrown/position1.htm.**
- **[103] M. Faidi, Feasability of Bacterial Cellulose in Furniture Design, Thesis, Aalto University School of Arts, Design and Architecture Interior architecture, 2017.**
- **[104] http://www.isplora.com/news/algaetecture-1/9/2018.**
- **[105] https://www.dezeen.com/2012/03/06/xylinum-by-jannis-hulsen.**
- **[106] http://news.mit.edu/2014/engineers-design-living-materials-1/30/2018.**

- **[107] A. T. Estévez, Learning from Nature: architecture and design in the first biodigital age- "Aprendiendo de la naturaleza", 2nd International Conference of Biodigital Architecture & Genetics, ESARQ (UIC), Barcelona, 2014.**
- **[108] T. F. Hansen, The Evolution of Genetic Architecture, Annual Review of Ecology, Evolution, and Systematics, 37, 1, 123–157, 2006.**
- **[109] C. Miles, M. Wayne, Quantitative trait locus (QTL) analysis, Nature Education, 2008.**
- **[110] T. F. C. Mackay, The Genetic Architecture of Quantitative Traits, Annual Review of Genetics, 35, 1, 303–339, 2001.**
- **[111] B. Kolarevic, Digital Architectures, University of Pennsylvania, USA, ACADIA, Eternity, Infinity and Virtuality, 254, 2000.**
- **[112] http://syndebio.com/algaezebo/SyndebioAlga(e)zebo by marcosandmarjan with richard beckett » - 1/9/2018.**
- **[113] https://www.ucl.ac.uk/bartlett/architecture/research/algaezebo/23/2/2018.**
- **[114] http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=162-2-2-2018.**
- **[115] http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=20&idproj=163-2-2-2018.**
- **[116] http://www.ecologicstudio.com/v2/project.php?idcat=3&idsubcat=71&idproj=148-2-2-2018.**
- **[117] http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=59&idproj=35-2-2-2018.**
- **[118] http://www.ecologicstudio.com/v2/project.php?idcat=7&idsubcat=71&idproj=127-2-2-2018.**
- **[119] N. M. Luscombe, D. Greenbaum, M. Gerstein, Review -What is bioinformatics? An introduction and overview, USA, Yearbook of Medical Informatics, 2001.**
- **[120] S. Kalantari, M. Hassn, S. Tabari, GROWMORPH: Bacteria Growth Algorithm and Design, Conference: Proceedings of the 22nd CAADRIA Conference, At: Xi'an Jiaotong-Liverpool University, Suzhou, China, 479-488, 2017.**
- **[121] P. W. Murdoch, Mutually Reinforcing Design & Symbiosis, Architectural Design, Master of Architecture, Thesis, 2012. https://digital.library.ryerson.ca/islandora/object/RULA%3A1490.**
- **[122] T. Wilson, J. W. Hastings, Bioluminescence, Annual Review of Cell and Developmental Biology, 14, 197-230, 1998.**
- **[123] M. Zimmer, Glowing genes: A revolution in biotechnology, Prometheus Books, Amherst New York, 196, 2005.**
- **[124] I. G. Diez, E. M. Gonzalez, M. A. V. Gonzalez, P. Ortiz Calderon, Design of prototype BIOSYNTHETIC material for use as construction material, https://seguridadypromociondelasalud.fundacionmapfre.org/n132/docs/Article4.pdf.**
- **[125] G. Parashar, N. S. Gavali, T. Patil, Isolation and Characterization of Bioluminescent Bacteria, IJSART, 2, 5, 2016.**
- **[126] H. J. Weitz, Naturally bioluminescent fungi, Mycologist, Cambridge University Press, UK, 18, 1, 2004.**
- **[127] S. C. Sabharwal, S. P. Kathuria, M. M. Dhingra, A new bioluminescent fungal system, J. Biosci, 5, 1, 53–62, 1983.**
- **[128] J. G .Morin, J .Hastings, Energy transfer in a bioluminescent system, J Cell Physiol, 77, 3, 313–318, 1971.**
- **[129] K. Jia, R. E. Ionescu, Measurement of Bacterial Bioluminescence Intensity and Spectrum: Current Physical Techniques and Principles, Adv Biochem Eng Biotechnol, Springer International Publishing Switzerland, 154, 19–45, 2015.**
- **[130] J. Hastings, J. Morin, Bioluminescence. In: Prosser CL (ed) Neural and integrative animal physiology, Wiley, New York, NY, 131–170, 1991.**
- **[131] A. K. Campbell, Living light: biochemistry, function and biomedical applications, Essays Biochem, 24, 41-76. 1989.**
- **[132] W. D. McElroy, M. DeLuca, Firefly luminescence, In Chemi- and Bioluminescence, ed. JG Burr, New York, Dekker, 387–99, 1985.**
- **[133] W. D. McElroy, H. H. Seliger, Origin and evolution of bioluminescence, In Horizons in Biochemistry, ed, MKasha & B Pullman, New York, Academic, 91–101, 1962.**

- **[134] V. C. Bode, J. W. Hastings. The purification and properties of the bioluminescent system in Gonyaulax polyedra, Arch. Biochem. Biophys, 103, 488, 4, 1963.**
- **[135] J. B. Buck, J. F. Case, F. E. Hanson, Control of flashing in fireflies, III. Peripheral excitation. Biol. Bull, 125-234, 1963.**
- **[136] J. Malave-Orengo, E. N. Rubio-Marrero, C. Rios-Velazquez, Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico, Microbial Biotechnology and Bioprospecting laboratory, University of Puerto Rico, Mayaguez, Puerto Rico.**
- **[137] E. M. Ilondu, A. A. Okiti, Bioluminescence in Mushroom and its Application Potentials, Nigerian Journal of Science and Environment, 14, 134, 2016.**
- **[138] J. W. Hastings, C. J. Potrikas, S. C. Gupta, M. Kurfurst, J. C. Makemson, Biochemistry and physiology of bioluminescent bacteria, Ado. Microbiot Physiol, 26, 235-291, 1985.**
- **[139] V. Nunes-Halldorson, N. Duran, Bioluminescent Bacteria: LUX Genes as Environmental Biosensors, Brazil, 2USDA-ARS US Water Conservation Laboratory, U.S.A.**
- **[140] L. Y. C. Len, E. Meighen, Bacterial bioluminescence, biochemistry and molecular biology, progen Biotech inc Richmond, 2018.**
- **[141] C. Miyamoto, M. Boylan, L. Cragg, E. Meighen, Comparison of the lux systems in Vibrio harveyi and Vibrio fischeri, J. Biolumin. Chemilumin, 3, 193-199, 1989.**
- **[142] C. Miyamoto, E. A. Meighen, A. F. Graham, Transcriptional regulation of lux genes transferred into Vibrio harveyi, J. Bacteriol, 172, 2046-2054, 1990.**
- **[143] T. O. Baldwin, M. M. Ziegler, The biochemistry and molecular biology of bacterial bioluminescence, Chem. Biochem. Flavoenzymes, 3, 467-524, 1992.**
- **[144] G. Mitchell, J. W. Hastings, The effect of flavin isomers and analogues upon the color of bacterial bioluminescence, J Biol Chem, 244, 10, 2572–2576, 1969.**
- **[145] G. Thouand, P. Daniel, H. Horry, P. Picart, M. J. Durand, K. Killham, O. G. Knox, M. S. DuBow, M. Rousseau, Comparison of the spectral emission of lux recombinant and bioluminescent marine bacteria, Luminescence, 18, 3, 145–155, 2003.**
- **[146] E. G. Ruby, K. H. Nealson, A luminous bacterium that emits yellow light, Science, 196, 4288, 432–434, 1977.**
- **[147] J. Hastings, Chemistries and colors of bioluminescent reactions: a review, Gene, 173, 1, 5–11, 1996.**
- **[148] T. W Cline, J. W Hastings, Mutated luciferases with altered bioluminescence emission spectra, J Biol Chem, 249, 14, 4668–4669, 1974.**
- **[149] L. Y Lin, C.R. Szittner, R. Friedman, E. A. Meighen, Changes in the kinetics and emission spectrum on mutation of the chromophore-binding platform in Vibrio harveyi luciferase, Biochemistry, 43, 11, 3183–3194, 2004.**
- **[150] J. Lee, DJ .O'Kane, BG. Gibson, Bioluminescence spectral and fluorescence dynamics study of the interaction of lumazine protein with the intermediates of bacterial luciferase bioluminescence, Biochemistry, 28, 10, 4263–4271, 1989.**
- **[151] H. Karatani, J. Hastings, Two active forms of the accessory yellow fluorescence protein of the luminous bacterium vibrio fischeri strain Y1., J Photochem Photobiol B: Biol, 18, 2, 227–232, 1993.**
- **[152] H. Karatani, T. Wilson, J. Hastings, A blue fluorescent protein from a yellow-emitting luminous bacterium, Photochem Photobiol, 55, 2, 293–299, 1992.**
- **[153] V. N. Petushkov, M. Ketelaars, B. G. Gibson, J. Lee, Interaction of photobacterium leiognathi and vibrio fischeri Y1 luciferases with fluorescent (antenna) proteins: bioluminecence effects of the aliphatic additive, Biochemistry, 35, 37, 12086–12093, 1996.**
- **[154] D. Ke, S. C. Tu, Activities, kinetics and emission spectra of bacterial luciferase-fluorescent protein fusion enzymes, Photochem Photobiol, 87, 6, 1346–1353, 2011.**
- **[155] A. Eberhard, A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, N. J. Oppenheimer, Structural identification of autoinducer of Photobacterium fischeri luciferase, J.Biochemistry, 20, 2444-2449, 1981.**

- **[156] M. Kannahi, S. Sivasankari, Isolation and Identification of Bioluminescent Bacteria from Marine Water at Nagapattinam Sea Shore Area, International Journal of Pharmaceutical Sciences Review and Research, 26, 2, 346-351, 2014.**
- **[157] T. Charrier, M. J. Durand, S. Jouanneau, M. Dion, M. Pernetti, D. Poncelet, G. Thouand, A multi-channel bioluminescent bacterial biosensor for the on-line detection of metals and toxicity. Part I: design and optimization of bioluminescent bacterial strains, Anal Bioanal Chem, 400, 4, 1051–1060, 2011.**
- **[158] V. S. Kulkarni, B. S. Kulkarni, Isolation of Bioluminescent Bacteria and Their Application in Toxicity Testing of Chromium in Water, International Journal of current microbiology & applied science, 4, 10, 23-32, 2015.**
- **[159] P. V. Dunlap, E. P. Greenberg, Control of Vibrio fischeri lux gene transcription by a cyclic AMP receptor protein LuxR protein regulatory circuit, J. Bacteriol, 170, 4040-4046, 1988.**
- **[160] P. V. Dunlap, E. P. Greenberg, Control of Vibrio fischeri luminescence gene expression in Escherichia coli by cyclic AMP and cyclic AMP receptor protein, J. Bacteriol, 164, 45-50, 1985.**
- **[161] J. Engebrecht, K. H. Nealson, M. Silverman, Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri, Cell, 32, 773-781, 1983.**
- **[162] R. K. Gupta, S. S. Patterson, S. Ripp, M. L. Simpson, G. S. Sayler, Expression of the Photorhabdus luminescens lux genes (lux A, B, C, D, and E) in Saccharomyces cerevisiae, FEMS Yeast Res, 4, 3, 305–313, 2003.**
- **[163] D. Close, T. Xu, A. Smartt, A. Rogers, R. Crossley, S. Price, S. Ripp, G. Sayler, The evolution of the bacterial luciferase gene cassette (lux) as a real-time bioreporter, Sensors, 12, 1, 732–752, 2012.**
- **[164] D. M. lose, S. S. Patterson, S. Ripp, S. J. Baek, J. Sanseverino, G. S. Sayler, Autonomous bioluminescent expression of the bacterial luciferase gene cassette (lux) in a mammalian cell line, PLoS One, 5, 8, 2010.**
- **[165] V. S. Bondar, O. Shimomura, J. I. Gitelson, Luminescence of Higher Mushrooms, Journal of Siberian Federal University, Biology, 4, 331-351, 2012.**
- **[166] R. L. Airth, W. D. McElroy, Light emission from extracts of luminous fungi, J. Bacteriol, 77, 249–250. 1959.**
- **[167] R. Dubois, Soc. Biol, 37, 559 – 562, 1885.**
- **[168] K. V. Purtov, V. N. Petushkov, M. S. Baranov, K. S. Mineev, N. S. Rodionova, Z. M. Kaskova, A. S. Tsarkova, A. I. Petunin, V. S. Bondar, E. K. Rodicheva, S. E. Medvedeva, Y. Oba, Y. Oba, A. S. Arseniev, S. Lukyanov, J. I. Gitelson, I. V. Yampolsky, The Chemical Basis of Fungal Bioluminescence, Chem. Int, 54, 8124 –8128, 2015. Wiley.Weinheim-www.angewandte.org.**
- **[169] E. N. Harvey, A History of Luminescence From the Earliest Times Until 1900, American Philosophical Society, Philadelphia, USA, 1957.**
- **[170] R. L. Airth, Characteristics of cell-free fungal bioluminescence, Johns-Hopkins Press, Baltimore, 262 – 273, 1961.**
- **[171] R. L. Airth, G. E. Foerster, The isolation of catalytic components required for cell-free fungal bioluminescence, Arch.Biochem.Biophy, 97, 567 – 573, 1962.**
- **[172] A. C. Gonzalez Veron, M. M. Ibarria, E. Garcia Lopez, Bioluminescent algae and possible implications in architectural design, PLEA2013-29th Conference, Sustainable Architecture for a Renewable Future, Munich, Germany, 3-4, 2013.**
- **[173] https://www.sott.net/article/238256-Philips-Bio-Light-Concept-Lights-The-Home-Using-Bacteria.**
- **[174] R. A. Nastro, N. Jannelli, M. Minutillo, M. Guida, M. Trifuoggi, L. Andreassi, A. L. Facci, V. K. Krastev, G. Falcucci, Performance evaluation of Microbial Fuel Cells fed by solid organic waste: parametric comparison between three generations, The 8th International Conference on Applied Energy – ICAE2016, Energy Procedia, 105, 1102 – 1108, 2017.**
- **[175] M. Rahimnejad, A. Adhami, S. Darvari, A. Zirepour, S.E. Oh, Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Engineering Journal, 54, 745–756, 2015.**
- **[176] M. Falk, Z. Blum, S. Shleev, Direct electron transfer based enzymatic fuel cells, Electrochimica Acta, 82, 2012.**

- **[177] C. Santoro, C. Arbizzani, B. Erable, I. Ieropoulos, Microbial fuel cells: From fundamentals to applications. A review, Journal of Power Sources, 356, 2017.**
- **[178] M. Rahimnejad, A. A. Ghoreyshi, G. Najafpour, T. Jafary, Power generation from organic substrate in batch and continuous flow microbial fuel cell operations, Appl. Energy, 88, 3999–4004, 2011.**
- **[179] M. C. Potter, Electrical effects accompanying the decomposition of organic compounds, Proc. Royal Soc. London, Ser. B, Containing Pap. Biol. Charact, 84, 260–276, 1911.**
- **[180] K. Lewis, Symposium on bio electrochemistry of microorganisms. IV. Biochemical fuel cells, Bacteriol. Rev, 30, 101–113, 1966.**
- **[181] R. M. Allen, H. P. Bennetto, Microbial fuel-cells, Appl. Biochem. Biotech, 39, 27–40, 1993.**
- **[182] A. N. Z. Alshehria, K. M. Ghanema, S. M. Al-Garniaa, Application of a five level central composite design to optimize operating conditions for electricity generation in a microbial fuel cell, Egypt Science Direct, 2015.**
- **[183] B. E. Logan, Microbial Fuel Cells, John Wiley & Sons, Inc, 2008.**
- **[184] H. Liu, B. E. Logan, Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane, Environmental Science & Technology, 38, 4040–4046, 2004.**
- **[185] C. Y. Lai, C. H. Wub, C. T. Meng, C. W. Lin, Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode, Applied Energy, 188, 392–398, 2017.**
- **[186] J. M. Sonawane, A. Yadavb, P. C. Ghosha, S. B. Adelojua, Recent advances in the development and utilization of modern anode materials for high performance microbial fuel cells, Biosensors and Bioelectronics, 90, 558–576, 2017.**
- **[187] Z. Du, H. Li, T. Gu, A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy, Biotechnol. Adv, 25, 464–482, 2007.**
- **[188] M. Ghasemi, W. R. Wan, W. R. Ismail, M. Rahimnejad, A. F. Ismail, J. X. Leong, M. Miskan, K. Ben Liew, Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance, Int. J. Hydrogen Energy, 38, 5480–5484, 2012.**
- **[189] G. Antonopoulou, K. Stamatelatou, S. Bebelis, G. Lyberatos, Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell, Biochem. Eng. J, 50, 10–15, 2010.**
- **[190] M. Rahimnejad, G. Najafpour, A. A. Ghoreyshi, Effect of mass transfer on performance of microbial fuel cell, Intech, 5, 233–250, 2011.**
- **[191] Y. Sharma, B. Li, The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs), Bioresource. Technol, 101, 1844–1850, 2010.**
- **[192] H. Liu, R. Ramnarayanan, B. E. Logan, Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell, University Park, Pennsylvania.**
- **[193] G. Najafpour, M. Rahimnejad, A. Ghoreshi, The enhancement of a microbial fuel cell for electrical output using mediators and oxidizing agents, Energy Sourc, 33, 2239–2248, 2011.**
- **[194] M. Rahimnejad, N. Mokhtarian, G. Najafpour, W. Daud, A. Ghoreyshi, Low voltage power generation in a biofuel cell using anaerobic cultures, World Appl. Sci. J, 6, 1585–1588, 2009.**
- **[195] G. W. Chen, S.J. Choi, T. H. Lee, G. Y. Lee, J. H. Cha, C. W. Kim, Application of biocathode in microbial fuel cells: cell performance and microbial community, Appl. Microbiol. Biot, 79, 379–388, 2008.**
- **[196] G. Wang, L. Huang, Y. Zhang, Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells, Springer Science+Business Media B.V, Biotechnol Lett, 30, 1959–1966, 2008.**
- **[197] H. Liu, S. Cheng, B. E. Logan, Production of Electricity from Acetate or Butyrate Using a Single-Chamber Microbial Fuel Cell, Environ. Sci. Technol, 39, 658-662, 2005.**
- **[198] M. Di Lorenzo, T.P. Curtis, I. M. Head, K. Scott, A single-chamber microbial fuel cell as a biosensor for wastewaters, water research, 43, 3145–3154, 2009.**
- **[199] Y. Ahn, B. E. Logan, A multi-electrode continuous flow microbial fuel cell with separator electrode assembly design, Appl Microbiol Biotechnol, 93, 2241–2248, 2012.**

- **[200] B. Virdis, S. Freguia, R. A. Rozendal, K. Rabaey, Z. Yuan, J. Keller, Microbial Fuel Cells, Elsevier Science publisher, 2011.**
- **[201] B. E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, Environ. Sci. Technol, 40, 5181–5192, 2006.**
- **[202] B. H. Kim, I. S. Chang, G. M. Gadd, Challenges in microbial fuel cell development and operation, Appl. Microbiol. Biot, 76, 485–494, 2007.**
- **[203] U. Schroder, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency, Phys. Chem. Chem. Phys, 9, 2619–2629, 2007.**
- **[204] D. H. Park, S. K. Kim, I. H. Shin, Y. J. Jeong, Electricity production in biofuel cell using modified graphite electrode with Neutral Red, Biotechnology Letters, 22, 1301–1304, 2000.**
- **[205] D. Z. Khater, K. M. El-Khatib, H. M. Hassan, Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation, Journal of Genetic Engineering and Biotechnology, 15, 127–137, 2017.**
- **[206] D. Permana, D. Rosdianti, S. Ishmayana, S. D. Rachman, H. E. Putra, D. Rahayuningwulan, H. R. Hariyadi, Preliminary Investigation of Electricity Production Using Dual Chamber Microbial Fuel Cell (DCMFC) with Saccharomyces cerevisiae as Biocatalyst and Methylene Blue as an Electron Mediator, Procedia Chemistry, 17, 36-43, 2015.**
- **[207] D. H. Park, J. G. Zeikus, Improved Fuel Cell and Electrode Designs for Producing Electricity from Microbial Degradation, East Lansing, 2002.**
- **[208] H. Liu, S. Cheng, L. Huang, B. E. Logan, Scale-up of membrane-free single-chamber microbial fuel cells, 2008.**
- **[209] C. Yuvraj, V. Aranganathan, Enhancement of Voltage Generation Using Isolated Dissimilatory Iron-Reducing (DIR) Bacteria Klebsiella pneumoniae in Microbial Fuel Cell, Arab J Sci Eng, 42, 65–73, 2017.**
- **[210] D. H. Park, J. G. Zeikus, Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens, Appl Microbiol Biotechnol, 59, 58–61, 2002.**
- **[211] http://www.learnabout-electronics.org/resistors_18.php-1/6/2018.**
- **[212] R. G. Lerner, G.L. Trigg, Encyclopedia of Physics (2nd Edition), VHC publishers, 1991.**
- **[213] http://www.fuelcellstore.com/blog-section/polarization-curves-1/6/2018.**
- **[214]http://batteryuniversity.com/learn/article/bu_808c_coulombic_and_energy_efficiency_with_the_battery-1/6/2018.**
- **[215] A. R, Ferracina, G.Caldwell, I. Ieropoulos, G. Rimbu, A. Adamatzky, N. Phillips, D. De Lucrezia, B. Imhof, M.M. Hanczyc, J. Nogales, J.Garcia, Living Architecture (LIAR): Metabolically engineered building units, In: Heisel, F; Hebel, D, ed, Cultivated Building Materials: Industrialized Natural Resources for Architecture and Construction, Berlin, Germany, Birkhauser, 170- 177, 2017.**
- **[216] http://livingarchitecture-h2020.eu/project-description.**
- **[217] http://emciblab.com/-www.liquifer.at-www.explora-biotech.com.**
- **[218] https://federicoborello.com/2014/10/09/bioclad-adaptive-biodigital-cladding-system.**
- **[219] https://federicoborello.files.wordpress.com/2014/10/presentation_12.png.**
- **[220] http://www.architesi.polito.it/de_aglio_tesi.asp?id_tesi=24528.**
- **[221] http://www.architectmagazine.com/practice/bacteria-activated-wallpaper-that-generates-electricity.**
- **[222] S. H. Kacem, S. Galai, A. P. De los Ríos, F. J. H. Fernández, I. Smaali, New efficient laccase immobilization strategy using ionic liquids for bio-catalysis and microbial fuel cells applications, Journal of Chemical Technology & Biotechnology, 93, 174- 183, 2017.**
- **[223] U. N. Dwivedi, P. Singh, V. P. Pandey, A. Kumar, Review: structure-function relationship among bacterial, fungal and plant laccases, Journal of Molecular Catalysis B: Enzymatic, 68, 117–128, 2011.**
- **[224] L. F. Johnson, E. A. Curl, J. H. Bond, Fribourg, Methods for Studying Soil Mycoflora: Plant Diseases Relationships, Burgess Publishing Co, Minneapolis, 179, 1960.**
- **[225] A. H. Moubasher, Soil Fungi in Qatar and other Arab Countries, The Scientific and Applied Research Centre, First ed, University of Qatar, Doha, Qatar, 566, 1993.**

- **[226] T. Watanabe, Soil and Seed Fungi. Pictorial Atlas of Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species, Second ed, CRC Press, Boca Raton London New York Washington D.C, 2002.**
- **[227] V. K. S. Olga, V. S. Elena, P. G. Valeria, V. M. Olga, V. L. Natalia, N. D. Aida, I. J. Alexander, M. Alexander, Purification and characterization of the constitutive form of laccase from basidiomycete Coriolus hirsutus and effect of inducers on laccase synthesis, Biotechnology and Applied Biochemistry, 28, 47- 54, 1998.**
- **[228] P. Das, S. Mukherjee, R. Sen, Improved bioavailability and biodegradation of a model poly aromatic hydrocarbon by a biosurfactant producing bacterium of marine origin, Chemosphere, 72, 1229–1234, 2008.**
- **[229] M. I. A. Ali, S. A. Ouf, N. M. Khalil, M. N. Abd El-Ghany, Biosynthesis of laccase by Aspergillus flavus NG85 isolated from Saint Catherine protectorate, Egypt. J. Bot, 55, 2015.**
- **[230] N. Bhuneshwari, C. Rachana, M.G. Roymom, Lignocellulolytic Fungal Isolation and Screening for Their Laccase Producing Ability, Indian J.Sci.Res, 13, 2, 188-191, 2017.**
- **[231] http://www.thermoscientificbio.com/.**
- **[232] S. Cheng, H. Liu, B. Logan, Increased performance of single chamber microbial fuel cells using an improved cathode structure, Electrochemistry Communications, 8, 489–494, 2006.**
- **[233] G. S. Jadhav, M. M. Ghangrekar, Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration, Bioresource Technology, 100, 717-723, 2009.**
- **[234] https://www.energyavenue.com/LED-Light-Bulbs/Festoon/08-Watt, 2019.**
- **[235] P. Ghosh, U. Ghosh, Statistical optimization of laccase production by Aspergillus flavus PUF5 through submerged fermentation using agro-waste as cheap substrate, Acta Biologica Szegediensis, 61, 25-33, 2017.**
- **[236] K. Brijwani, A. Rigdon, P. V. Vadlani, Fungal Laccases: Production, Function, and Applications in Food Processing, Enzyme Research, 10, 2010.**
- **[237] H. V. Prajapati, F. P. Minocheherhomji, Laccase - a wonder molecule: a review of its properties and applications, International Journal of Pure & Applied Bioscience, 6, 766-773, 2018.**
- **[238] G. M. Scott, M. Lentz, M. Akhtar, M. Sykes, S. Abubakr, Environmental aspects of biosulfite pulping, Proceedings of 1995 international environmental conference Atlanta: TAPPI, 1155-1161, 1995.**
- **[239] M. Hazuchová, D. Chmelová, M. Ondrejovič, The optimization of propagation medium for the increase of laccase production by the white-rot fungus Pleurotus ostreatus, Nova Biotechnologica et Chimica, 16, 113-123, 2017.**
- **[240] J. F. Osma, U. Moilanen, L. J. Toca-Herrera, S. Rodrıguez-Couto, Morphology and laccase production of white-rot fungi grown on wheat bran flakes under semi-solid-state fermentation conditions, FEMS Microbiol Lett, 318, 1, 27-34, 2011.**
- **[241] T. Robinson, G. McMullan, R. N. Marchant, P. Remediation of dyes in textile effluent, a critical review on current treatment technologies with a proposed alternative, Bioresource Technology, 77, 247-255, 2001.**
- **[242] J. L. Toca-Herrera, J. F. Osma, S. Rodríguez Couto, Potential of solid-state fermentation for laccase production, Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Méndez-Vilas, 2014.**
- **[243] V. Vivekanand, P. Dwivedi, N. Pareek, R. P. Singh, Banana Peel: A potential substrate for laccase production by Aspergillus fumigatus VkJ2.4.5 in solid-state fermentation, Applied Biochemistry and Biotechnology, 165, 204–220, 2011.**
- **[244] S. S. Bhattacharya, V. K. Garlapati, R. Banerjee, Optimization of laccase production using response surface methodology coupled with differential evolution, New Biotechnology, 28, 31–39, 2011.**
- **P. M. Kirk, P. F. Cannon, D. W. Minter, J. A. Stalpers, Dictionary of the fungi (10th ed.), CABI, Wallingford, UK, 2008.**
- **[245] B. Cong, N. Wang, S. Liu, F. Liu, X. Yin, J. Shen, Isolation, characterization and transcriptome analysis of a novel Antarctic Aspergillus sydowii strain MS-19 as a potential lignocellulosic enzyme source, BMC Microbiology, 17, 129, 2017.**
- **[246] S. S. More, P. S. Renuka, K. Pruthvi, M. Swetha, S. Malini, S. M. Veena, Isolation, purification, and characterization of fungal laccase from Pleurotus sp., Enzyme Research, 2011.**

- **[247] S. B. Pointing, E. B. G. Jones, L. L. P. Vrijmoed, Optimization of laccase production by Pycnoporus sanguineus in submerged liquid culture, Mycologia, 92, 1, 139-144, 2000.**
- **[248] N. M. Khalil, M. Ali, S. Ouf, M. Abdelghany, Characterization of Aspergillus flavus NG 85 laccase and its dye decolorization efficiency. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7, 817-826, 2016.**
- **[249] R. C. Minussi, G.M. Pastore, N. Duran, Potential applications of laccase in the food industry, Trends in Food Science & Technology, 13, 205–216, 2002.**
- **[250] R. Couto, S. S. Maria, Application of solid-state fermentation to ligninolytic enzyme production, Biochemical Engineering Journal, 36, 211-219, 2005.**
- **[251] D. Pant, G. Van Bogaert, L. Diels, K. Vanbroekhoven, A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production, Bioresource Technology, 101, 1533-1543, 2010.**
- **[252] P. Tanikkula, N. Pisutpaisala, Performance of a membrane-less air-cathode single chamber microbial fuel cell in electricity generation from distillery wastewater, Energy Procedia, 79, 646 – 650, 2015.**
- **[253] S. E. Oh, B. E. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, Water Research, 39, 4673-4682, 2005.**
- **[254] A. Tardast, M. Rahimnejad, G. Najafpour, A. A. Ghoreyshi, H. Zare, Fabrication and operation of a novel membrane-less microbial fuel cell as a bioelectricity generator, Int. J. Environ. Eng, 3, 2012.**
- **[255] Aspergillus: Habitat, Reproduction and Importance, Ascomycotina-http://www.biologydiscussion.com/fungi/aspergillus-habitat-reproduction-and-importance-ascomycotina/24000.**
- **[256] B. Ingalls, Mathematical Modelling in Systems Biology: An Introduction, Applied Mathematics, University of Waterloo, 2012.**
- **[257] H. Hatzikirou, A. Deutsch, A. G. Hoekstra, Lattice-Gas Cellular Automaton Modeling of Emergent Behavior in Interacting Cell Populations, Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer, Berlin Heidelberg, 2010.**
- **[258] K. Krawczyk, W. Dzwinel, D. A. Yuen, Nonlinear Development of Bacterial Colony Modeled with Cellular Automata and Agent Objects, International Journal of Modern Physics C, 2003.**
- **[259] P. Hogeweg, Cellular Automata as a Paradigm for Ecological Modeling, Bioinfnmutica Paduulaan 8-Utrecht, The Netherlands.**
- **[260] P. Gerlee, A. R. Anderson, Stability Analysis of a Hybrid Cellular Automaton Model of Cell Colony Growth, Division of Mathematics, University of Dundee, Scotland, 2018.**
- **[261] M. S. Alber, M. A. Kiskowski, J. A. Glazier, Y. Jiang, On Cellular Automaton Approaches To Modeling Biological Cells, https://www2.seas.gwu.edu/~simhaweb/iisc/Alber.pdf.**
- **[262] M. De Domenico, F. B. Kessler, Multilayer Network Modeling of Integrated Biological Systems-Network Science of Biological Systems at Different Scales A Review, Physics of Life Reviews, 24, 149-152, 2018.**
- **[263] S. Kelleter, Simulation of Fungal Growth and Structure- Development of an Extendable Basic Model of Coprinopsis cinerea, Master Thesis, University of Gottingen, Faculty of Forest Sciences and Forest Ecology, 2017.**
- **[264] M. Fricker, D. Bebber, L. Boddy, Mycelial Networks: Structure and Dynamics, British Mycological Society Symposium Series, 2008.**
- **[265] G. P. Boswell, H. Jacobs, K. Ritz, G. M. Gadd, F. A. Davidson, The Development of Fungal Networks in Complex Environments, Society for Mathematical Biology, Bulletin of Mathematical Biology, 69, 605–634, 2007.**
- **[266] F. A. Davidson, G. P. Boswell, M. W. F. Fischer, L. Heaton, D. Hofstadler, M. Roper, Mathematical modelling of fungal growth and function, IMA Fungus, 2, 1, 33-37, 2011.**
- **[267] J. M. Halley, H. N. Comins, J. H. Lawton, M. P. Hassell, Competition, succession and pattern in fungal communities: towards a cellular automaton model, OIKOS, 70, 435-442, 1994.**
- **[268] A. Meškauskas, M. D. Fricker, D. Moore, Simulating colonial growth of fungi with the Neighbour-Sensing model of hyphal growth, Mycological research, 108, 1241-1256, 2004.**

- **[269] A. Meškauskas, A. McNulty, D. Moore, Concerted regulation of tropisms in all hyphal tips is sufficient to generate most fungal structures, Mycological research, 108, 341-353, 2004.**
- **[270] F. Lagarde, N. Jaffrezic-Renault, Cell-based electrochemical biosensors for water quality assessment, Anal. Bioanal. Chem, 400, 947–964, 2011.**
- **[271] J. H. Lee, R. J. Mitchell, B. C. Kim, D. C. Cullen, M. B. Gu, A cell array-biosensor for environmental toxicity analysis, Biosens Bioelectron, 21, 3, 500–7, 2005.**
- **[272] M. Park, S. L. Tsai, W. Chen, Microbial Biosensors: Engineered Microorganisms as the Sensing Machinery, Sensors, 13, 5777-5795, 2013.**
- **[273] B. F. Pfleger, D.J. Pitera, J.D. Newman, V. J.J. Martin, J. D. Keasling, Microbial sensors for small molecules: Development of a mevalonate biosensor, Metab. Eng, 9, 30–38, 2007.**
- **[274] R. Daniel, R. Almog, A. Ron, S. Belkin, Y. S. Diamand, Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode, Biosensors and Bioelectronics, 24, 888–893, 2008.**
- **[275] L. Su, W. Jia, C. Hou, Y. Lei, Microbial biosensors: A review, Biosens. Bioelectron, 26, 1788–1799, 2011.**
- **[276] S. Daunert, G. Barrett, J. S. Feliciano, R. S. Shetty, S. Shrestha, W. Smith-Spencer, Genetically engineered whole-cell sensing systems: Coupling biological recognition with reporter genes, Chem. Rev, 100, 2705–2738, 2000.**
- **[277] S. Topp, J. P. Gallivan, Emerging applications of riboswitches in chemical biology, ACS Chem. Biol, 5, 139–148, 2010.**
- **[278] M. N. Win, C. D. Smolke, A modular and extensible RNA-based gene-regulatory platform for engineering cellular function, Proc. Natl. Acad. Sci, USA, 104, 14283–14288, 2007.**
- **[279] J. C. Liang, R. J. Bloom, C. D. Smolke, Engineering biological systems with synthetic RNA molecules, Mol. Cell, 43, 915–926, 2011.**
- **[280] J. Sinha, S. J. Reyes, J. P. Gallivan, Reprogramming bacteria to seek and destroy an herbicide. Nat. Chem. Biol, 6, 464–470, 2010.**
- **[281] A. Roda, L. Cevenini, E. Michelini, B. R. Branchini, A portable bioluminescence engineered cell-based biosensor for on-site applications, Biosens Bioelectron, 26, 8, 3647–53, 2011.**
- **[282] J. Huang, M. Hooijmans, C. A. Briasco, S. G. M. Geraats, K. M. Luyben, D. Thomas, Effect of free-cell growth parameters on oxygen concentration profiles in gel-immobilized recombinant Escherichia coli, Appl Microbiol Biotechnol, 33, 6, 619–23, 1990.**
- **[283] M. Affi, C. Solliec, P. Legentillomme, J. Comiti, J. Legrand, G. Thouand, Numerical design of a card and related physicochemical phenomena occurring inside agarose-immobilized bacteria: a valuable tool for increasing our knowledge of biosensors, Sensors Actuators B Chem, 138, 1, 310–7, 2009.**
- **[284] M. Affi , C. Solliec , P. Legentilhomme , J. Comiti, J. Legrand , S. Jouanneau, G. Thouand, Numerical modeling of the dynamic response of a bioluminescent bacterial biosensor, Anal Bioanal Chem, 408, 8761–8770, 2016.**
- **[285] E. A. Meighen, Bacterial bioluminescence: organization, regulation, and application of the lux genes, Faseb J, 7, 11, 1016–22, 1993.**
- **[286] J. M. Radovich, Mass transfer limitations in immobilized cells, Biotechnol Adv, 3, 1, 1–12, 1985.**
- **[287] C. Michan, M. Manchado, G. Dorado, C. Pueyo, In vivo transcription of the Escherichia coli oxyR regulon as a function of growth phase and in response to oxidative stress, J Bacteriol, 181, 9, 2759–64, 1999.**
- **[288] R. P. PINTO, Dynamic Modelling and Optimization Of Microbial Fuel Cells and Microbial Electrolysis Cells, Université de Montréal, Doctoral Thesis, 2011.**
- **[289] https://sciencing.com/various-types-microscopes-biology-5949595.html.**
- **[290] J. J. Tyson, K. C. Chen, B. Novak, Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell, Curr Opin Cell Biol, 15, 2, 221-31, 2003.**
- **[291] D. Mullassery, C. A. Horton, C. D. Wood, M. R. H. White, Single Live Cell Imaging for Systems Biology, Essays Biochem, 45, 121–133, 2008.**
- **[292] https://www.researchgate.net/figure/Light-microscopy-of-Aspergillus-spp-with-lactophenol-cotton-blue-stain-cultured-from-burn_fig1_261753683.**

- **[293] https://www.carolina.com/fungi-microscope-slides/aspergillus-conidiophores-slide-w-m/297872.pr.**
- **[294] Germination of conidia of Aspergillus niger is accompanied by major changes in RNA profiles- https://www.researchgate.net/figure/Germination-of-A-niger-conidia-as-observed-by-bright-field-A-and-fluorescence_fig1_235756427.**
- **[295] Sensitivity of Aspergillus nidulans to the Cellulose Synthase Inhibitor Dichlobenil: Insights from Wall-Related Genes' Expression and Ultrastructural Hyphal Morphologies- https://www.researchgate.net/figure/Bright-field-microscopy-pictures-40x-in-A-B-and-C-and-60x-in-D-of-mycelium-grown-in_fig2_258997623.**
- **[296] Screening of Microorganisms Isolated from some Enviro-Agro-Industrial Wastes in Saudi Arabia for Amylase Production, https://www.researchgate.net/figure/A-microscopic-picture-by-phase-contrast-microscope-of-the-most-potent-fungal-isolate_fig1_317169982.**
- **[297] http://www.microlabgallery.com/gallery/Asper2.aspx.**
- **[298]https://www.researchgate.net/figure/Photomicrographs-of-Aspergillus-terreus-isolate-A-4634-showing-a-normal-hyphae_fig6_265865019.**
- **[299] M. H. Lee, G. Wiedman, S. Park, A. Mustaev, Y. Zhao, D. S. Perlin, A novel, tomographic imaging probe for rapid diagnosis of fungal keratitis, Medical Mycology, 0, 1–7, 2017**
- **[300] S. Gurung, P. P. Yegneshwaran, P. Y. Bhutia, A. Gupta, I. Bairy, P. Jagat, P. Uttam, D. Sharma, T. Peggy, Onychomycosis in two geographically distinct regions in India, 2018. https://www.researchgate.net/publication/235330794-Onychomycosis-in-two-geographically-distinct-regions-in-India.**
- **[301] A. A. Ishijima, K. Hayama, J. P. Burton, G. Reid, M. Okada, Y. Matsushita, S. A. Teikyo, Effect of Streptococcus salivarius K12 on the In Vitro Growth of Candida albicans and its Protective Effect in an Oral Candidiasis Model, Appl Environ Microbiol, 78, 7, 2190-9, 2012.**
- **[302] A. Orell, V. Tripp, V. Aliaga-Tobar, S. V. Albers, V. Maracaja-Coutinho, L. Randau, A regulatory RNA is involved in RNA duplex formation and biofilm regulation in Sulfolobus acidocaldarius, Nucleic Acids Res, 18, 46, 9, 4794-4806, 2018.**
- **[303] http://www.bitplane.com/biofilms.aspx#prettyPhoto/0/11, 2018.**
- **[304] J. Kikuta, M. Ishii, Recent advances in intravital imaging of dynamic biological systems, J Pharmacol Sci, 119, 3, 193-7, 2012.**
- **[305] http://molpharm.aspetjournals.org/content/60/1/26.**
- **[306] A. Sergei, A. Aseyev, P. M. Weber, A. Anatoli, Ultrafast Electron Microscopy for Chemistry, Biology and Material Science, Journal of Analytical Sciences, Methods and Instrumentation, 3, 30-53, 2013.**
- **[307] J. E. McGregor, L. T. Staniewicz, S. E. Guthrie Neé Kirk, A. M. Donald, Environmental scanning electron microscopy in cell biology, Methods Mol Biol, 931, 493-516, 2013.**
- **[308]https://www.researchgate.net/figure/Development-of-A-niger-monitored-by-scanning-electron-microscopy-The-vegetative_fig6_235763153.**
- **[309] Australian Dust Storm Associated with Extensive Aspergillus sydowii Fungal "Bloom" in Coastal Waters, https://www.researchgate.net/figure/Aspergillus-sydowii-conidiospores-and-hyphae-from-Australian-coastal-waters-A-and-B_fig2_261033408.**
- **[310] https://www.keyence.com/ss/products/microscope/bz-x700/study/principle/002/index.jsp.**
- **[311] R. D. Phair, T. Misteli, Kinetic modelling approaches to in vivo imaging. Nature Reviews Molecular Cell Biology, 2, 12, 898-907, 2001.**
- **[312] K. W. Eliceiri, M. R. Berthold, I. G. Goldberg, L. Ibáñez, B. S. Manjunath, M. E. Martone, R. F. Murphy, H. Peng, A. L. Plant, B. Roysam, N. Stuurman, J. R. Swedlow, P. Tomancak, A. E. Carpenter, Biological Imaging Software Tools, 2012. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3659807/- Nat Methods.**
- **[313] S. A. Jones, S. H. Shim, J. He, X. Fast, Three-dimensional super-resolution imaging of live cells, Nat. Methods, 8, 499–508, 2011.**
- **[314] T. A. Planchon, L. Gao, D. E. Milkie, M. W. Davidson, J. A. Galbraith, C. G. Galbraith, E. Betzig, Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination, Nat. Methods, 8, 417–423, 2011.**

- **[315] A. Edelstein, N. Amodaj, K. Hoover, R. Vale, S. N. C. Protoc, Computer control of microscopes using μManager. Mol Biol, 2010.**
- **[316] H. P. Lin, C. Vincenz, K. W. Eliceiri, T. K. Kerppola, B. M. Ogle, Bimolecular fluorescence complementation analysis of eukaryotic fusion products, Biol Cell. 102, 9, 525-37, 2010.**
- **[317] T. A. Pologruto, B. L. Sabatini, K. Svoboda, Scan Image: flexible software for operating laser scanning microscopes, BioMedical Engineering, 2, 2003.**
- **[318] M. Linkert, C. T. Rueden, C. Allan, J. M. Burel, W. Moore, A. Patterson, B. Loranger, J. Moore, C. Neves, D. Macdonald, A. Tarkowska, C. Sticco, E. Hill, M. Rossner, K. W. Eliceiri, J. R. Swedlow, Metadata matters: access to image data in the real world, J Cell Biol, 189, 5, 777-82, 2010.**
- **[319] E. Glory, R. F. D. Murphy, Automated subcellular location determination and high-throughput microscopy, Cell. 12, 1, 7-16, 2007.**
- **[320] J. R. Lakowicz, Principals of Fluorescence Spectroscopy, New York: Academic Press, 1999.**
- **[321] P. Kankaanpää, L. Paavolainen, S. Tiitta, M. Karjalainen, J. Päivärinne, J. Nieminen, V. Marjomäki, J. Heino, D. J White, BioImageXD: an open, general-purpose and high-throughput image-processing platform, Nature Methods, 9, 683–689, 2012.**
- **[322] F. Chaumont, S. Dallongeville, N. Chenouard, N. Hervé, S. Pop, T. Provoost, V. Meas-Yedid, P. Pankajakshan, T. Lecomte, Y. Le Montagner, T. Lagache, A. Dufour, J. C. Olivo-Marin, Icy: an open bioimage informatics platform for extended reproducible research, Nat Methods, 9, 7, 690-6, 2012.**
- **[323] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, an open-source platform for biological-image analysis, Nat Methods, 28, 9, 7, 676-82, 2012.**
- **[324] H. Peng, Z. Ruan, F. Long, J. H. Simpson, E. W. Myers, V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets, Nat Biotechnol, 28, 4, 348-53, 2010.**
- **[325] A. E. Carpenter, T. R. Jones, M. R. Lamprecht, C. Clarke, I. H. Kang, O. Friman, D. A. Guertin, J. H. Chang, R. A. Lindquist, J. Moffat, P. Golland, D. M. Sabatini, Cell Profiler: image analysis software for identifying and quantifying cell phenotypes, Genome Biol, 7, 10, 2006.**
- **[326] S. Pieper, B. Lorensen, W. Schroeder, R. Kikinis, The NA-MIC Kit: ITK, VTK, pipelines, grids and 3D slicer as an open platform for the medical image computing community, Proceedings of the 3rd IEEE International Symposium on Biomedical Imaging: From Nano to Macro, 1, 698–701, 2006.**
- **[327] J. C. Fiala, Reconstruct a free editor for serial section microscopy, 218, Pt 1, 52-61, 2005.**
- **[328] Y. Wan, H. Otsuna, C. B. Chien, C. Hansen, Fluo Render: An Application of 2D Image Space Methods for 3D and 4D Confocal Microscopy Data Visualization in Neurobiology Research, IEEE Pac Vis Symp, 201-208, 2012.**
- **[329] D. Feng, D. Marshburn, D. Jen, R. J. Weinberg, R. M. Taylor, A. Burette, Stepping into the third dimension, J Neurosci, 27, 47, 12757-60, 2007.**
- **[330] A. Rosset, L. Spadola, O. J. Ratib, OsiriX: an open-source software for navigating in multidimensional DICOM images, Digit Imaging, 17, 3, 205-16, 2004.**
- **[331] C. L. Tsai, J. P. Lister, C. S. Bjornsson, K. Smith, W. Shain, C. A. Barnes, B. Roysam, Robust, globally consistent and fully automatic multi-image registration and montage synthesis for 3-D multi-channel images, J. Microsc, 243, 154–171, 2011.**
- **[332] S. Reibisch, S. Saalfeld, P. Tomančák, Globally optimal stitching of tiled 3D microscopic image acquisitions, Bioinformatics, 25, 1463–1465, 2009.**
- **[333] S. Saalfeld, Elastic Volume Reconstruction from series of ultra-thin microscopy sections, Nat. Methods.**
- **[334] A. H. Zewail, J. M. Thomas, 4D Electron Microscopy. Imaging in Space and Time, Imperial College Press, London, 2010.**
- **[335] D. J. Flannigan, O. Lourie, 4D ultrafast electron microscopy sheds light on dynamic processes from the micrometer to the atomic scale, Microscopy and Analysis, 2013.**
- **[336] D. J. Flannigan, B. Barwick, A. H. Zewail, Biological imaging with 4D ultrafast electron microscopy, PNAS, 107, 22, 9933-9937, 2010.**

- **[337] M. Giunti, C. Mazzola, Dynamical systems on monoids: Toward a general theory of deterministic systems and motion, Methods, Models, Simulations and Approaches Towards a General Theory of Change, 173-185, 2012.**
- **[338] S. Banach, (1932), Théorie des opérations linéaires, Monografie Matematyczne 1, Warszawa, 1932.**
- **[339] P. Y. L. Francesca, R. Nardi, Probabilistic Cellular Automata Theory, Applications and Future Perspectives, Emergence, Complexity and Computation.**
- **[340] H. Men, X. Zhao, Microbial Growth Modeling and Simulation Based on Cellular Automata , Research Journal of Applied Sciences, Engineering and Technology, 6, 11, 2061-2066, 2013.**
- **[341] L. Wei1, X. Lin, M. Wang, W. Huang, Low artificial anisotropy cellular automaton model and its applications to the cell-to-dendrite transition in directional solidification, Materials Discovery, 3, 17-28, 2016.**
- **[342] T. Toffoli, N. Margolus, Cellular Automata Machines: A New Environment for Modeling, MIT Press, 60, 1987.**
- **[343] A. Ben-Menahem, Historical Encyclopedia of Natural and Mathematical Sciences, Springer, 4632, 2009.**
- **[344] J. N. Wilson, G. X. Ritter, Handbook of Computer Vision Algorithms in Image Algebra (2nd ed.), CRC Press, 177, 2000.**
- **[345] http://mathworld.wolfram.com/vonNeumannNeighborhood.html.**
- **[346] P. E. Black, Manhattan distance, in Dictionary of Algorithms and Data Structures, 2006. https://www.nist.gov/dads/HTML/manhattanDistance.html.**
- **[347] R. Breukelaar, T. H. Bäck, Using a Genetic Algorithm to Evolve Behavior in Multi-Dimensional Cellular Automata: Emergence of Behavior, Proceedings of the 7th Annual Conference on Genetic and Evolutionary Computation (GECCO '05), New York, NY, USA, ACM, 107–114, 2005.**
- **[348] http://www.wikiwand.com/en/Moore_neighborhood.**
- **[349] M. A. Tsompanas, A. Adamatzky, G. C. Sirakoulis, J. Greenman, I. Ieropoulos, Towards implementation of Cellular Automata in Microbial Fuel Cells, PLoS ONE, 12, 5.**
- **[350] https://www.researchgate.net/figure/A-simple-glider-in-Conways-Game-of-Life-GOL-The-GOL-universe-is-a-two-dimensional_fig6_257811192- INTERLUDE - Time, Dynamics, Life, and the Emergence of Complexity, 2013.**
- **[351] https://sourceforge.net/projects/smoothlife/files/.**
- **[352] K. Pearson, The Problem of the Random Walk, Nature, 72, 1865, 294, 1905.**
- **[353] E. Wirth, Pi from agent border crossings by NetLogo package, Wolfram Library Archive, 2015.**
- **[354] E. Wirth, G. Szabó, A. Czinkóczky, MEASURE LANDSCAPE DIVERSITY WITH LOGICAL SCOUT AGENTS, ISPRS – International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, XLI-B2, 491–495, 2016.**
- **[355]https://www.semanticscholar.org/paper/A-Composite-Random-Walk-for-Facing-Environmental,C. A. Piña-García, D. Gu, H. Hu, A Composite Random Walk for Facing Environmental Uncertainty and Reduced Perceptual Capabilities, ICIRA, 2011.**
- **[356] D. H. Le, Y. K. Kwon, GPEC: A Cytoscape plug-in for random walk-based gene prioritization and biomedical evidence collection, Computational Biology and Chemistry, 37, 17-23, 2012.**
- **[357] P. A. Gagniuc, Markov Chains: From Theory to Implementation and Experimentation, USA, NJ: John Wiley & Sons, 1–235, 2017.**
- **[358] Markov chain; Definition of Markov chain in US English by Oxford Dictionaries". Oxford Dictionaries, English. Retrieved 2017-12-14.**
- **[359] R. Serfozo, Basics of Applied Stochastic Processes, Springer Science & Business Media, 2, 2009.**
- **[360] Y. A. Rozanov, Markov Random Fields, Springer Science & Business Media, 58, 2012.**
- **[361] B. B. Mandelbrot, The Fractal Geometry of Nature, Freeman, New York, 1982.**
- **[362] P. Révész, Random walk in random and nonrandom environments, World Scientific "Pólya's Random Walk Constants", 1990.**
- **[363] M. Kohls, T. Hernandez, Expected Coverage of Random Walk Mobility Algorithm, 2016. arxiv.org.**
- **[364] https://en.wikipedia.org/wiki/Branching_random_walk.**

- **[365] https://www.probstat.math.uni-mainz.de/.**
- **[366] N. Madras, G. Slade, The Self-Avoiding Walk, Birkhäuser, 1996.**
- **[367] G. F. Lawler, Intersections of Random Walks. Birkhäuser, 1991.**
- **[368] N. Madras, A. D. Sokal, The pivot algorithm–A highly efficient Monte-Carlo method for the self-avoiding walk, Journal of Statistical Physics, 50, 109, 18, 1988.**
- **[369] N. Clisby, B. Dünweg, High-precision estimate of the hydrodynamic radius for self-avoiding walks, Physical Review E, 2016. http://www.mpip-mainz.mpg.de/publications_2016.**
- **[370] C. S. Hamilton, S. Barkhofen, L. Sansoni, I. Jex, C. Silberhorn, Driven Discrete Time Quantum Walks, IOP Publishing Ltd, 2016.**
- **[371] G. B. Ermentrout, L. Edelstein-Keshet, Cellular automata approaches to biological modeling, J Theor Biol, 7, 160, 1, 97-133, 1993.**
- **[372] R. Feynman, The Brownian Movement, The Feynman Lectures of Physics, I, 41-1, 1964.**
- **[373]https://www.mathworks.com/matlabcentral/fileexchange/44733-simulation-for-brownian-motion-within-an-harmonic-potential.**
- **[374] P. Langevin, Sur la théorie du mouvement brownien [On the Theory of Brownian Motion], C. R. Acad. Sci. Paris, 146, 530–533, 1908, reviewed by D. S. Lemons, A. Gythiel, Am. J. Phys, 65, 1079, 1997.**
- **[375] http://mathworld.wolfram.com/MooreNeighborhood.html-http://cell-auto.com/neighbourhood/moore.**
- **[376] T. Li, S. Kheifets, D. Medellin, M. Raizen, Measurement of the instantaneous velocity of a Brownian particle, Science, 328, 5986, 1673 -1675, 2010.**
- **[377] A. Deutsch, S. Dormann, Cellular Automaton Modeling of Biological Pattern Formation Characterization, Applications and Analysis, Mathematical Biosciences, 200, 118–123, 2006.**
- **[378] J. Kroc, P. M. A. Sloot, A. G. Hoekstra, Simulating Complex Systems by Cellular Automata, Understanding Complex Systems, Springer, Verlag Berlin Heidelberg, 2010.**
- **[379] D. Worster, Nature's Economy, Cambridge University Press, 398–401, 1994.**
- **[380] M. Uyenoyama, R. Singh, The Evolution of Population Biology, Cambridge University Press, 1–19, 2004.**
- **[381] M. Rani, R. Haq, D. Verma, Variants of Koch curve: A Review. National Conference on Development of Reliable Information Systems, Techniques and Related Issues (DRISTI), Proceedings published in International Journal of Computer Applications, 2018.**
- **[382] P. Prusinkiewicz, J. Hanan, M. Hammel, R. Mech, L-systems: from the Theory to Visual Models of Plants, 2nd CSIRO Symposium on Computational Challenges in Life Sciences, CSIRO Publishing, 1996.**
- **[383] http://algorithmicbotany.org/papers/abop/abop-ch1.pdf.**
- **[384] E. Bonabeau, Agent-based modeling: Methods and techniques for simulating human systems, PNAS, 99, 3, 2002.**
- **[385] N. Gilbert, AGENT-BASED MODELS, SAGE Publications, UK, 2007.**
- **[386] S. Matsuura, Growth and Colony Patterning of Filamentous Fungi, Biology Published, 2000.**
- **[387] H. V. D. Parunak, R. Savit, R. L. Riolo, Agent-Based Modeling vs. Equation-Based Modeling:-A Case Study and Users' Guide, International Workshop on Multi-Agent Systems and Agent-Based Simulation, MABS: Multi-Agent Systems and Agent-Based Simulation, 10-25, Lecture Notes in Computer Science book series, 1998.**
- **[388] T. E. Gorochowski, Agent-based modelling in synthetic biology, Essays in Biochemistry, 60, 325–336, 2016.**
- **[389] M. Birbaumer, F. Schweitzer, Agent-Based Modeling of Intracellular Transport, European Physical Journal B, 82, 3-4, 245-255, 2011.**
- **[390] F. Schweitzer, Active Brownian Particles: Artificial Agents in Physics, Stochastic Dynamics, 358-371, Part of the Lecture Notes in Physics book series (LNP, 484), 2007.**
- **[391] J.L. Schiff, Introduction to Cellular Automata, 2005. psoup.math.wisc.edu**
- **[392] R. Malhotra, N. Singh, Y. Singh, Genetic Algorithms: Concepts, Design for Optimization of Process Controllers, Computer and Information Science, 4, 2, 2011.**

- **[393] P. Kumar, D. Gospodaric, P. Bauer, Improved genetic algorithm inspired by biological evolution, Soft Comput, 11, 923–941, 2007.**
- **[394] K. C. Clarke, Cellular Automata and Agent-Based Models 62, Handbook of Regional Science, 1217-1233, 2013.**
- **[395] http://www.iseesystems .com.**
- **[396] http://ccl.northwestern.edu/netlogo.**
- **[397] H. V. Westerhoff, A. N. Brooks, E. Simeonidis, R. García-Contreras, F. He, F. C. Boogerd, V. J. Jackson, V. Goncharuk, A. Kolodkin, Macromolecular networks and intelligence in microorganisms, Front Microbiol, 5, 379, 2014.**
- **[398] A. Stephan, Varieties of Emergentism, Evol. Cogn, 5, 49–59, 1999.**
- **[399] A. Stephan, The dual role of 'emergence' in the philosophy of mind and in cognitive science, Synthese, 151, 485–498, 2006.**
- **[400] F. C. A. Kolodkin, P.N. Boogerd, F. J. Bruggeman, V. Goncharuk, J. Lunshof, Emergence of the silicon human and network targeting drugs, Eur. J. Pharm. Sci, 46, 190–197, 2012.**
- **[401] R. Loosemore, Complex Systems, Artificial Intelligence and Theoretical Psychology, Proceedings of the 2007 conference on Advances in Artificial General Intelligence: Concepts, Architectures and Algorithms: Proceedings of the AGI Workshop, 159–173, 2007.**
- **[402] S. Johnson, Emergence: The Connected Lives of Ants, Brains, Cities, and Software, Scribner: New York, NY, USA, 2001.**
- **[403] S. Fox, Beyond AI: Multi-Intelligence (MI) Combining Natural and Artificial Intelligences in Hybrid Beings and Systems, Technologies, 5, 38, 2017.**
- **[404] L. Chittka, S. J. Rossiter, P. Skorupski, C. Fernando, What is comparable in comparative cognition?, Philos. Trans. R. Soc, 367, 2677–2685, 2012.**
- **[405] M. Wilson, Six Views of Embodied Cognition, Psychon. Bull. Rev, 9, 625–636, 2002.**
- **[406] F. C. Boogerd, F.J. Bruggeman, R. C. Richardson, A. Stephan, H. V. Westerhoff, Emergence and its place in nature: a case study of biochemical networks, Synthese, 145, 131–164, 2005.**
- **[407] A. G. Wouters, Viability explanation, Biol. Philos, 10, 435–457, 1995.**
- **[408] A. G. Wouters, Design explanation: determining the constraints on what can be alive, Erkenntnis, 67 65–80, 2007.**
- **[409] H. V. Westerhoff, K. Van Dam, Thermodynamics and Control of Biological Free Energy Transduction, Amsterdam: Elsevier Science Ltd, 1987.**
- **[410] F. Shu-Yang, B. Freedman, R. Cote, Principles and practice of ecological design, Environmental Reviews, 12, 2, 2004.**
- **[411] S. K. Kellert, J. H. Heerwagen, M. L. Mador, Biophilic design: The theory, science and practice of bringing buildings to life, Hoboken, NJ: John Wiley & Sons, 432, 2008.**
- **[412] A. Almusaed, Biophilic architecture (Towards a new potential of healthy architecture), Conference: Rethinking Sustainable Construction, 12thRinker International Conference, USA, 1, 2006.**
- **[413] R. Milo, S. Shen-Orr, S. Itzkovitz, N. Kashtan, D. Chklovskii, U. Alon, Network motifs: simple building blocks of complex networks, Science, 298, 824–827, 2002.**
- **[414] U. Alon, Network motifs: theory and experimental approaches, Nat. Rev. Genet, 8, 450–461, 2007.**
- **[415] N. Kashtan, S. Itzkovitz, R. Milo, U. Alon, Topological generalizations of network motifs, Phys. Rev. E Stat. Nonlin. Soft Matter Phys, 70, 2004.**
- **[416] F. He, V. Fromion, H. V. Westerhoff, (Im)Perfect robustness and adaptation of metabolic networks subject to metabolic and gene-expression regulation: marrying control engineering with metabolic control analysis, BMC Syst. Biol, 7, 131, 2013.**
- **[417] W. C. van Heeswijk, H. V. Westerhoff, F. C. Boogerd, Nitrogen assimilation in Escherichia coli: putting molecular data into a systems perspective, Microbiol. Mol. Biol. Rev, 77, 628–695, 2013.**
- **[418] M. E. Csete, J. C. Doyle, Reverse engineering of biological complexity, Science, 295, 1664–1669, 2002.**
- **[419] H. Samad, J. P. Goff, M. Khammash, Calcium homeostasis and parturient hypocalcemia: an integral feedback perspective, J. Theor. Biol, 214, 17–29, 2002.**
- **[420] D. Kahn, H. V. Westerhoff, Control theory of regulatory cascades, J. Theor. Biol, 153, 255–285, 1991.**

- **[421] D. A. Fell, Understanding the Control of Metabolism, London: Portland Press, 1997.**
- **[422] R. Bonneau, M. T. Facciotti, D. J. Reiss, A. K. Schmid, M. Pan, A. Kaur, A predictive model for transcriptional control of physiology in a free-living cell, Cell, 131, 1354–1365, 2007.**
- **[423] M. Sorek, N. Q. Balaban, Y. Loewenstein, Stochasticity, bistability and the wisdom of crowds: a model for associative learning in genetic regulatory networks, PLoS Comput. Biol, 2013.**
- **[424] S. McGregor, V. Vasas, P. Husbands, C. Fernando, Evolution of associative learning in chemical networks, PLoS Comput. Biol, 2012.**
- **[425] J. Carrera, S. F. Elena, A. Jaramillo, Computational design of genomic transcriptional networks with adaptation to varying environments, Proc. Natl. Acad. Sci, 2012.**
- **[426] K. F. Jarrell, M. J. McBride, The surprisingly diverse ways that prokaryotes move, Nat. Rev. Microbiol, 6, 2008.**
- **[427] R. B. Bourret, A. M. Stock, Molecular information processing lessons from bacterial chemotaxis, J Biol Chem, 277, 12, 9625-8, 2002.**
- **[428] J. M. Skotheim, L. Mahadevan, Physical Limits and Design Principles for Plant and Fungal Movements, Science, 308, 1308-1310, 2005.**
- **[429] S. Upadhyay, B. D. Shaw, The role of actin, fibrin and endocytosis in growth of hyphae in Aspergillus nidulans, Mol Microbiol, 68, 690–705, 2008.**
- **[430] S. D. Harris, Hyphal morphogenesis: an evolutionary perspective, Fungal Biol, 115, 475–484, 2011.**
- **[431] J. Chant, Cell polarity in yeast, Annu Rev Cell Dev Biol, 15, 365–391, 1999.**
- **[432] F. Chang, S. G. Martin, Shaping fission yeast with microtubules, Cold Spring Harb Perspect Biol, 2009.**
- **[433] G. Steinberg, Hyphal growth: a tale of motors, lipids, and the Spitzenkörper, Eukaryot Cell, 6, 351–360, 2007.**
- **[434] C. Brand, N.A Gow, Mechanisms of hypha orientation of fungi, Current opinion in microbiology, 12, 4, 350-7, 2009.**
- **[435] F. Chang, M. Peter, Yeasts make their mark, Nat Cell Biol, 5, 294–299, 2003.**
- **[436] G. J. Celio, M. Padamsee, B. T. Dentinger, R. Bauer, D. J. McLaughlin, Assembling the fungal tree of life: constructing the structural and biochemical database, Mycologia, 98, 850–859, 2006.**
- **[437] G. Steinberg, M. A. Peñalva, M. Riquelme, H. A. Wösten, S. D. Harris, Cell Biology of Hyphal Growth, Microbiology Spectrum, 2017.**
- **[438] S. D. Harris, Morphogenesis is coordinated with nuclear division in germinating Aspergillus nidulans conidiospores, Microbiology, 145, 2747–2756, 1999.**
- **[439] S. Konzack, P. E. Rischitor, C. Enke, R. Fischer, The role of the kinesin motor KipA in microtubule organization and polarized growth of Aspergillus nidulans, Mol Biol Cell, 16, 497–506, 2005.**
- **[440] S. Bartnicki-Garcia, E. Lippman, Fungal morphogenesis: cell wall construction in Mucor rouxii, Science, 165, 302–304, 1969.**
- **[441] S. Bartnicki-Garcia, Fundamental aspects of hyphal morphogenesis, 245–267, In Ashworth JM, Smith E (ed), Microbial differentiation, Cambridge University Press, Cambridge, United Kingdom, 1973.**
- **[442] J. G. H. Wessels, A steady-state model for apical wall growth in fungi, Acta Bot Neerl, 37, 3–16, 1988.**
- **[443] T. M. Bourett, R.J. Howard, Ultrastructural immunolocalization of actin in a fungus, Protoplasma, 163, 199–202, 1991.**
- **[444] S. Bartnicki-Garcia, F. Hergert, G. Gierz, Computer simulation of fungal morphogenesis and the mathematical basis for hyphal tip growth, Protoplasma, 153, 46–57, 1989.**
- **[445] D. H. Jennings, Translocation of solutes in fungi, Biol Rev Camb Philos Soc, 62, 215–243, 1987.**
- **[446] D. L. Delgado-Álvarez, S. Bartnicki-García, S. Seiler, R. R. Mouriño-Pérez, Septum development in Neurospora crassa: the septal ctomyosin tangle, PLoS One, 9, 2014.**
- **[447] https://proteopedia.org/wiki/index.php/Myosin.**
- **[448] C. Fiddy, A. P. Trinci, Mitosis, septation, branching and the duplication cycle in Aspergillus nidulans, J Gen Microbiol, 97, 169–184, 1976.**
- **[449] A. Simonin, J. Palma-Guerrero, M. Fricker, N. L. Glass, Physiological significance of network organization in fungi, Eukaryot Cell, 11, 1345–1352, 2012.**

- **[450] X. Xiang, M. Plamann, Cytoskeleton and motor proteins in filamentous fungi, Curr Opin Microbiol, 6, 628–633, 2003.**
- **[451] M. J. Egan, M. A. McClintock, S. L. Reck-Peterson, Microtubule based transport in filamentous fungi, Curr Opin Microbiol, 15, 637–645, 2012.**
- **[452] C. Lin, M. Schuster, S. C. Guimaraes, P. Ashwin, M. Schrader, J. Metz, C. Hacker, S. J. Gurr, G. Steinberg, Active diffusion and microtubule based transport oppose myosin forces to position organelles in cells, Nat Commun, 7, 11814, 2016.**
- **[453] A. Abadeh, R. R. Lew, Mass flow and velocity profiles in Neurospora hyphae: partial plug flow dominates intra-hyphal transport, Microbiology, 159, 2386–2394, 2013.**
- **[454] R. R. Lew, Mass flow and pressure-driven hyphal extension in Neurospora crassa, Microbiology, 151, 2685–2692, 2005.**
- **[455] P. Markham, Occlusions of septal pores in filamentous fungi, Mycol Res, 98, 1089–1106, 1994.**
- **[456] R. T. Moore, J. H. McAlear, Fine structure of mycota 7. Observations on septa of ascomycetes and basidiomycetes, Am J Bot, 49, 86–94, 1962.**
- **[457] A. F. van Peer, W. H. Müller, T. Boekhout, L. G. Lugones, H. A. Wösten, Cytoplasmic continuity revisited: closure of septa of the filamentous fungus Schizophyllum commune in response to environmental conditions, PLoS One, 4, 2009.**
- **[458] N. A. Gow, A. J. Brown, F. C. Odds, Fungal morphogenesis and hostinvasion, Curr Opin Microbiol, 5, 366-371, 2002.**
- **[459] https://www.media.mit.edu/projects/living-mushtari/overview.**
- **[460] S. Hoehme, D. Drasdo, A cell-based simulation software for multi-cellular systems, Systems biology Advance, 2010.**
- **[461] https://www.sciencedirect.com/topics/materials-science/youngs-modulus.**
- **[462] https://www.sciencedirect.com/topics/materials-science/poisson-ratio.**
- **[463] https://www.media.mit.edu/projects/wanderers/overview.**
- **[464] D. J Moore, L. J. McNulty, A. Meskauskas, Branching in Fungal Hyphae and Fungal Tissues: Growing Mycelia in a Desktop Computer, Branching Morphogenesis, 75-90, 2004.**
- **[465] https://en.wikipedia.org/wiki/Budding#cite_note-smyth-1.**
- **[466] http://biology-pictures.blogspot.com/2011/10/budding-yeast-picture.html.**
- **[467] https://www.britannica.com/science/yeast-fungus/media/652395/203798.**
- **[468] D. Liakopoulos, An auxiliary, membrane-based mechanism for nuclear migration in budding yeast-Marisa Kirchenbauer, Molecular biology of the cell, 24, 9, 2013. https://www.researchgate.net/figure/Model-showing-events-during-nuclear-migration-spindle-positioning-in-budding-yeast-Early_fig7_235750939.**
- **[469] https://mkmra2.blogspot.com/2019/01/robot-programming-with-kukaprc-v3.html.**
- **[470]https://www.behance.net/gallery/22536831/ROBOTIC-EXTRUSION(6-Axis-KUKAABS-3D-Printing).**
- **[471] M. Kayser, L. Cai, S. Falcone, C. Bader, N. Inglessis, B. Darweesh, N. Oxman. Design of a multi-agent, fiber-composite digital fabrication system, Sci. Robot, 3, 2018. https://link.springer.com/chapter/10.1007/978-3-319-92294-2_22.**
- **[472] https://www.media.mit.edu/projects/fiberbots/overview.**
- **[473] D. Singh, D. Thomas, Advances in medical polymer technology towards the panacea of complex 3D tissue and organ manufacture, American Journal of Surgery, 2018.**
- **[474] T. J. Hinton, Q. Jallerat, R. N. Palchesko, J. H. Park, M. S. Grodzicki, H. J. Shue, M. H. Ramadan, A. R. Hudson, A. W. Feinberg, Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels, Science Advances, 1, 9, 2015.**
- **[475] D. J. Thomas, Could 3D bioprinted tissues offer future hope for microtia treatment?, International Journal of Surgery, 32, 43–44, 2016.**
- **[476] Y. Nakashima, K. Okazak, K. Nakayama, S. Okada, H. Mizu-uchi, Bone and Joint Diseases in Present and Future. Fukuoka Igaku Zasshi = Hukuoka Acta Medica, 108, 1, 1–7, 2017.**

- **[477] A. Shafiee, A. Atala, Printing Technologies for Medical Applications, Trends in Molecular Medicine, 22, 3, 254–265, 2016.**
- **[478] C. K. Chua, W. Y. Yeong, Bioprinting: Principles and Applications. Singapore: World Scientific Publishing Co, 296, 2016.**
- **[479] M. Cooper-White, How 3D Printing Could End The Deadly Shortage Of Donor Organs, Huffpost Science, TheHuffingtonPost.com, 2016.**
- **[480] K. Harmon, A sweet solution for replacing organs, Scientific American, 308, 4, 54–55, 2013.**
- **[481] L. A. Hockaday, K. H. Kang, N. W. Colangelo, P. Y. C. Cheung, B. Duan, E. Malone, J. Wu, L.N. Girardi, L. J. Bonassar, H. Lipson, C. C. Chu, J. T. Butcher, Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds, Biofabrication, 4, 3, 2012.**
- **[482] S. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nature Biotechnology, 32, 773–85, 2014.**
- **[483] J. Yoo, A. Atala, Bioprinting: 3D printing comes to life, Manufacturing Engineering, 2015.**
- **[484] D. Thomas, D. Singh, Novel techniques of engineering 3D vasculature tissue for surgical procedures, The American Journal of Surgery, 2018.**
- **[485] T. jiang, J.G. Munguia-Lopez, S. Flores-Torres,J. Kort-Mascort, J. M. Kinsella, Extrusion bioprinting of soft materials: An emerging technique for biological model fabrication, Appl. Phys. Rev, 2019.**
- **[486] J. J. Manappallil, Basic Dental Materials, JP Medical Ltd, 2015.**
- **[487] 3D Printing Technology At The Service Of Health, healthyeve. Retrieved 4 August2016.**
- **[488] Z. Ali, A. Z. Kouzani, S. Y. Khoo, B. Nasri-Nasrabadi, A. Kaynak, Development and analysis of a 3D printed hydrogel soft actuator, Sensors and Actuators A: Physical, 2017.**
- **[489] Z. Ali, A. Z. Kouzani, S. Y. Khoo, I. Gibson, A. Kaynak, 3D printed hydrogel soft actuators, Region 10 Conference (TENCON), IEEE, 2272–2277, 2016.**
- **[490] F. A. Auger, L. Gibot, D. Lacroix, The Pivotal Role of Vascularization in Tissue Engineering, Annual Review of Biomedical Engineering, 15, 177–200, 2013.**
- **[491] P. Bajaj, R. M. Schweller, A. Khademhosseini, J. L. West, R. Bashir, 3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine, Annual Review of Biomedical Engineering, 16, 247–76, 2014.**
- **[492] M. Kesti, M. Müller, J. Becher, M. Schnabelrauch, M. d'Este, D. Eglin, M. Zenobi-Wong, A versatile bioink for three-dimensional printing of cellular scaffolds based on thermally and photo-triggered tandem gelation, Acta Biomaterialia, 11, 162–72, 2015.**
- **[493] A. D. Augst, J. Kong, Hyun, D. J. Mooney, Alginate Hydrogels as Biomaterials, Macromolecular Bioscience, 6, 8, 623–33, 2006.**
- **[494] E. Axpe, M. L. Oyen, Applications of Alginate-Based Bioinks in 3D Bioprinting, International Journal of Molecular Sciences, 17, 12, 1976, 2016.**
- **[495] S. Kriegman, D. Blackiston, M. Levin, J. Bongard, A scalable pipeline for designing reconfigurable organisms, PNAS, 117, 4, 1853-1859, 2020.**
- **[496] S. Hamada, K. G. Yancey, Y. Pardo, M. Gan, M. Vanatta, D. An, Y. Hu, T. L. Derrien, R. Ruiz, P. Liu, J. Sabin, D. Luo, Dynamic DNA material with emergent locomotion behavior powered by artificial metabolism, Science Robotics, 2019.**