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Hybrid systems for wastewater treatment in the framework of circular economy.

Coupling biological and membrane technologies for a sustainable water cycle

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Abstract

The increasing water demand coupled to the depletion of natural water sources has raised the need to investigate and develop in wastewater treatment and reuse. Even more, the application of circular economy principles to water cycle has highlighted the need to see wastewater as a source of water and resources. Therefore, hybridization of already developed technologies can help achieve circular economy goals. Moreover, these hybrid systems that take the best of each technology are capable to gain to the limitations of current conventional treatments. Thus, in this thesis, different hybrid systems have been developed and tested (at bench and pilot scales) for wastewater treatment, both urban and industrial.

On one hand, three upflow anaerobic sludge blanket (UASB) reactors with different configurations: flocculent biomass, flocculent biomass and membrane solids separation and granular biomass and membrane solids separation (UASB-AnMBR), were operated to compare start-up, solids hydrolysis and effluent quality. The challenges of this work were both the low temperature and the low COD content. A really quick start-up was observed for the three reactors and was attributed to the previous acclimation of the seed sludge. The UASB configurations with membrane retained the solids in the reactor increasing solids hydrolysis efficiency. Moreover, flocculent biomass promoted slightly higher hydrolysis than granular one. Therefore, a configuration based on flocculent UASB-AnMBR was appropriate for the treatment of urban wastewater with low COD content at 10°C.

On the other hand, a single-stage AnMBR for the treatment of cheese whey and its co-digestion with cattle slurry was investigated with the aim of potentially recovering water and energy. High COD removal (91% ± 7%) was achieved with a biogas production of 0.2 – 0.9 m^3 biogas/kg COD removed. Therefore, high energy recovery could potentially be obtained when using

this process with a mean value of 2.4 kWh/kg COD removed. Although energy recovery was directly validated, several limitations were detected regarding water reuse. Those limitations comprised high salt concentration in the permeate, which should be removed prior to its reuse.

Moreover, petrochemical wastewater pre-treatment was optimised with the final objective of water recycling. It consisted in a coagulation-flocculation (CF) step followed by a moving bed biofilm reactor (MBBR) aimed to decrease suspended solids (SS) and organic content. In this case, only the first part of the hybrid system was optimised, membrane units were not included in this work. CF tests showed a decrease in wastewater turbidity but no significant DOC removal. Wastewater was then treated by MBBR. In MBBR, high sCOD removal efficiency (80-90%) was maintained. The MBBR proved to be also effective when treating raw wastewater as well as when feed wastewater effluent proportions were changed. The obtained results showed that MBBR was a suitable technology for petrochemical wastewater pre-treatment.

Finally, a novel treatment strategy for landfill leachate aimed to decrease its environmental impact was studied. The system consisted in a membrane bioreactor (MBR) pre-treatment aimed to remove COD, N and SS. It was followed by a combined reverse osmosis – electrodialysis reversal (RO-EDR) treatment aimed to remove salts and decrease brine volume. MBR decreased inorganic carbon by 92 ± 8% and achieved N removal of 85%. RO achieved a recovery of 84% and rejections of above 95%. EDR unit treating RO brine achieved a recovery of 67%. Thus, average recovery of the whole system was above 90%. It is important to highlight that end-of-life RO regenerated membranes were used in this study. This fact, together with the low volume of brine (<10%) helped decrease the environmental impact of leachate treatment.

Hence, this thesis was conducted from an applied research approach, aimed to reduce the gap between basic technology development and industrial implementation.

Keywords: anaerobic membrane bioreactor (AnMBR); membrane bioreactor (MBR); microbial community; moving bed biofilm reactor (MBBR); regenerated membranes; resource recovery; reverse osmosis (RO); upflow anaerobic sludge blanket (UASB); wastewater; water recovery.

Resum

La creixent demanda d'aigua i l'esgotament de les fonts naturals ha generat la necessitat d'investigar i desenvolupar nous tractaments d'aigua així com la seva reutilització. L'aplicació dels principis de l'economia circular al cicle de l'aigua ha posat de manifest la necessitat de percebre les aigües residuals com a font d'aigua i recursos. Així doncs, la hibridació de tecnologies ja desenvolupades pot ajudar a complir els objectius de l'economia circular. A més, aquests sistemes híbrids són capaços de superar les limitacions dels tractaments convencionals. Així doncs, en aquesta tesi, s'han desenvolupat i provat diferents sistemes híbrids (a escala de banc de proves i pilot) per al tractament d'aigües residuals urbanes i industrials.

D'una banda, s'han operat tres configuracions de reactors UASB (*Upflow Anaerobic Sludge Blanket*) per comparar la posada en marxa, la hidròlisi dels sòlids i qualitat de l'efluent. Aquestes configuracions eren: biomassa flocular, biomassa flocular amb separació per membrana i biomassa granular amb separació per membrana (UASB-AnMBR). Els reptes d'aquest treball han estat tant la baixa temperatura com el baix contingut en DQO. La posada en marxa ha estat molt ràpida per als tres reactors, atribuïda a l'aclimatació prèvia dels fangs. Els resultats mostren que una configuració basada en UASB-AnMBR amb biomassa flocular ha estat adequada per al tractament d'aigües residuals urbanes amb baix contingut en DQO a 10°C.

D'altra banda, s'ha investigat un AnMBR per al tractament de xerigot i la seva codigestió amb purí amb l'objectiu de recuperar aigua i energia. S'ha aconseguit una elevada eliminació de DQO (91% ± 7%) amb una producció de biogàs de 0,2 a 0,9 m^3 de biogàs/kg de DQO eliminada. Per tant, es calcula que es podria obtenir una elevada recuperació d'energia amb un valor mitjà de 2,4 kW/kg de DQO eliminada. Tot i que s'ha validat directament la recuperació d'energia, s'han detectat diverses limitacions en relació amb la reutilització de l'aigua. Aquestes limitacions inclouen una elevada

concentració de sal en el permeat, que caldria eliminar abans de la seva reutilització.

A més, s'ha optimitzat el pretractament per a aigües residuals petroquímiques amb l'objectiu de reciclar l'aigua. Aquest ha consistit en una coagulació-floculació (CF) seguida d'un MBBR (*Moving Bed Biofilm Reactor*) per tal de disminuir els sòlids en suspensió (SS) i el contingut orgànic. En aquest cas, només s'ha optimitzat la primera part del sistema híbrid ja que no s'han inclòs les etapes de membrana en aquest treball. Les proves de CF han mostrat una disminució de la terbolesa de les aigües residuals sense eliminació significativa de DQO. Aquest efluent s'ha tractat per MBBR. A l'MBBR s'ha mantingut una elevada eficiència d'eliminació de DQOs (80-90%). Els resultats obtinguts mostren que el MBBR és una tecnologia adequada per al pretractament de les aigües residuals petroquímiques.

Finalment, s'ha estudiat una nova estratègia de tractament de lixiviats d'abocador per disminuir el seu impacte ambiental. El sistema s'ha basat en un pretractament amb bioreactor de membrana (MBR) per a l'eliminació de DQO, N i SS seguit d'un tractament combinat d'osmosi inversa–electrodiàlisi reversible (OI-EDR) per a l'eliminació de sals i disminució el volum de salmorra. L'MBR ha disminuït el carboni inorgànic en un 92 ± 8% i ha aconseguit una eliminació de N del 85%. Gràcies a les etapes d'OI i EDR, la recuperació mitjana de tot el sistema ha superat el 90%. És important destacar que en aquest estudi s'han utilitzat membranes regenerades d'OI al final de la seva vida útil. Aquest fet, juntament amb el baix volum de salmorra (<10%) ha contribuït a disminuir l'impacte ambiental del tractament de lixiviats.

Per tant, aquesta tesi s'ha dut a terme des d'un enfoc de recerca aplicada, amb l'objectiu de reduir la bretxa entre el desenvolupament tecnològic bàsic i la implementació industrial.

Paraules clau: bioreactor de membrana anaerobi (AnMBR); bioreactor de membrana (MBR); comunitat microbiana; reactor de biofilm de llit mòbil (MBBR); membranes regenerades; recuperació de recursos; osmosi directa (OI); reactor anaerobi de flux ascendent (UASB); aigua residual; recuperació d'aigua.

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Abbreviation list

AD	Anaerobic Digestion
AnMBR	Anaerobic Membrane Bioreactor
AOP	Advanced Oxidation Processes
AOX	Adsorbable Organic Halides
ARC	Agència de Residus de Catalunya
AS	Activated Sludge
bCOD	Biodegradable Oxygen Demand
BOD_5	Biological Oxygen Demand
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
BW	Brackish Water
CAPEX	Capital Expenditures
CASP	Conventional Activated Sludge Process
CBGR	Consorci del Bages per la Gestió de Residus
CED	Cumulative Energy Demand
CF	Coagulation-Flocculation
CN	Cyanide
COD	Chemical Oxygen Demand
COS	Centre for Omic Sciences
CSTR	Continuously Stirred Tank Reactor
CTBD	Cooling Tower Blowdown
cWW	Cleaning Wastewater
DCI	Dow Chemical Ibérica
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DT-RO	Disc Tube Reverse Osmosis

EC	Electrical Conductivity
ED	Electrodialysis
EDR	Electrodialysis Reversal
EIP Water	European Innovation Partnerships Water
F-AnMBR	Flocculent Anaerobic Membrane Bioreactor
FE	Freshwater Eutrophication
FET	Freshwater Ecotoxicity
FO	Forward Osmosis
FS	Fossil Resource Scarcity
FU	Functional Unit
F-UASB	Flocculent Upflow Anaerobic Sludge Blanket
GAC	Granular Activated Carbon
G-AnMBR	Granular Anaerobic Membrane Bioreactor
GT	Gas Transfer
GW	Global Warming
HPLC	High Performance Liquid Chromatography
HRGC-FID	High-Resolution Gas Chromatography With Flame Ionization Detection
HRGC-MS	High-Resolution Gas-Phase Chromatography And Mass Spectrometry
HRT	Hydraulic Retention Time
HT-C	Human Carcinogenic Toxicity
HT-NC	Human Non-Carcinogenic Toxicity
IER	Ion Exchange Resins
ITS	Internal Transcribed Spacer
JRC	Joint Research Centre
KWR	KWR Water Cycle Research Institute
LCA	Life Cycle Assessment

LCC	Life Cycle Costing
LCV	Lower Calorific Value
LMH	$L/(m^2 \cdot h)$
MBBR	Moving Bed Biofilm Reactor
MBR	Membrane Bioreactor
mcrA	methyl coenzyme M reductase
MD	Membrane Distillation
ME	Membrane Extraction
MF	Microfiltration
MSW	Municipal Solid Waste
NF	Nanofiltration
O&G	Oil And Grease
OD	Stratospheric Ozone Depletion
OFWRP	Old Ford Water Recycling Plant
OLR	Organic Loading Rate
OPEX	Operating Expense
OTU	Operational Taxonomic Unit
PAC	Powdered Activated Carbon
PAH	Polycyclic Aromatic Hydrocarbon
PC	Principal Component
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyl
pCOD	Particulate Chemical Oxygen Demand
PCR	Polymerase Chain Reaction
PM	Fine Particulate Matter Formation
PSD	Particle Size Distribution
PT	Proposed Treatment

PVDF	Polyvinylidene Fluoride
pWW	Process Wastewater
QEOP	Queen Elisabeth Olympic Park
RDP	Ribosomal Database Project
RNA	Ribonucleic Acid
RO	Reverse Osmosis
rRNA	Ribosomal Ribonucleic Acid
SBR	Sequencing Batch Reactor
sCOD	Soluble Chemical Oxygen Demand
SDI	Silt Density Index
SOUR	Specific Oxygen Uptake Rates
SRB	Sulphate Reducing Bacteria
SRT	Sludge Retention Time
SS	Suspended Solids
SW	Seawater
tCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solids
TIC	Total Inorganic Carbon
TKN	Total Kjeldahl Nitrogen
TMP	Transmembrane Pressure
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TPH	Total Petroleum Hydrocarbons
TSS	Total Suspended Solids
tWW	Cooling Towers Wastewater
UASB	Upflow Anaerobic Sludge Blanket

UF	Ultrafiltration
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
V_{up}	Upflow Velocity
WRD	Water Consumption
WssTP	Water Supply and Sanitation Technology Platform
WW	Wastewater
WWTP	Wastewater Treatment Plant

CHAPTER 1

Introduction

1.1. Circular economy in the water cycle

The world runs on water. In the same manner as materials, water is traditionally looked at from a linear point of view. This linear approach is based on the Take-Make-Dispose strategy, mostly employed for materials consumption, and is reflected by the Take-Use-Discharge strategy that is generally embraced in the water sector (Ellen MacArthur Foundation et al., 2018). Subsequently and regrettably, linear take-use-discharge management together with climate change and natural water depletion are causing water crises all over the world. Freshwater resources are more and more under stressful conditions. It already exists a huge mismatch between available water resources and water demand in many parts of the world. According to the report "Policy Options for Decoupling Economic Growth from Water Use and Water Pollution" (UNEP, 2015) by 2030, global freshwater demand could exceed by 40% water viable sources if no changes are made on water management. Nowadays, in Europe, water scarcity affects at least 11% of the population and 17% of the territory (EC, 2012, 2007). For this reason, preservation of water resources is one of the main milestones on environmental protection in Europe (EEA, 2017; EC, 2012).

Circular water management is a promising solution to the water challenge. According to Ellen MacArthur Foundation (2013), circular economy targets a redefinition of growth by approaching society-wide positive benefits. It involves progressively decoupling economic activity from the consumption of finite resources and disposal of waste outside the system. Supported by a transition to renewable energy sources, the circular model is based on three principles:

- Designing out waste and pollution
- Keeping products and materials in use
- Regenerating natural systems

The application of circular economy principles to water management will help mitigate and prevent a global water crisis. Ellen MacArthur Foundation et al. (2018) related circular economy principles to water management (Table 1.1) and presented a circular economy diagram for the management and supply of water based on the principle of "restorative by design" (Figure 1.1). This diagram is divided into two halves: the "nature managed" and "human managed". On the nature managed side, water is represented in its natural state in which no human driven uses are taking place. Within a given basin, the natural water cycle acts on re-optimizing, reusing, and replenishing water. On the right side, water circularity is impacted by human actions that modify the natural water cycle such as freshwater abstraction, water loss through inefficient water management methods and water pollution. By applying circular economy to water, the human water cycle can be better aligned with the natural water cycle. The following measures, sorted by priority, have been identified: to avoid use, to reduce use, to reuse, to recycle and to replenish.

Table 1.1. Relation between Circular Economy Principles and Water Systems Management from Ellen MacArthur Foundation et al. (2018).

Circular Economy Principles ***(Ellen MacArthur Foundation)***	**Water Systems Management**
Principle 1: **Design out waste externalities**	• Optimise the amount of energy, minerals, and chemicals use in operation of water systems in concert with other systems. • Optimise consumptive use of water within sub-basin in relation to adjacent sub-basins (e.g. use in agriculture or evaporative cooling) • Use measures or solutions which deliver the same outcome without using water

Circular Economy Principles *(Ellen MacArthur Foundation)*	Water Systems Management
Principle 2: **Keep Resources in Use**	• Optimise resource yields (water use & reuse, energy, minerals, and chemicals) within water systems. • Optimise energy or resource extraction from the water system and maximise their reuse. • Optimise value generated in the interfaces of water system with other systems.
Principle 3: **Regenerate Natural Capital**	• Maximise environmental flows by reducing consumptive and non-consumptive uses of water. • Preserve and enhance the natural capital (e.g. river restoration, pollution prevention, quality of effluent, etc.) • Ensure minimum disruption to natural water systems from human interactions and use.

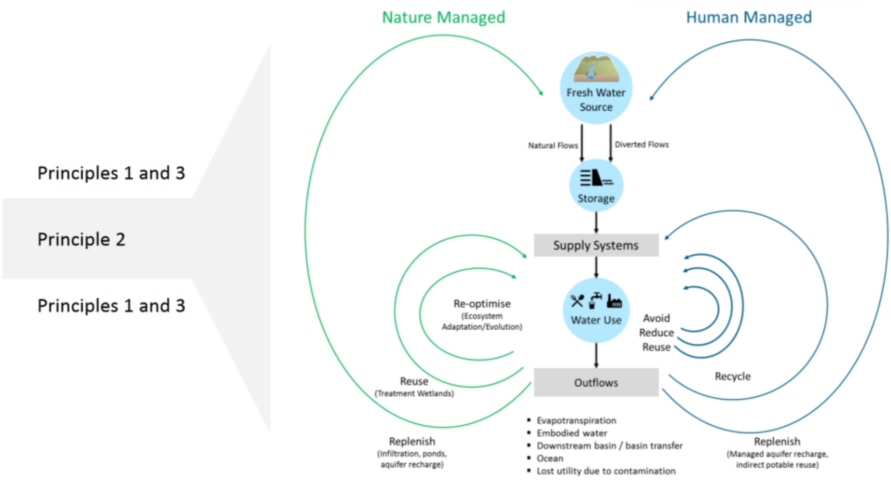

Figure 1.1. Circular Economy Systems Diagram specific to Water System from Ellen MacArthur Foundation et al. (2018).

Subsequently, water resource challenges can be faced from many points which include efficient rainwater management and efficient irrigation systems in the agricultural sector, leakage reduction and improvements to household water reuse efficiency in the municipal sector, and water saving schemes in the industrial sector (UNEP, 2015). Among them, water reclamation and its reuse is considered a strategic option to supplement water supplies and protect natural resources (Alcalde Sanz and Gawlik, 2014). For this reason, water reuse and recycling has been identified as one of the five top priorities of the European Innovation Partnerships Water (EIP Water).

Treated wastewater (also referred to as reclaimed wastewater or recycled water) can be used for various purposes. The main applications of treated wastewater include agricultural irrigation, landscape irrigation, industrial reuse, and groundwater recharge as published in the Joint Research Centre (JRC) report "Water Reuse in Europe. Relevant guidelines, needs for and barriers to innovation" (Alcalde Sanz and Gawlik, 2014). Within the industrial sector, the most popular applications include cleaning purposes and cooling towers make-up. The use of reclaimed water within the industrial sector provides economic, social and environmental benefits (Wintgens et al., 2013). Besides granting a reliable, locally-controlled water supply, water recycling can help decrease water abstraction from sensitive ecosystems. Other benefits include reducing wastewater discharges and decreasing and preventing pollution.

Various water reuse schemes have been implemented all over Europe. Old Ford Water Recycling Plant (OFWRP) located next to the Queen Elisabeth Olympic Park (QEOP, London) provides water for urban non-potable applications in London. In Germany, Braunschweig is one of very few large-scale agricultural reuse sites in Europe. In this case, the effluent of Steinhof wastewater treatment plant (WWTP) is used for agricultural restricted irrigation (fodder and industrial crops). Regenerated wastewater from Riu Sec WWTP is currently being used for urban purposes in Sabadell (Spain), mainly street cleaning, public parks and garden irrigation and urban uses in commercial areas. Regions suffering from extreme water scarcity, such as Israel, have also implemented water reuse schemes. The Shafdan WWTP, located in the heavily populated Dan Region near Tel Aviv, produces high quality water for unrestriced irrigation enabling agricultural activities under extreme water scarcity in the Negev. Additionally, several projects have been focused on water reuse within several industry sectors. As an example, Demoware project (Demoware, 2014-2016) implemented water reuse technologies by enhancing their performance, ensuring their safety, demonstrating how benefits overcome risks, helping on the development of a correct governance strategy and providing a single identity for the whole sector. Currently, the ongoing NextGen project (NextGen, 2018-2022) will demonstrate innovative technological, business and governance solutions for water in the circular economy in ten high-profile, large-scale, demonstration cases across Europe.

Large amounts of energy and materials have been traditionally utilised in wastewater treatment to meet discharge standards. Nonetheless, wastewater contains resources that can be recovered for secondary uses when treated properly. Thus, water reuse can be also managed not only from the water perspective but also as a source of energy and nutrients mainly approached from onsite energy generation, nutrient recycling and water reuse as reviewed by Mo and Zhang (2013). Accordingly, resource recovery strategies considered by utilities and industry decision makers should include both nutrients, energy, and water.

Assessment of the suitability and sustainability of the different water treatment strategies can be evaluated using the Life Cycle Assessment (LCA) tool. It provides measurements on environmental performance of different water reuse technologies according to the associated potential environmental impacts. LCA results can support decision making in selecting the most suitable technology for a given case or identifying opportunities to enhance the environmental performance of water recycling systems (Tangsubkul et al., 2005).

1.2. Types of wastewater

Large amounts of wastewater are generated daily. According to Metcalf & Eddy et al. (2007), wastewater can be defined as "used water discharged from homes, businesses, industry, cities and agriculture". Consistent with this definition, there are as many wastewater types as water uses (i.e. urban wastewater, industrial wastewater, etc.). Thus, quality and characteristics of wastewater depend on their source, the way they are collected and the treatment they receive. Typically, wastewater may contain organic material, suspended solids, dissolved salts, microbial load or toxic compounds. Therefore, wastewater treatment is meant to convert wastewater into an effluent that can be sent back to the environment with minimum impact, or directly reused.

This thesis presents different approaches for the treatment of four representative wastewater types, which include urban wastewater and three industrial effluents that are described in the following sections.

1.2.1. Urban wastewater

Anthropocentric activities unavoidably generate waste that, at some extent, ends up as wastewater. According to Mateo-Sagasta et al. (2015), data of the current values of wastewater generation and treatment is frequently not systematically monitored or not reported in many countries. Those authors reported values ranging from 390 km^3 to 477 km^3 of global annual domestic water withdrawals.

Wastewater characteristics are influenced by: behaviour, lifestyle, technical and juridical framework, design of sewer systems and climate conditions, among others. Main constituents of domestic wastewater include organic matter, microorganisms, nutrients and, to a lesser extent, metals and other inorganic constituents (Henze et al., 2008). Organic matter is the major pollutant. Typical composition of domestic/municipal wastewater is shown in Table 1.2, where high represents concentrated wastewater (low water consumption and/or infiltration) and low represents diluted wastewater (high water consumption and/or infiltration). It is noteworthy to point out that locations with frequent rain present diluted wastewater.

Table 1.2. Circular Economy Systems Diagram specific to Water System from Ellen MacArthur Foundation et al. (2018).

Parameter	High	Medium	Low
COD total	1,200	750	500
COD soluble	480	300	200
COD suspended	720	450	300
BOD	560	350	230
VFA (as acetate)	80	30	10
N total	100	60	30
NH_3-N	75	45	20
P total	25	15	6
PO_4^{3-}	15	10	4
TSS	600	400	250
VSS	480	320	200

To treat urban wastewater, the most widely used technology is based on the activated sludge process. Although this technology provides good quality effluents, it implies high costs for aeration as well as the generation of high amounts of biomass that needs further management (Metcalf & Eddy et al., 2003).

Therefore, from the circular economy perspective, challenges faced in urban wastewater treatment include decrease of energy consumption and decrease of sludge generated. The low organic content of urban wastewater has traditionally prevented its treatment via anaerobic processes, which have been conventionally used for the treatment of highly organic loaded effluents. However, in the recent years, anaerobic treatment of municipal wastewater has drawn attention since it presents several advantages over aerobic processes. One of these advantages is that, from the resource recovery perspective, organic content contained in wastewater can be transformed into energy (in form of biogas) through anaerobic processes. Anaerobic processes have no need for aeration and the production of biogas turns this technology into energetically self-sufficient. Moreover, anaerobic processes have a significant lower production of excess sludge, thus, less sludge management is required. These biological treatments are further described in section 1.3.1.

1.2.2. Industrial effluents

Unlike municipal wastewater, industrial effluents typically present high organic strength and extreme physicochemical nature (i.e., pH, temperature, salinity), and may contain substances that can be toxic to or inhibit biological treatment processes (Lin et al., 2012). Industrial effluents such as from food processing industry, landfilling, chemical industry, petroleum industry and tannery can present extremely different compositions among them.

This thesis covers the treatment of three representative industrial effluents with different characteristics. On one hand, food industry wastewater is represented by cheese whey, a highly biodegradable effluent presenting high organic load. On the other hand, an industrial wastewater with lesser organic content but highly refractory (non-biodegradable), such as petrochemical wastewater is studied. Finally, landfill leachate, with high recalcitrant organic content, high salinity and potentially toxic compounds is also investigated.

1.2.2.1. Food processing wastewater: cheese whey

Dairy industry is one of the main sources of generation of industrial effluent in Europe. This industry is based on the processing and manufacturing of raw milk into products such as yogurt, ice cream, butter, cheese and various types of desserts by means of different processes, such as pasteurization, coagulation, filtration, centrifugation and chilling among others (Rivas et al., 2010). Dairy effluents have variable composition, with regard of final products, system type, and operation methods used in the manufacturing plant. These effluents are mainly composed by different dilutions of milk (or transformed products), and cleaning water containing alkaline and acidic chemicals from the cleaning processes performed. Due to the high amount of dairy effluents generated, treating them is of crucial importance not only for the environment, but also for the purpose of recycling water for its reuse in industrial processes (Qasim and Mane, 2013).

Among such effluents is cheese whey, the liquid fraction obtained during cheese manufacturing which has a water content greater than 90% (Rivas et al., 2010). Specifically, cheese whey is the liquid effluent resulting from the precipitation and removal of milk casein in cheese making processes (Siso, 1996). As reviewed by Carvalho et al. (2013), the greater part of the milk lactose remains in the cheese whey, comprising the main fraction (90%) of the organic load. This organic load also contains fat and protein. The BOD_5/COD ratio is generally higher than 0.5 making this effluent highly biodegradable. Therefore, biological cheese whey treatment systems are preferred over physicochemical ones. Cheese whey inorganic fraction is attributable to presence of mineral salts, mainly NaCl and KCl and calcium salts (mostly phosphates). This inorganic content is the result of NaCl addition during cheese production. Additionally, cheese whey presents a significant risk of eutrophication to natural waters because of its nitrogen and phosphorus contents.

Thus, after the prior description, the main concerning pollutant is its organic content. This high organic strength, from the resource recovery perspective, makes it suitable for energy recovery using anaerobic digestion processes (Carvalho et al., 2013; Ergüder et al., 2001; Kalyuzhnyi et al., 1997; Prazeres et al., 2012). However, from the biological treatment point of view, high sodium contents may adversely affect biological digesters operation. Moreover, acidic pH (3.8–6.5) and low alkalinity may also affect its biological treatment efficiency inhibiting methane production. Other inhibiting parameters of the biological processes can be cited, such as free ammonia, potassium and volatile fatty acids (Appels et al., 2008). These challenges in anaerobic processes for the treatment of cheese whey need to be overcome to successfully apply this technology. Moreover, within the water cycle circular economy approach, and besides the energy recovery from the wastewater, cheese whey water content can potentially be reused by coupling anaerobic process with membrane technology using AnMBR configuration. Deeper explanation of the mentioned treatment technologies can be read in section 1.3.

1.2.2.2. Petrochemical wastewater

Industry sector is one of the main water users in Europe, accounting for about 40% of total water abstractions. Within this sector, industry of refined petroleum products is, in most European countries, the manufacturing industry with the highest water demand. Based on data from 2010, the petrochemical industry uses annually up to 2,725 hm^3 in Germany, 797 hm^3 in Belgium, 511 hm^3 in Norway and 201 hm^3 in Spain (Förster, 2014). Among the main industrial sectors, the manufacturing industry, also presents the highest wastewater production.

In the case of petrochemical industry, with oil as raw material, the industrial processes comprise a combination of organic matter sequential transformation processes primarily based on cracking, refining, distillation, reforming and synthesis. Most petrochemical companies in Europe have crackers by which complex organic molecules are broken down into simpler molecules such as ethylene and propylene. The estimated cracker capacity in Europe in 2015 was 23,303,000 Kt ethylene/year (Petrochemicals Europe, 2016). Significant large volumes of freshwater, primarily for processing and cooling, are needed for the cracking process, requiring an average amount of freshwater of 5.7 hm^3/year (Barthe et al., 2015).

Petrochemical wastewater composition varies depending on the processes performed at the refinery as well as on the type of crude oil used, therefore, wastewater presents huge fluctuations in quality and quantity (Wu et al., 2017). Moreover, the processes involved in this industry make petrochemical wastewater especially complex. It typically contains high concentrations of oil, salts, heavy metals, volatile phenols, polycyclic aromatic hydrocarbons and lipids (Wu et al., 2016). Generally, it is characterised by high organic strength, usually containing organic pollutants some of them refractory that can be toxic to or inhibit biological treatment (Wu et al., 2015). Those recalcitrant pollutants include aromatic and aliphatic hydrocarbons, phenolic substances, naphthenic acids and sulphides among others.

The nature of petrochemical wastewater implies several challenges for its treatment being recalcitrant and toxic compounds those that draw more attention. Usually the selection of the most suitable treatment system depends on the specific characteristics of the wastewater including composition and concentration of the pollutants. In many cases, effluents released from separate processing units are mixed prior to its treatment.

Typically, petrochemical wastewater treatments include physical and mechanical pre-treatment stages followed by a biological stage using activated-sludge (Bahri et al., 2018). Among these treatments, the biological ones, either suspended or immobilised, have been reported to successfully treat wastewater contaminated with various pollutants such as phenols or chlorophenols (Sanchez-Salas et al., 2016). Nevertheless, biological treatments still present several drawbacks including high organic shocks and the presence of toxic compounds. Thus, so far, different wastewater treatment approaches have been considered depending on wastewater composition. Flotation, coagulation, biological treatment and membrane separation processes as well as advanced oxidation processes for treating oily wastewater were reviewed in terms of treatment efficiency by Yu et al. (2017). In this review, combined processes were recommended due to the complexity of oily wastewater. From the water cycle circular economy perspective, treated petrochemical wastewater can potentially be reused within the same facilities either for cooling systems, cleaning or even used as process water. Therefore, combination of technologies that allow water reclamation in this industry sector need to be tested and verified prior to its use in full-scale systems.

1.2.2.3. Landfill leachate

Current global production of municipal solid waste (MSW) approaches roughly 1.3 billion tonnes per year, and it is expected to double to about 2.2 billion tonnes per year by 2025 according to the World Bank's report "What a Waste: A Global Review of Solid Waste Management" (Hoornweg and Bhada-Tata, 2012). The amount of municipal solid waste (MSW), one of the most important by-products of an urban lifestyle, is growing even faster than the rate of urbanization. In 2016, European Union generated 246,515 thousand tons of MSW, 24% of which was managed through landfilling. In southern European countries, this percentage was increased up to double or three-fold as is the case of Spain (57%), Croatia (77%) or Greece (82%) (Eurostat, 2018). Landfilling is a widely used process for disposing industrial and municipal solid waste thanks to its low exploitation and capital costs (Renou et al., 2008a). The sanitary landfilling method for the disposal of solid waste material is widely accepted and used thanks to its economic advantages. Besides its economic advantages, landfilling minimizes environmental impact and other inconveniences, and it allows waste to decompose under controlled conditions until its final transformation into relatively inert, stabilized material. Hence, the worldwide trend is for controlled sanitary landfilling as the preferred method of disposing of both solid urban waste and a large proportion of solid industrial waste. However, landfilling inevitably implies the generation of leachate which consists of a strongly polluted wastewater.

Landfill leachate is produced as a consequence of rainwater percolation through the landfill, biochemical processes and water content of wastes themselves. Landfill leachates typically contain high amounts of organic matter, ammonium, heavy metals, chlorinated organic and inorganic salts, although their composition may vary (Alvarez-Vazquez et al., 2004). The quality of leachates is affected by several factors: age, precipitation, seasonal weather variation, waste type and composition. Particularly, composition of landfill leachates changes significantly depending on the age of the landfill (Baig et al., 1999) as the composition of the landfilled material is transformed with time. Accordingly, three types of leachates were already defined in 1976 according to landfill age (Table 1.3). Landfill leachate properties can usually be represented by the basic parameters COD, BOD_5, the ratio BOD_5/COD, pH, suspended solids, ammonium, total Kjeldahl nitrogen and heavy metals. In young landfills, containing large amounts of biodegradable organic matter, a rapid fermentation occurs, resulting in volatile fatty acids (VFA) as the main fermentation products.

Acid fermentation is enhanced by high water content in the solid waste (Renou et al., 2008a). This early phase of a landfill's lifetime is called the acidogenic phase, and leads to the release of large quantities of free VFA, as much as 95% of the organic content. As a landfill matures, the methanogenic phase occurs. Methanogenic microorganisms develop in the waste and VFA are converted to biogas (CH_4, CO_2). The organic fraction in the leachate becomes dominated by refractory compounds such as humic substances. Hence, old landfill leachate is characterized by its low BOD_5/COD ratio and rather high ammonium content. For this reasons, the existing relation between the age of the landfill and the organic matter composition may provide useful criteria to choose a suitable treatment process.

Table 1.3. Landfill leachate classification vs. age (Chian and Dewalle, 1976).

	Recent	**Intermediate**	**Old**
Age (years)	<5	5–10	>10
pH	6.5	6.5–7.5	>7.5
COD (mg/L)	>10,000	4,000–10,000	<4,000
BOD_5/COD	>0.3	0.1–0.3	<0.1
Organic compounds	80% VFA	5–30% VFA + humic and fulvic acids	Humic and fulvic acids
Heavy metals	Low-medium	Low-medium	Low
Biodegradability	Important	Medium	Low

Accordingly, leachate treatment faces several challenges as its high organic load, mostly recalcitrant, as well as its high inorganic strength and potentially toxic compounds require from very efficient treatment technologies. Traditionally, leachate management and treatment options include recirculating the leachate back to the landfill, treating for sanitary sewer discharge, or treating for local surface water discharge. This management and treatment involve biological, chemical and physical methods. All these technologies were extensively reviewed by Renou et al. (2008a). Biological treatments can help partially remove its organic content as well as its nitrogen load. However, as mentioned before, success of the biological treatment highly depends on the leachate age, therefore, its biodegradability. However, in order to meet strict quality standards for direct discharge of leachate into the surface water, the development of integrated methods of treatment, that is, a combination of chemical, physical and biological steps, is required. From the water cycle circular economy approach, landfill leachate can be seen as a potential effluent for water reuse schemes. However, given the nature of the landfill, in this case, circular economy could mostly be focused on the treatment itself rather than in resource recovery. That is, for example, including the use of regenerated membranes in its treatment.

1.3. Wastewater treatment

Wastewater treatment has traditionally included two sorts of treatment: primary and secondary. The primary treatment is aimed to remove suspended solids, both organic and inorganic. This primary treatment is usually based on physicochemical systems such as settling and coagulation-flocculation, among others. The secondary treatment, based in biological systems, is aimed to degrade soluble organic materials. Typical secondary treatments include activated sludge, trickling filters, constructed wetlands or anaerobic processes. Conventional wastewater treatment generally ends with secondary treatment, which usually does not efficiently remove all compounds targeted but supplies an adequate quality for discharging into natural water bodies.

Consequently, when water reuse is targeted, tertiary treatment is usually required. Tertiary treatment aims at polishing wastewater before its reuse or discharge and may consist in the removal of nutrients, toxic compounds, residual suspended matter or microorganisms. In most cases, tertiary treatment includes membrane filtration, activated carbon or disinfection (i.e. chlorination, UV radiation, etc.), among others. As mentioned before and according to Voulvoulis (2018), water reuse offers the possibility to change the linear human water cycle towards a circular flow by closing the loop, aiming at decoupling human water consumption from the depletion and pollution of water reserves.

In the following sections, wastewater treatment technologies which have been studied in this thesis are described.

1.3.1. Biological wastewater treatment

The main objective of biological treatment is to remove or reduce the concentration of organic and inorganic compounds (nitrogen and phosphorous) from wastewater. Biological operations may be carried out in aerobic, anoxic or anaerobic environments and the choice will depend on the wastewater composition as well as the required effluent quality. Aerobic processes (oxygen is required) include conventional activated sludge (AS) or attached-growth configurations while anaerobic processes (oxygen is absent) include continuous stirred tank reactor (CSTR) and upflow anaerobic sludge blanket (UASB) reactor, among others. Anoxic processes (oxygen is not required) are typically used for nitrogen removal from wastewater and they can occur in attached growth or suspended growth reactors.

Among aerobic processes, activated sludge, developed in 1913, is the most representative of the suspended growth aerobic systems and it is currently the most widely used biological wastewater treatment process in the developed world (Scholz, 2016). AS process types include conventional continuous flow reactors and sequencing batch reactor (SBR), among others. Activated sludge systems can effectively remove organic matter content from wastewater, however, it has been widely recognised that aeration and post-treatment of the excess activated sludge are the major energy consumers of the process (Liu et al., 2018).

In addition, activated sludge systems also give goof results for nitrification, that is conversion of ammonium into nitrate. However, complete ammonium removal requires coupling of nitrification and denitrification processes, that is the coupling of aerobic and anoxic processes. In recent years, SBR systems have attracted big interest for domestic and industrial wastewater treatment as SBR can carry out biological nitrogen removal in a single reactor by maintaining aerobic and anoxic stages sequentially (Guo et al., 2007).

Contrary to suspended growth, in attached-growth systems, sessile microorganisms grow on a surface creating a biofilm. Among attached-growth systems there are trickling filters, rotating biological contactors and moving bed biofilm reactors (MBBR). Compared to conventional AS, MBBRs present several advantages. The biomass growing in biofilm instead of suspended flocs enables the decrease of reactor volume producing a very compact technology. Moreover, biofilm formation allows different microbial groups to compete and co-exist in different niches, even those microorganisms with lower growth rates (Piculell, 2016). In general, fixed-film processes are less sensitive to environmental variations and, thus, to toxic compounds (Renou et al., 2008b; Schneider et al., 2011). Thus, MBBR systems are potentially adequate for the treatment of potentially toxic effluents such as petrochemical wastewater. Regarding aeration, MBBR systems need higher dissolved oxygen (DO) concentration and higher mixing intensity due to diffusion limitation compared to activated sludge processes. The enhanced mixing both prevents carrier clogging and increases substrate availability. In addition, oxygen diffusion limitation creates a concentration gradient in the biofilm that, instead of being considered a drawback, permits the growth of different microbial niches in the different depths of the biofilm (Piculell, 2016). The biofilm formation process itself compresses four steps, namely attachment, accumulation, re-generation and maturation (Zhu et al., 2015). Once the steady state is reached, the detachment of biomass causes that part of this biomass remains in suspension. This suspended biomass can contribute to the overall performance of the reactor but it may vary due to growth rate, specific activity, loading rate and hydraulic retention time (HRT) (Piculell, 2016).

High organic loading rates and low sludge production are among the many advantages that anaerobic digestion (AD) processes show over other biological operations. Nevertheless, the energy production is the key feature for the increased application of anaerobic processes. Aerobic wastewater treatment involves high costs of aeration and sludge handling which are noticeably lower in anaerobic treatment as no oxygen is needed and there is less production of sludge. Moreover, greenhouse gas emissions during anaerobic treatment are lower compared to aerobic technologies if methane produced is used as an energy source (Lew et al., 2009). For these reasons, the treatment of wastewater is likely to be performed using anaerobic digestion processes. Anaerobic digestion assumes, however, that long solids retention times (SRTs) are necessary due to its slow growth rate. Anaerobic processes have been traditionally used for high organic loaded wastes such as excess sludge produced in AS, food wastewater or industrial wastewater, etc. However, recent works have studied its application to municipal wastewater, much diluted, which has traditionally been treated using AS systems (Song et al., 2018).

Anaerobic digestion is a multi-stage process, in which organic matter is degraded sequentially in several biological steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1.2**¡Error! No se**

encuentra el origen de la referencia.). Hydrolysis is carried out by hydrolytic bacteria. Acidifying bacteria then convert hydrolysis products into VFAs, alcohols, aldehydes, CO_2 and H_2. The next step is acetogenesis, in which all these products are transformed into acetate. Finally, methane and CO_2 are generated by acetotrophic methanogens. However, methane can also be produced directly from H_2 and CO_2 that are produced in the different steps of the anaerobic digestion process by the microorganisms called hydrogenotrophic methanogens. Anaerobic digestion removes organic wastewater content to produce biogas but does not have an effect on the removal of nitrogen or phosphorus.

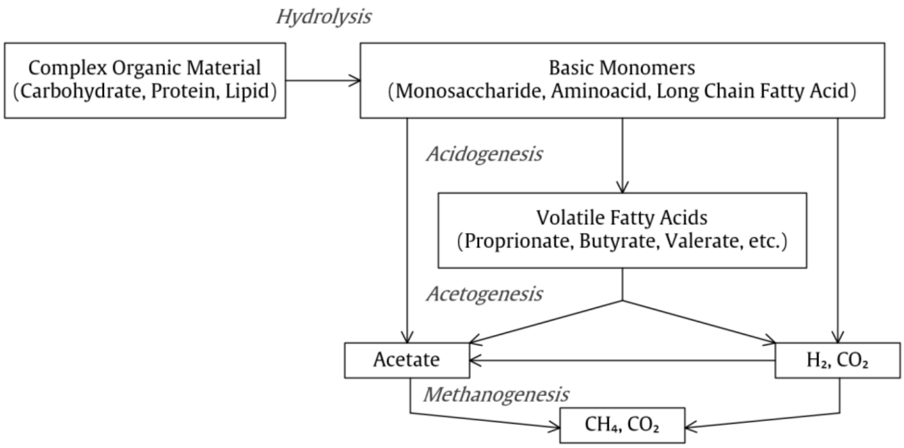

Figure 1.2. Steps of anaerobic digestion process (Evren et al., 2011).

Process instabilities such as inhibition, acidification, and foaming (especially at high organic loading rates (OLR)) are commonly linked to the microbial communities. Any imbalance in a single degradation step disrupts the whole process (Li et al., 2016). Under mesophilic and thermophilic conditions, VFA accumulation in anaerobic reactors indicates process imbalance since it is the main pre-methanogenic intermediate (Boe et al., 2010). Contrarily, the key bottleneck of anaerobic processes under low temperatures (<20°C) is the hydrolysis of the organic matter into soluble molecules, which causes the accumulation of suspended solids in the reactor and thus decreases the efficiency of the process (Ozgun et al., 2015, 2013; Petropoulos et al., 2017).

As stated before, anaerobic processes have been developed in different configurations such as conventional CSTR and UASB. On one hand, CSTR reactors are the most common low rate digesters for large scale application. In CSTR solids and liquid retention times are equal, thus, CSTR effluent consists of a digestate containing high amount of solids. On the other hand, UASB reactors, developed in the early 1970s by Lettinga and his coworkers (1980) allow the retention of high concentrated biomass thanks to the formation of a dense sludge bed as well as clever design and operation. Since UASB reactors are fed in upflow mode they act as settling devices in which non-settable biomass is released and settable biomass is kept in the reactor. This characteristic allows a better exploitation of the reactor working volume (Metcalf & Eddy et al., 2003).

Within the circular economy framework, energy self-sufficient biological wastewater has been of great interest for wastewater reclamation. This approach has been applied to municipal wastewater treatment schemes as reviewed by Liu et al. (2018). According to this review, the way towards energy self-sufficient operation of biological processes is based on maximising energy recovery, while minimizing energy consumption. Such process configurations are known as A-B processes (Wan et al., 2016). In this scheme, A-stage is aimed at capturing COD content from wastewater by means of anaerobic digestion, while B-stage is designed for nutrient removal or recovery (Figure 1.3). Thus, A-B processes are based on energy recovery via anaerobic digestion while minimizing energy consumption in the nutrients removal and recovery stage. Potentially used processes include chemically enhanced primary treatment, high rate activated sludge or anaerobic processes for A-stage and shortcut nitrification-denitrification or partial nitrification and anammox for B-stage.

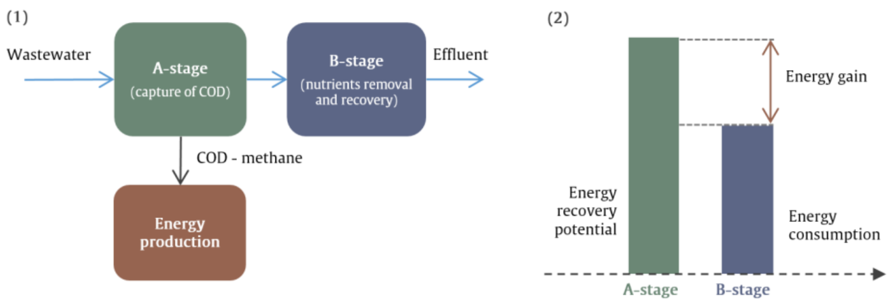

Figure 1.3. General configuration of an A-B process. (1) Scheme of an A-B process; (2) Energy balance of an A-B process (Wan et al., 2016).

1.3.1.1. Microbial ecology in biological treatment systems

Recent development of high-throughput sequencing technologies has pushed the study of microbial communities. Analysis of high-throughput sequencing data through suitable bioinformatics tools has played a significant role in the investigation of microbial metagenome. This knowledge is of special importance as there is a close relation between the performance and stability of biological wastewater treatment systems and the microbial community structure and dynamics of the bioreactor (Chen et al., 2017). Operational parameters such as influent composition, sludge retention time or dissolved oxygen can highly affect the microbial community structure. Hence, understanding the effect of different factors on microbial community can have a great impact on improving process performance.

1.3.2. Membrane technology

Membrane technology is employed in the water industry to improve the quality of water for use, reuse, or discharge to the environment. A membrane is a material which can be used in water and wastewater treatment that allows some physical or chemical components to cross more easily through it than others. It is therefore perm-selective, as long as it is more permeable to those constituents passing through it (which become permeate) than those which are rejected by it (which become retentate). The nature and structure of the membrane defines the degree of selectivity (Judd, 2011).

Membrane based separations have gained an increasing popularity over the last three decades and have become one of the most promising technologies for the 21st century (Guo et al., 2012). The different types of membrane processes currently available are shown in Table 1.4. In some processes it can be that membranes are not necessarily used to retain the contaminants and allow water to pass through, but they can instead be used to: either selectively extract constituents (extractive); or introduce a component in the molecular form (diffusive) (Judd, 2011). Mature commercial membrane applications in water and wastewater treatment are limited to the pressure-driven processes (reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF)) and electrodialysis (ED), which can extract problem ions such as nitrate and those ions associated with hardness or salinity using an electric field. Membrane technologies employed in the municipal sector are mainly pressure driven and, whilst the membrane perm-selectivity and separation mechanism may vary from one process to another, such processes have the common elements of a purified permeate product and a concentrated retentate waste (Figure 1.4).

Table 1.4. Dense and Porous Membranes for Water Treatment (Judd, 2011).

Pressure-driven/rejection	Extractive/diffusive
Reverse Osmosis (RO) Separation achieved by virtue of differing solubility and diffusion rates of water (solvent) and solutes in membrane.	**Forward Osmosis (FO)** Separation driven by difference in osmotic pressure across the membrane set up by employing an inert and recoverable "draw" solution on the permeate side.
Nanofiltration (NF) Separation achieved through combination of charge rejection, solubility-diffusion and sieving through micropores (<2 nm).	**Electrodialysis (ED)** Separation achieved by virtue of differing ionic size, charge and charge density of solute ions, using ion-exchange membranes.
Ultrafiltration (UF) Separation by sieving through mesopores (2-50 nm)	**Membrane Distillation (MD)** Separation driven by employing a partial vacuum on the permeate side to provide a difference in partial pressure.
Microfiltration (MF) Separation of suspended solids from water by sieving through macropores (>50 nm).	**Membrane Extraction (ME)** Constituent removed by virtue of a concentration gradient between retentate and permeate side of membrane.
	Gas Transfer (GT) Gas transferred under a partial pressure gradient into or out of water in molecular form.

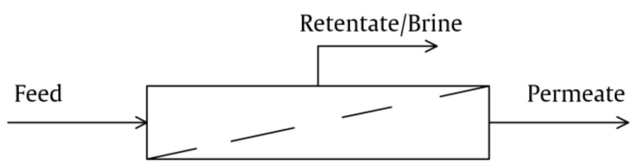

Figure 1.4. Scheme of a membrane process (Judd, 2011).

RO, NF, UF and MF are the four key membrane separation processes in which water forms the permeate product (Figure 1.5). Thus, membranes can be defined depending on the type of separation they are able to perform, which then gives an indication of the pore size. For the key membrane processes identified, pressure is applied to force water through the membrane. MF and UF are low-pressure processes that can effectively remove suspended solids, microorganisms (MF) and colloids (UF). Otherwise, NF and RO are high-pressure membrane processes and they can remove soluble salts and metal ions.

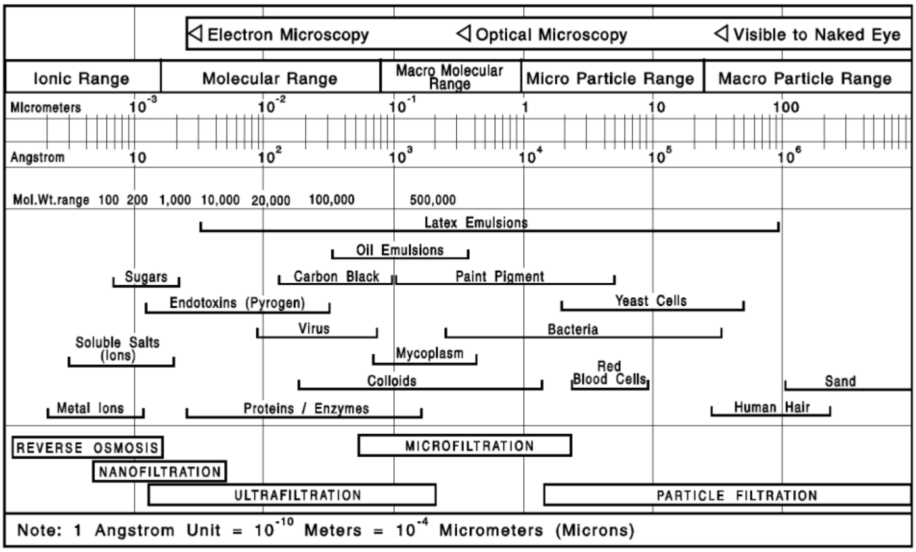

Figure 1.5. . Range of filtration processes (Dow Water & Process Solutions, 2016).

Rejection of pollutants is eventually a key limiting factor in all membrane processes. Rejected elements in the retentate have a tendency to accumulate at the membrane surface, causing a decrease in the flow of water through the membrane (flux) at a given transmembrane pressure (TMP), or, on the contrary, an increase in the TMP for a given flux, thus reducing the permeability (ratio of flux to TMP). These phenomena are collectively referred to as fouling. Membrane fouling represents the major limitation to membrane process operation. Fouling can occur due to a series of physicochemical and biological mechanisms all associated to increased accumulation of solid material onto the membrane surface (blinding) and within the membrane structure (pore restriction or pore plugging/occlusion). This should be differentiated from clogging, which occurs when the membrane channels are filled with solids due to poor hydrodynamic performance, a more common phenomenon than fouling in membrane bioreactors (MBRs).

Thus, the major obstacle for the application of membrane processes is membrane fouling. That is to say, membrane efficiency and membrane fouling are the significant challenges for RO, NF, UF and MF related technologies. This means that, to achieve a continuous and reliable operation, a water pre-treatment is usually required. Inappropriate pre-treatment requires frequent cleaning of the membrane elements to restore productivity and salt rejection. It is important to mention that the cost of membrane cleaning, downtime and lost system performance can be significant (Dow Water & Process Solutions, 2016).

1.3.2.1. Regenerated membranes

A sort of RO membranes, the spiral-wound ones, have been abundantly applied and established in the municipal and industrial sector for freshwater production and salt concentration in desalination of seawater (SW) and brackish water (BW). As the number of large desalination plants using membrane technology is increasing in the last years, the resulting number of old RO modules to be discarded is expected to become a critical challenge in the near future (Lawler et al., 2012). Membranes replaced at the end-of-life stage, together with the continuous growth of RO technologies, derives to a vast accumulation of end-of-life modules that are disposed in landfills (Goh et al., 2016), which are classified as inert solid waste. It has been reported that in brackish water treatment facilities there is an average replacement of modules between 10-20% per year, depending on the pre-treatment. In industrial and tertiary wastewater treatment facilities, the replacement is around 30% per year (Burn et al., 2015). As tendency towards circular economy, membrane reuse has been the focus of recent research on finding new purposes to the end-of-life membranes as summarized in Figure 1.6 (Landaburu-Aguirre et al., 2016).

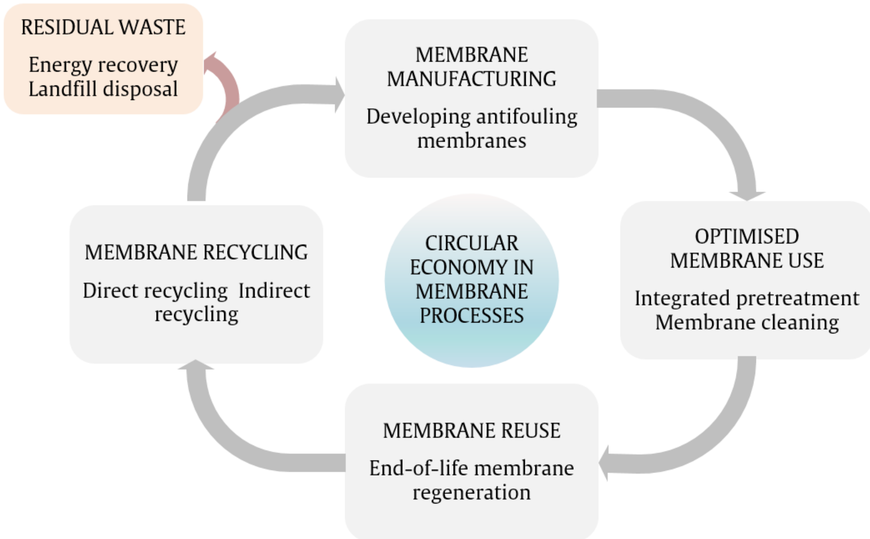

Figure 1.6. Circular economy in desalination (Landaburu-Aguirre et al., 2016).

An alternative to landfill management is recycling end-of life membranes by conditioning them for their reuse which can be either indirect or direct. On one hand, indirect reuse implies deconstructing the membrane element and reusing its parts for the assembly of other membranes. On the other hand, direct reuse consists of cleaning the membranes and recovering their commercial properties (Coutinho de Paula and Santos Amaral, 2017). Among other options, it is possible to chemically modify with an oxidative agent the membrane polymeric active layer leading to a membrane with new properties and uses. The direct reuse or recycle of membrane modules after treating them with an oxidative agent to grant them with new uses is also known as membrane regeneration (Coutinho De Paula et al., 2017; Lawler et al., 2013). Recently Lawler et al. (2015) performed a Life Cycle Assessment (LCA) of several end-of-life membrane disposal options (landfill, incineration, gasification, energy recovery, direct reuse and recycling) to quantify and compare their environmental impact. Results of this study showed that direct reuse is the most environmentally favourable option, whilst the current landfill disposal is the least favourable one. Membrane reuse options involve direct application of the old membranes within lower throughput systems (i.e. brackish water treatment) and chemical conversion into porous, ultrafiltration-like filters. Other options include, direct recycling of some of the module components, and energy recovery through incineration.

1.3.3. Hybrid systems

Increased challenges in wastewater treatment such as higher environmental standards as well as complex effluents to be treated and reused have raised interest for hybrid treatment systems. Those systems are based on the combination of two or more technologies which can include biological and physicochemical processes. Hybrid systems are meant to enhance significantly the efficiency and operability of the wastewater treatment synergistically, that is, reinforcing the two processes. The synergy of this treatments comes either from the increased pollutant removal, decreasing or eliminating adverse effects of some treatments or increasing the global functionality of the whole process. Hybrid processes are intended to be compact requiring less space and potentially decrease the economic impact of the treatment.

Hybrid systems that include biological processes are widely used and are very versatile in the treatment of different effluents. There are multiple combinations of bioreactors with membrane processes for the treatment of a wide range of urban and industrial wastewater. The coupling of both biological and membrane processes can enhance wastewater treatment strategies by providing excellent quality of the treated water, which can be reused for various purposes.

Within hybrid systems, membrane bioreactors (MBR) are wastewater treatment processes integrating a perm-selective membrane with a biological process. All currently available commercial MBR processes utilise a membrane for the rejection of the solid materials generated in the biological process to provide a clarified and disinfected product (Judd, 2011). MBRs can be either configured side-stream, in which the membrane is out of the reactor and it is operated in a cross-flow mode, or submerged, in which the membrane is submerged in the mixed liquor and it is operated in dead end mode. A classical MBR comprises a conventional activated sludge process (CASP) coupled with membrane separation to retain the biomass. In addition, it concentrates the biomass and therefore the reactor volume can be reduced and the treatment efficiency increased. MBRs, thus, tend to generate treated waters of higher purity with respect to dissolved constituents such as organic matter and ammonium, both of which are significantly removed by a biological treatment.

In the case of anaerobic treatments, the slow growth rate of the anaerobic microorganisms has been limiting the efficiency of anaerobic digestion, needing large reactor volumes for wastewater treatment by anaerobic digestion. Thus, anaerobic MBRs (AnMBR) in which the suspended solids retention time is increased, the degradation efficiency is increased. AnMBR provides short hydraulic retention times while keeping high solids retention time as no particulate matter can exit the system. Therefore, particulate organics retained in the reactor can be hydrolysed and decomposed because of the long solids retention time. Also AnMBR allows anaerobic microorganisms, which have relatively low growth rates compared with the aerobes, to grow without being washed out from the process. In the literature, AnMBRs are described for the treatment of a wide variety of wastewater types ranging from municipal wastewater and raw domestic wastewater, to white water from pulp and paper mills or petrochemical effluents (Chang, 2014; Musa et al., 2018; Peña et al., 2015). Regarding municipal wastewater in particular, both conventional MBRs and AnMBRs being operated under similar conditions ended up producing similar soluble COD removal efficiencies with AnMBRs avoiding at the same time all costs for aeration (Baek and Pagilla, 2006). AnMBR have been also reviewed as A-processes for energy self-sufficient wastewater treatment systems (Song et al., 2018).

MBR and AnMBR are configurations already implemented in full-scale. As an example of AnMBR, there is Memthane® technology developed by Veolia. This technology consists in an anaerobic digester coupled to an external ultrafiltration membrane configuration. Full-scale AnMBRs are constructed from one of three configurations; flat sheet submerged from Kubota-ADI, multi-tube (side-stream) serial from Veolia-Pentair and multi-tube (side-stream) parallel from Veolia-Pentair. According to Veolia's information in its web, to date seven full-scale Memthane AnMBR installations have been implemented to treat industrial wastewater from dairy, food and ethanol industries achieving COD removals from 96 to 99% from relatively high COD concentration.

A key step in the recent MBR development for food industry wastewater treatment was the idea of submerged AnMBR since it implies a reduction in implementation and operation investment costs. A submerged AnMBR system named "KSAMBR" was developed by Kubota Membrane Technology Inc. in the last decade, and it has been successfully applied for food and beverage wastewater treatments in 15 full-scale plants (14 in Japan, 1 in North America as of August 2008) (Kanai et al., 2010). This configuration consists in a separate chamber with a vacuum driven-membrane. Permeate from KSAMBR is subjected to aerobic treatment to obtain high-quality effluent. The main features of KSAMBR include stable operation, only one third or one-fifth footprint requirement, and the production of 3–5 times less biomass concentration than conventional digesters.

Nutrient removal has also been targeted within MBR technology. Ersu et al. (2008) evaluated the integration of an anaerobic and anoxic compartment with a submerged MBR and investigated various mixed liquor and permeate recirculation configurations for biological nutrient removal. Fu et al. (2009) modified a lab-scale MBR to evaluate the efficiency of nutrient removal in treating high strength synthetic water.

The coupling of biological and membrane processes has also been studied in different configurations for landfill leachate treatment. Bohdziewicz et al. (2008) studied landfill leachate treatment efficiency at lab scale using fermentation process in UASB reactor and post-treatment in RO process. Wang et al. (2014) tested at pilot scale anoxic/aerobic granular active carbon (GAC) assisted membrane bioreactors (A/O-GAC-MBR) integrated with nanofiltration – reverse osmosis. MBRs showed excellent and stable removal efficiency with average above 80% for COD and ammonium and the final permeate from RO was proved to accomplish the limits for reutilization in industry. Zhang et al. (2013) combined an aerobic submerged MBR/RO system enhanced by Fenton oxidation for treatment of old municipal landfill leachate. Another configuration was tested by Hasar et al. (2009) in which ammonia stripping was followed by a coagulation–flocculation process, COD and suspended solids (SS) were removed 36% and 46%, respectively. After pre-treatment, an aerobic/anoxic membrane bioreactor (Aer/An MBR) accomplished COD and total inorganic nitrogen (total-N) removals above 90% and 92%. Finally, RO was applied to the collected Aer/An MBR effluents.

Hybrid systems have also been tested for petrochemical industry effluents. Hansen et al. (2016) showed the feasibility for the reuse of the petrochemical internal streams as makeup water in cooling towers, in a cascade based system. Venzke et al. (2017) used an RO system for water reclamation in the petrochemical industry after a conventional AS and stabilisation ponds. Although it showed promising results, calcium concentration of reclaimed water was too high for the water being used in high-pressure boilers. Furthermore, different pre-treatment methods such as coagulation-filtration and UF, and two final membrane treatment technologies, NF and RO, for desalination of a cooling tower blowdown (CTBD) were investigated by Davood Abadi Farahani et al. (2016). MBR systems have also been studied for the treatment and reuse of petrochemical wastewater (Bayat et al., 2015; Lin et al., 2011).

The combination of membrane bioreactor and reverse osmosis (MBR–RO) is becoming an attractive technology for wastewater reclamation and reuse. In their work, Xiao et al. (2014), tested the treatment of semiconductor industry wastewater using a hollow fibre PVDF membrane followed by a spiral-wound reverse osmosis membrane in a pilot scale system. Cartagena et al. (2013) studied the reduction of emerging micropollutants by combining MBR-NF/RO treatment. In their study, municipal wastewater was treated in a MBR pilot plant with flat sheet and hollow fibre membranes coupled with NF/RO membranes. Results showed that the quality of water obtained was appropriate for reuse and it had salinity removal efficiencies higher than 97%, 96% for total organic carbon (TOC), 91% for nitrates and 99% for total phosphorous (TP). Also, Alturki et al. (2010), tested at lab scale a 9L MBR system with submerged hollow fibre UF membranes coupled to a cross-flow membrane filtration test unit in which NF and RO membranes were tested. An enhanced removal efficiency of a wide range of trace organic contaminants in indirect potable water was obtained.

Subsequently, hybrid systems for wastewater treatment are promising technologies intended for water reuse and resource recovery. Different configurations of hybrid systems are studied in this thesis for the treatment of different urban and industrial effluents in the framework of circular economy in the water cycle.

1.4. Research motivation and thesis overview

This thesis is framed in the Sustainability Unit at Eurecat, the Catalan Technological Centre (former CTM Centre Tecnològic). This group focuses its research activity in different areas of the water cycle, which include separation processes (particularly membranes), biological processes, life cycle analysis, process modelling, quality assurance in water distribution networks and health risk analysis, among others. The Sustainability Unit is provided by technological and scientific tools including chemical and microbiological analytic equipment, equipment to simulate, at lab and bench scales, a whole series of water treatment processes. These water treatment processes include biological reactors, pressure-driven membrane processes (MF, UF, NF and RO), current-driven membrane processes (electrodialysis), advanced oxidation processes, among others.

This thesis was carried out within the framework of three European projects from Eurecat and one project from Crandfield University (during a research stay that took place at the beginning of 2018). UASB technology intended for urban wastewater treatment (Chapter 3) was researched in Cranfield University under an agreement with the utility Severn Trent Water. In addition, AnMBR technology for cheese whey treatment (Chapter 4) was developed within Demoware FP7 project (Grant agreement no. 619040, 2014-2016). Demoware was led by Eurecat (CTM at that moment) and it was aimed to enhance the availability and reliability of innovative water reuse solutions in both agriculture, industrial and urban sectors.

Project consortium included 27 partners from 10 different countries working in 10 demonstration sites located all over Europe and dealing with different research activities within the water reuse sector. Regarding petrochemical wastewater treatment (Chapter 5), it was researched within the project LIFE Rewatch (LIFE15 ENV/ES/000480, 2017-2020). LIFE Rewatch had the objective of demonstrating an innovative recycling scheme to increase the water efficiency in the petrochemical industry. Project consortium, led by Eurecat, also included Dow Chemical Ibérica, Veolia Water Systems Ibérica, KWR and WssTP. Finally, landfill leachate treatment (Chapter 6) was developed in the framework of LIFE+ Releach project (LIFE13 ENV/ES/000970, 2014-2017). Its main objective was to demonstrate and disseminate the technical and economic feasibility of decreasing the overall environmental impact of waste management by adopting new landfill leachate treatment strategies. Both public and private Spanish entities, including TYPSA, Protecmed, ARC and CBGR, were part of the project consortium. As in the other projects, Eurecat (CTM at that moment) was the leader partner of Releach project.

1.4.1. Research motivation

The increasing water demand coupled to the depletion of natural water sources has raised the need to investigate and develop in the field of wastewater treatment and reuse. Even more, the application of circular economy principles to water cycle has highlighted the need to see wastewater as a source of water and valuable compounds. Thus, more efficient and sustainable treatment systems should be developed. Nowadays, besides the interest in recovering resources from wastewater, current wastewater treatment technologies (i.e. activated sludge, separation processes, etc.) face several difficulties when treating challenging wastewater. Such difficulties include high organic loads, inhibitions due to toxic compounds, etc. In this sense, this thesis covers both urban and industrial wastewater treatments. Industrial wastewater is represented by cheese whey from food industry (high organic load), petrochemical wastewater (lesser organic content but highly refractory) and landfill leachate (high recalcitrant organic content, high salinity and potentially toxic compounds).

Both to recover resources from wastewater and to overcome challenges related to wastewater treatment, it is required to develop and test hybrid processes (section 1.3.3) that take the best of each technology and are capable to gain to the limitations of current conventional treatment. Therefore, this thesis is conducted from an applied research approach, aimed to reduce the gap between basic technology development and industrial implementation. It is crucial to demonstrate the treatment technologies at a scale between lab and full scale to obtain understanding on technologies that will contribute to overcome the aforementioned limitations.

Based on these principles, this thesis aims to use novel hybrid systems for wastewater treatment to help close the loop of the circular economy in wastewater treatment field.

1.4.2. Thesis overview

This document is divided into seven chapters. The first chapter (*Introduction*), in which this section is included, comprises the introduction to the subject plus an extensive state of the art. The second chapter (*Objectives*) states the objectives of this thesis. Chapters 3 to 6 (Figure 1.7) include the main part of the thesis and describe the studies performed using hybrid systems for wastewater treatment aimed at water reuse, resource recovery and decrease of the environmental impact. Finally, last chapter (*Conclusions and future perspectives*) gives an overview of the main achievements of this work and points out the topics for future research derived from this thesis.

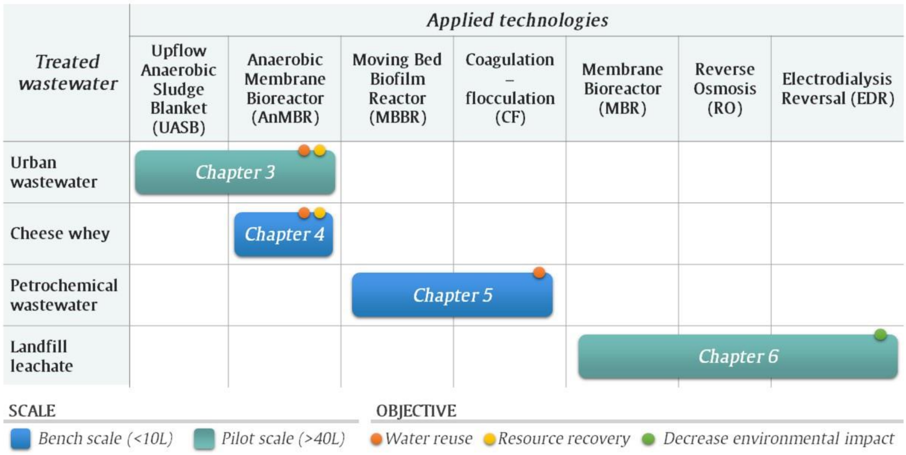

Figure 1.7. Overview of the thesis content.

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CHAPTER 2

Objectives

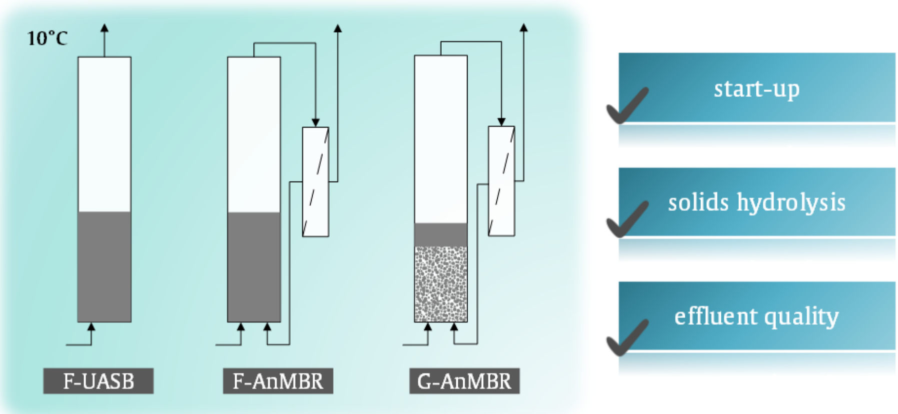
The main objective of this thesis is to study and enhance the efficiency of hybrid systems for wastewater treatment. This efficiency is related to either water reuse, resource recovery or decrease of the environmental impact for both urban and industrial water cycles.

Specific objectives for each chapter are the following:

- To improve the performance of UASB systems in terms of start-up, solids hydrolysis and effluent quality by innovative configurations for municipal wastewater treatment under psychrophilic conditions (Chapter 3).
- To demonstrate the technical feasibility of AnMBR for the recovery of resources (energy and water) in the treatment of cheese whey (Chapter 4).
- To optimise the pre-treatment of petrochemical wastewater for in-situ water reclamation (Chapter 5).
- To minimise the environmental impact in landfill leachate treatment by increasing the recovery of current membrane-based systems and using tailor-made regenerated membranes (Chapter 6).
- To characterize the microbial community in the bioreactors (Chapters 3, 4 and 5).

CHAPTER 3

Hydrolysis and start-up of anaerobic municipal wastewater treatment under psychrophilic conditions: the importance of reactor configuration



Abstract

Three upflow anaerobic sludge blanket (UASB) pilot scale reactors with different configurations: with flocculent biomass (F-UASB), with flocculent biomass and membrane solids separation (F-AnMBR) and granular biomass and membrane solids separation (G-AnMBR), were operated to compare start-up, solids hydrolysis and effluent quality. The reactors were fed in parallel with settled wastewater in a temperate climate at 9.7 ± 2.4. Both the low temperature and the low COD content (sCOD 54.1 ± 10.3 mg/L and pCOD 84.1 ± 48.5 mg/L), made the work challenging and not previously reported. The seed sludge had been stored for 5 months and it was previously used in pilot scale AnMBR for a period of three years at temperatures between 7-22°C. A really quick start-up during the acclimation period (days 0-16) was observed for the three reactors and could be attributed to the previous acclimation of the seed sludge to the settled wastewater and temperate climate temperatures. The results obtained for the first 45 days of operation showed that solids management was critical to achieve a high effluent quality. The UASB configurations with membrane retained the solids in the reactor increasing solids hydrolysis efficiency. Flocculent biomass promoted slightly higher hydrolysis than granular biomass since flocculent sludge acted as a fine filter whilst the granular acted as a coarse filter. The microbial diversity of the biomass was also assessed. *Proteobacteria*, *Bacteroidetes* and *Firmicutes* phyla accounted for an abundance of around 70% in the three reactors but its distribution was slightly different. *Bacteroidales* and *Clostridiales* were the major bacterial fermenter orders detected and a relative high abundance of syntrophic bacteria, represented by *Syntrophobacterales* (9.5-11.2%), was also detected. Additionally, an elevated abundance of sulphate reducing bacteria (SRB) (i.e. *Desulfovibrionales* and *Desulfobacterales*) was also identified and was attributed to the low COD/SO_4^{2-} ratio of the wastewater

(0.5). The coexistence of acetoclastic and hydrogenotrophic methanogenesis is suggested due to the high abundance of *Methanosaeta* spp. (50-53%) as well as *Methanomicrobiales* (13-22%) and *Methanobacteriales* (9-16%). Overall, the F-AnMBR showed the higher rates of hydrolysis per solid removed (38 ± 25%) among the three different UASB configurations tested under psychrophilic conditions.

3.1. Introduction

Low strength municipal wastewater is characterised by its low organic content (COD 500 mg/L) and high solids content (TSS 250 mg/L) (Henze et al., 2008). The most widely used technology for wastewater is based on the activated sludge process, but this implies a high cost for aeration as well as the generation of high amounts of biomass that needs to be further managed (Metcalf & Eddy et al., 2003). In the recent years, anaerobic treatment of municipal wastewater has drawn attention since it presents several advantages over aerobic processes. Anaerobic processes have no need for aeration and the production of biogas makes this technology potentially self-sufficient in terms of energy. Moreover, anaerobic processes have a significant lower production of excess sludge. The upflow anaerobic sludge blanket (UASB) reactors, developed in the early 1970s by Lettinga and his coworkers (Lettinga et al., 1980) allowed the retention of high concentrated biomass thanks to the formation of a dense sludge bed as well as clever design and operation. Since UASB reactors are fed in upflow mode they act as settling devices in which non-settable biomass is released and settable biomass is kept in the reactor. This characteristic allows the better exploitation of the reactor working volume (Metcalf & Eddy et al., 2003). The use of UASB reactors for municipal wastewater treatment is common practice in semi tropical climates (Chong et al., 2012; Ozgun et al., 2013). However, the characteristics of municipal wastewater still constitute a challenge for anaerobic systems in temperate climates (Stazi and Tomei, 2018).

The key bottleneck of anaerobic processes under low temperatures (<20°C) is the hydrolysis of the organic matter into soluble molecules, which causes the accumulation of suspended solids in the reactor and thus decreases the efficiency of the process (Ozgun et al., 2013, 2015b; Petropoulos et al., 2017). Besides, it is difficult to achieve a low effluent chemical oxygen demand (COD) due to low substrate affinity of the anaerobic biomass (Ozgun et al., 2013). To overcome these limitations, anaerobic membrane bioreactor (AnMBR) technology has been investigated. The main success of AnMBRs for municipal wastewater treatment at low temperatures is the complete decoupling of hydraulic retention time (HRT) and sludge retention time (SRT). Hence, this configuration allows the complete retention of biomass inside the reactor and produces higher quality effluent in terms of COD, TSS and pathogen counts (Liao et al., 2006). Furthermore, recent studies have shown how intermittent sparging can reduce the energy demand for controlling membrane fouling (K. M. Wang et al., 2018a). In the case of UASB configured AnMBR, sludge bed traps most of the most of the particulate matter by adsorption and biodegradation, thus the TSS in the membrane tank is lower than in continuously stirred tank reactor (CSTR) configured AnMBR reactors, potentially decreasing the fouling propensity of the membrane (Ozgun et al., 2015a). However, membrane integration removes the hydraulic selection pressure required for granulation, by preventing the washout of flocculent sludge with low settling properties, which could potentially decrease the settleability of the biomass in the long-term operation (Ozgun et al., 2015a).

Each stage of the anaerobic wastewater degradation process is executed by different microbial communities. The connections between microbial community structures and operational conditions are under study (Ali Shah et al., 2014; Park et al., 2017; Svojitka et al., 2017; P. Wang et al., 2018; Zhu et al., 2017). Microbial communities in anaerobic digesters have remained unknown for a long time (Morris et al., 2014). The recent application of molecular technologies, such as next-generation sequencing, has increased the knowledge and understanding of the complex microbial interactions in the anaerobic process (Fischer et al., 2016). While bacterial structures and functions are known, with elevated functional redundancy despite variable taxonomic composition, numerous methanogen groups remain unidentified or poorly understood, and changes between digesters have not been examined in detail (Wilkins et al., 2015).

Anaerobic UASB reactors can use flocculent or granular biomass. From the superior settling capacity of granular sludge, it could be assumed that granular sludge could be advantageous for UASB based AnMBR. However, to date, few studies have compared granular and flocculent biomass with the purpose of evaluating the two inoculums in UASB configured AnMBR treating municipal wastewater. Martin et al. (2013) compared a granular UASB configured AnMBR with a flocculent CSTR configured AnMBR and confirmed the lower fouling propensity of the granular UASB while the biological performance was similar. Nevertheless, given the different reactor configuration applied, the impact of the flocculent or granular biomass in UASB configured AnMBR could not be directly inferred. On the other side (Wang et al., n.d.) compared granular and flocculent UASB configured AnMBRs concluding that flocculent biomass could be utilised as an alternative to granular biomass since similar permeability was obtained when sludge blanket was controlled. While Wang et al. (Wang et al., n.d.) focused their research in settleability of the sludge blanket, hydrolysis and microbial diversity still need to be investigated.

Both the low temperature and the low COD content, make the current work challenging and not previously reported. Thus, the aim of this work was to compare start-up, solids hydrolysis and effluent quality of three UASB configurations for municipal wastewater treatment under psychrophilic conditions (9.7 ± 2.4°C).

3.2. Materials and methods

3.2.1. Experimental set-up

Three reactors were operated in parallel in this study; two 70 L cylindrical UASB (0.2 m diameter x 2.2 m height) and one 42.5 L UASB (0.19 m diameter x 1.5 m height) with lamella settlers for solid/liquid/gas separation at the top of the column (Figure 3.1). One of the 70L reactor was operated as an UASB with flocculent biomass (F-UASB), while the other two reactors were operated as AnMBR but with flocculent and granular biomass (F-AnMBR and G-AnMBR) by coupling them to a submerged hollow fibre membrane. The flocculent 70L reactors (F-AnMBR and F-UASB) were inoculated with 16L of municipal digested sludge treating a mixture of primary and secondary sludges. The granular 42.5L reactor (G-AnMBR) was inoculated with 16L granular sludge from a mesophilic UASB used for pulp and paper industry. Both inoculums had a previous acclimation of 3 years treating the same wastewater and had been left without feeding for five months (K. M. Wang et al., 2018b).

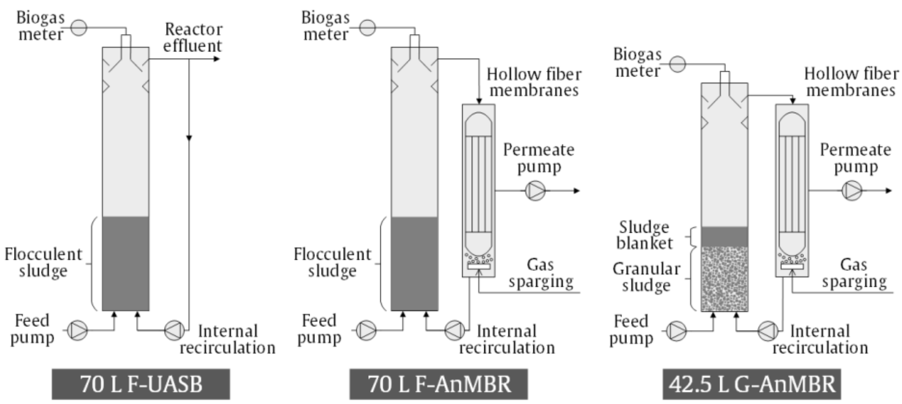


Figure 3.1. Schematics of pilot scale F-AnMBR, F-UASB and G-AnMBR.

Settled wastewater from Cranfield University wastewater treatment plant with a capacity of 2840 population equivalent, was fed through the bottom of the three UASB reactors using peristaltic pumps (520U, Watson Marlow, Falmouth, UK). All three reactors were operated at HRT of 8 h. Peristaltic pumps (620S, Watson 117 Marlow, Falmouth, UK) were used for the internal recirculation to keep the upflow velocity (V_{up}) at 0.4 m/h (Metcalf & Eddy et al., 2014). In the three reactors, sludge expanded to about 30% of the column height. In the case of G-AnMBR, there was a sludge blanket layer above the granular sludge bed, which was composed of dispersed growth flocs from the influent, as previously described by Aiyuk et al. (2006) and Chong et al. (2012). Whilst for flocculent reactors there was no obvious differentiation between the sludge blanket and inoculum flocculent sludge bed. The sludge height in the UASB column was measured in a daily basis.

In the F-AnMBR and G-AnMBR configurations, the effluent was fed to 30L membrane tanks and from there, recycled to the base of the reactor to maintain upflow velocity. In F-AnMBR, the hollow-fibre membrane module (ZW-10) (GE Water & Process Technologies, Oakville, Ontario, Canada) comprised four elements, each 76 polyvinylidene fluoride (PVDF) hollow fibres (0.52 m in length and 1.9 mm outer diameter), providing a total surface area of 0.93 m². In G-AnMBR, the hollow-fibre membrane module (ZW-10) (GE Water & Process Technologies, Oakville, Ontario, Canada) comprised four elements, each 54 polyvinylidene fluoride (PVDF) hollow fibres (0.72 m in length and 1.9 mm outer diameter), providing a total surface area of 0.93 m². The membranes had a nominal pore size of 0.04 µm.

Permeate was driven using peristaltic pumps (520U, Watson Marlow, Falmouth, UK). In F-AnMBR, transmembrane pressure was monitored by a pressure transducer (-1 to 1 bar, Gems sensor, Basingstoke, UK) in the permeate line and recorded by a data logger (ADC-2006, Pico Technology, St Neots, UK). In the G-AnMBR, pressure transducers on the permeate line (-1 to 1 bar, PMC 131, Endress+Hauser, Manchester, UK) and at the bottom of the membrane tank (0 to 2.5 bar, 060G2418, Danfoss, Nordborg, Denmark) were used to monitor TMP and liquid level height respectively.

Nitrogen-enriched air, produced by a nitrogen generator (NG6, Noblegen gas generator, Gateshead, UK), was used for continuous gas sparging. Specific gas demand per surface area (SGD_m) of 2.0 $m^3/(m^2\ h)$ was kept along operation. Since the HRT was fixed to 8 h, it resulted in an initial normalised permeate flux of 13.2 LMH for F-AnMBR and 8.3 for G-AnMBR, normalised to 20°C according to Judd (2011):

$$J_T = J_{20} \cdot 1.025^{(T-20)} \tag{3.1}$$

3.2.2. Analytical methods

Alkalinity, pH, total suspended solids (TSS) and biological oxygen demand (BOD_5) were measured according to standard methods (APHA et al., 2012). Sulphate concentration, total and soluble chemical oxygen demand (COD) were analysed with Merck test kits (Merck KGaA, Darmstadt, Germany). Soluble COD was measured after filtering with 0.45 µm retention membrane filters (47 mm Cellulose Nitrate Membranes, Whatman, GE Healthcare Life Sciences, Little Chalfont, UK). Particle size distribution (PSD) was measured using Mastersizer 3000 laser diffraction particle size analyser (Malvern Instruments Ltd, Malvern, UK).

Samples for volatile fatty acids (VFAs) analysis were filtered (0.45 µm), acidified (H_2SO_4) and kept frozen at -20°C prior to its analysis. VFAs were quantified using high performance liquid chromatography (HPLC) by means of a Shimadzu HPLC system (Kyoto, Japan) with a Phenomenex Rezex ROA/Organic Acid 7.80mm×300mm column (Phenomenex, Macclesfield, UK) according to Parawira et al. (2004).

Biogas flow rate was measured by means of three gas meters (TG0.5, Ritter, Bochum, Germany). Biogas methane (CH_4) composition was analysed by a gas analyser (Servomex 1440, Crowborough, UK). Dissolved methane was calculated using unitless form of Henry′ s law for dissolved gases, which, in the reactor headspace will depend on temperature, partial pressure and solubility (Crone et al., 2016). The unitless form of Henry′ s law is written as:

$$\frac{C_g}{C_s} = H_u \tag{3.2}$$

Where C_g is the concentration of constituent in gas phase (mg/L), C_s is the saturation concentration of constituent in liquid (mg/L) and H_u is unitless Henry′ s law constant, which will vary with temperature (Metcalf & Eddy et al., 2014). Methane yield was calculated accounting for COD used for methanogenesis, that means taking into account COD used for sulphate reduction by sulphate reducing bacteria (Lens et al., 1998), and total methane produced. Reaction rates were calculated according to the equations presented by Elmitwalli et al. (2002):

$$Hydrolysis\ (\%) = 100 \times \frac{CH_4\ as\ COD + sCOD_{eff} - sCOD_{inf}}{tCOD_{inf} - sCOD_{inf}} \tag{3.3}$$

$$Methanogenesis\ (\%) = 100 \times \frac{CH_4\ as\ COD}{tCOD_{inf}} \tag{3.4}$$

In order to evaluate the differences in the measured parameters Tukey HSD tests for multiple comparison of means were performed ($p< 0.05$), whereby different subscript letters indicate statistically significant differences.

3.2.3. Microbial community analysis

3.2.3.1. Sludge sampling, DNA extraction and library preparation

Biomass samples from the three reactors were taken after 45 days of operation. Samples were frozen at −80 °C for further analysis. For DNA extraction, samples were centrifuged at 5000 x g for 10 min. DNA extraction from the obtained pellet was performed using PowerSoil® DNA Isolation Kit (Mo Bio Laboratory Inc., USA).

Library preparation was performed at Centre for Omic Sciences, COS (Reus, Spain). Partial bacterial 16S rRNA gene sequences were amplified from extracted DNA using primer pair 341F-532R (5'-CCTACGGGRSGCAGCAG-3'; 5'-ATTACCGCGGCTGCT-3'), which targets the V3 region of the 16S rRNA gene sequence and primer pair 515F-806R (5'-GTGCCAGCMGCCGCGGTAA-3'; 5'-GGACTACHVGGGTWTCTAAT-3') which targets the V4 region. Partial archaeal 16s rRNA gene sequence was amplified using primer pair S-D-Arch-0787-a-S-20 and S-D-Arch-1043-a-A-16 (5'-ATTAGATACCCSBGTAGTCC-3'; 5'-GCCATGCACCWCCTCT-3') (Fischer et al., 2016). All these primers were designed to include at their 5' end one of the two adaptor sequences used in the Ion Torrent sequencing library preparation protocol linking a unique Tag barcode of 10 bases to identify different samples. PCR cycle parameters are described elsewhere (Ellis et al., 2012; Fischer et al., 2016; Milani et al., 2013; Tridico et al., 2014). In short, PCR products were confirmed by a 2% agarose gel and specific bands were excised and then purified using Nucleospin Gel (Macherey-Nagel, Germany). The concentration of the PCR amplicons was analysed by electrophoresis on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and the kit Agilent High Sensitivity DNA (Agilent Technologies, USA). Equimolar pools (60 pM) of each fragment and sample were combined.

3.2.3.2. Ion Torrent PGM sequencing and sequenced-based microbiome analysis

Multiplexed samples were prepared for sequencing employing the Ion 520 & Ion 530 Kit-Chef (Life Technologies, USA) according to the manufacturer's instructions. Prepared samples were loaded on an Ion 530 Chip and then sequenced using Ion GeneStudio S5 (Life Technologies, USA) at 850 reads per run. After sequencing, individual sequence reads were filtered by the PGM software to remove low quality and polyclonal sequences. Those reads were analysed using QIIME (v1.9.1) (Caporaso et al., 2011), the analysis included OTU clustering, Alpha-diversity analysis, OTU analysis and species annotation. OTU assigning method was UCLUST and the taxonomy assigning method was BLAST. Sequence similarity threshold for both OTU and taxonomy assignments was 97%. The taxonomy database employed was *GreenGenes* for 16s rRNA gene sequences. Principal component analysis (PCA) was used to compare reactors microbial communities. PCA was performed using Matlab.

3.3. Results and discussion

3.3.1. Performance of UASB and UASB-AnMBR systems

The influent, effluent and membrane permeates characteristics are presented in Table 3.1. In this study an initial acclimation period took place from day 0-16 at a temperature of 7.1 ± 1.9 °C, followed by a steady state period (days 17-45) which was conducted at 10.3 ± 2.1 °C which represented a significantly higher temperature (Table 3.1). The average temperature of this study was significantly lower than temperatures of previous AnMBR studies for the treatment of municipal wastewater, Gouveia et al. (2015) operated at 18 ± 2 °C, Wang et al. (2018) at 16.3 ± 3.7 °C and Martin Garcia et al. (2013) worked in a range of 10 to 20 °C.

Table 3.1. Inlet wastewater and effluent characteristics (average + standard deviation). Temperature is in °C, pH in upH and the rest of the parameters are in mg/L.

Para-meter	Inlet	F-UASB			F-AnMBR					G-AnMBR				
		Reactor effluent			Reactor effluent		Permeate			Reactor effluent		Permeate		
		Days 0-16	Days 17-45	Days 0-45	Days 0-16	Days 17-45	Days 0-16	Days 17-45	Days 0-45	Days 0-16	Days 17-45	Days 0-16	Days 17-45	Days 0-45
Temp.	9.7±2.4	7.1±1.9	10.3±2.1	9.3±2.4	7.1±1.9	10.3±1.9	n.a.	n.a.	n.a.	7.1±1.9	10.3±2.0	n.a.	n.a.	n.a.
pH	7.8±0.2	8.1±0.2	8.0±0.1	8.0±0.1	8.0±0.2	7.9±0.1	8.6±0.2	8.7±0.1	8.7±0.1	8.1±0.2	8.3±0.1	8.6±0.1	8.7±0.1	8.7±0.1
sCOD	54.1±10.3	42.6±19.3	53.4±6.3	49.5±13.3	58.7±22.3	56.7±8.3	39.9±7.0	38.0±5.9	38.1±6.0	68.0±23.1	59.5±9.0	39.1±6.7	28.4±4.2	31.8±6.7
pCOD	84.1±48.5	49.8±16.8	36.0±16.7	39.4±19.4	86.8±62.6	182±93.8	1.3±1.3	0.4±0.9	0.7±1.1	443±152	266±106	1.5±0.8	0.7±1.3	0.8±1.4
BOD_5	67.8±25.7	71.6±27.9	68.2±11.4	69.9±17.5	107	103±12.4	10.9	9.9±1.3	11.1±1.9	143±73.5	131±32.8	13.2±6.9	5.9±0.2	6.5±1.2
TSS	47.7±30.1	13.1±14.0	27.2±14.8	22.0±15.7	29.3±18.9	121±58.4	<1	<1	<1	238±109	173±60.6	<1	<1	<1
Alk.	226±20.7	310±7.1	291±18.1	299±16.9	328±17.7	258±31.9	335±7.1	264±9.5	292±38.1	348±10.6	316±29.6	345±0.0	304±26.7	321±29.3
VFA	8.1±7.9	n.a.	0.4±0.7	n.a.	n.a.	n.a.	n.a.	3.6±4.4	2.6±4.4	n.a.	n.a.	n.a.	2.0±2.4	2.0±2.4
SO_4^{2-}	70.0±1.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

The settled municipal wastewater presented an average COD content of 153 ± 75.1 mg/L, representing a weak wastewater for anaerobic processes (Stazi and Tomei, 2018). Previous studies with settled domestic wastewater presented equal to higher COD contents from 221 to 976 mg/L showing it is possible to use AnMBR technology for the treatment of this weak wastewater (Gouveia et al., 2015; Martin Garcia et al., 2013; Shin et al., 2014; K. M. Wang et al., 2018a). Thus, both the low temperature and the low COD content, made the current work challenging and not previously reported.

Inlet pH was slightly alkaline 7.8 ± 0.2 and it did not vary significantly after treatment (Table 3.1). However, a pH increase was observed in permeate after membrane filtration similarly to what was observed by Wang et al. (2018). This increase could be attributable to CO_2 stripping due to nitrogen gas sparging. This CO_2 stripping would cause carbonate-bicarbonate buffer equilibrium displacement leading to a pH increase. Average BOD_5 content in feed wastewater was 67.8 ± 25.7 mg/L. F-UASB treatment showed a low BOD_5 removal (8.6 ± 7.7 %), while for F-AnMBR and G-AnMBR sensibly higher removal percentages were observed, specifically 80 ± 5.9 % and 89 ± 4.3% respectively. For AnMBR configurations, permeate COD and BOD_5 obtained are comparable to previous studies of AnMBR operated on the same sewage (Martin Garcia et al., 2013; K. M. Wang et al., 2018a).

COD removal efficiency was quite steady from the beginning of the operation as can be observed from Figure 3.2, achieving, during the acclimation period (days 0 to 16), sCOD (F-UASB – 13 ± 11%, F-AnMBR – 35 ± 6%, G-AnMBR – 31 ± 18%) and pCOD (F-UASB – 61 ± 19%, F-AnMBR – 99 ± 1%, G-AnMBR – 100 ± 0%) removals similar to those obtained for the rest of the period studied (days 17-45). sCOD removal from day 17 to 45 was (F-UASB – 11 ± 12%, F-AnMBR – 31 ± 12%, G-AnMBR – 48 ± 11%) while for pCOD it was (F-UASB – 60 ± 32%, F-AnMBR – 99 ± 2%, G-AnMBR – 98 ± 2%). The quick start-up according to COD removal efficiencies during acclimation period (days 0 to 16) can be attributed to the previous acclimation of the biomass to the temperate treatment conditions, even with the previous five-month period of storage without feeding. For the whole operation period, average sCOD removal in the F-UASB without the membrane was 11.0%, varying from 1-28%, while for the AnMBR configurations it was around 32 ± 11% for flocculent sludge and 43 ± 15% for granular sludge (Figure 3.2). Higher sCOD removals were obtained when using membrane configurations. The pore size of the filter used for sCOD determination was 0.45 µm while the average pore size of the membrane was 0.04 µm. This indicated that an important fraction of soluble COD would be retained by the ultrafiltration membrane (Gouveia et al., 2015). The same was observed previously by Ozgun et al. (2015) when comparing UASB and AnMBR performances. Similar sCOD removal efficiencies were observed for the AnMBRs, although a slightly better removal efficiency was observed for G-AnMBR. During the whole period, AnMBR configurations, as expected, achieved high pCOD removals for both sludge types, accounting for 99 ± 2% in both cases. On the other side, although F-UASB was capable of partially removing pCOD (57 ± 30%), its efficiency was lower than AnMBRs thanks to the solid retention capacity of the membranes. Similarly, Hejnic et al. (2016) reported an increase from 64% to 85% in the total COD removal after adding a membrane to a UASB system. Also, Peña et al. (2015) demonstrated that membrane effect increased 45% total COD removal efficiency. The higher COD removal efficiency observed for AnMBR configurations could be explained because of the complete retention of all particulate, colloidal and biomass matter into the system.

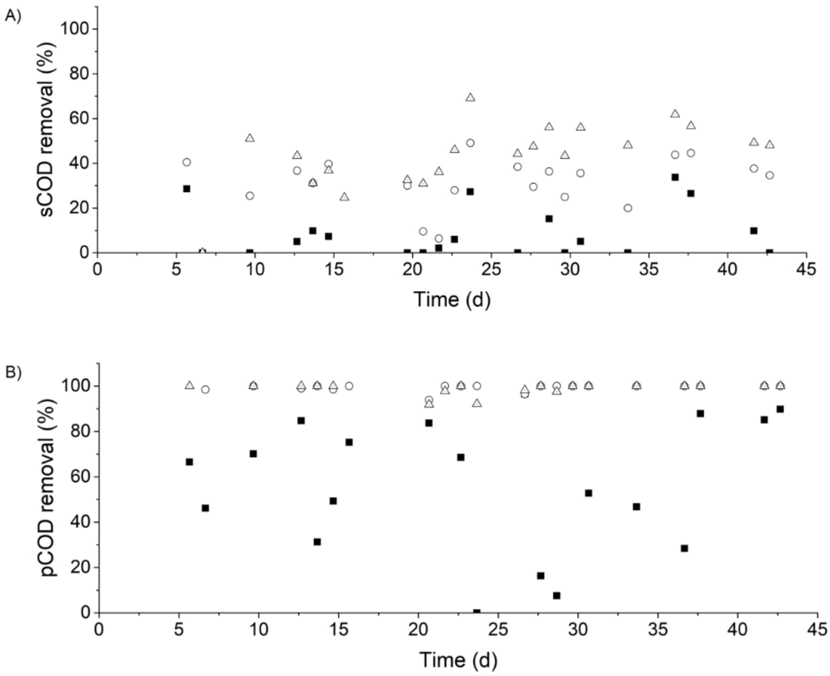


Figure 3.2. (A) sCOD and (B) pCOD removals. F-UASB (▪), F-AnMBR (○) and G-AnMBR (△).

After the acclimation period, the methane yields of the three configurations were compared, including both gas and dissolved methane contents. F-UASB methane yield (0.13 ± 0.12 m^3 CH_4/kg tCOD removed) was lower than methane yield for F-AnMBR (0.20 ± 0.14 m^3 CH_4/kg tCOD removed) and G-AnMBR (0.18 ± 0.09 m^3 CH_4/kg tCOD removed) although high standard deviations were obtained for this parameter. Thus, membrane had a positive effect since the retention of solids was supposed to enhance hydrolysis, solubilising the organic matter and increasing the methane yield of the system.

Membrane bioreactors have been widely known for its efficient retention of particulate matter into the system (Chang, 2014). As expected, TSS removal in the configuration including membranes was higher than for the

F-UASB not coupled to membrane, accounting for 99 ± 1% in F-AnMBR and G-AnMBR, while it was held at 57 ± 34% in F-UASB. Because of the inclusion of the membrane in the system, TSS concentration found at reactor effluents in AnMBR, prior to membranes, were significantly higher than TSS detected at UASB effluent (Table 3.1). This could be explained by the build-up of sludge and suspended particles into the AnMBR systems thanks to the complete retention of particles by membranes that, in the case of F-UASB were cleared from the system.

Turbulence created by the gas sparging in the membrane tank could also lead to particle break-up and disintegration that would also be retained in the system. This effect was previously described at lab scale by Ozgun et al. (2015) when comparing UASB and AnMBR performances. In spite of the increase of effluent solids, the UASB reactor still acts as a proper biofilter prior to membrane treatment, which prevents membrane from facing great concentrations of TSS. Although TSS were higher for AnMBR than for UASB, its concentration was still kept at <500 mg/L, which is lower than the concentrations faced by membranes in CSTR-based AnMBRs (Martin Garcia et al., 2013).

Figure 3.3 shows the total suspended solids mass balance for the three studied configurations. The mass balance for F-AnMBR and G-AnMBR is statistically similar, meaning biomass inoculum did not affect the TSS removal efficiency of the system, but lower TSS removal was obtained in the F-UASB. From these results it can be stated that the membrane becomes essential when it comes to TSS removal, since F-UASB TSS removed per day and per volume of reactor (27 ± 20 mg TSS/(d L)) were significantly lower than the AnMBR ones (111 ± 57 mg TSS/(d L) for F-AnMBR and 113 ± 59 mg TSS/(d L) for G-AnMBR).

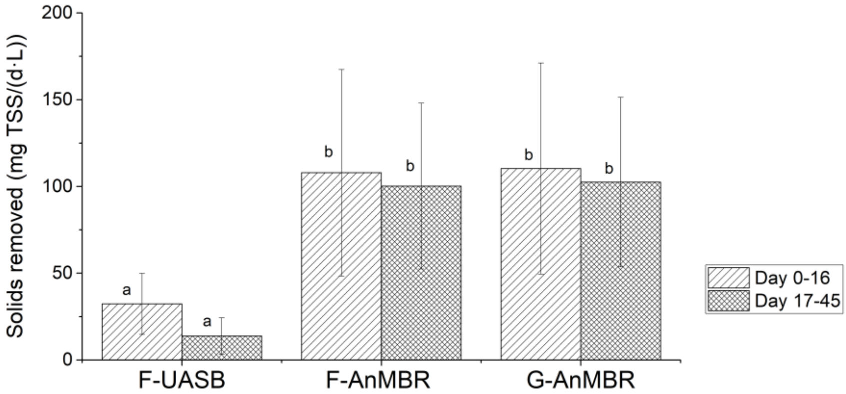


Figure 3.3. TSS mass balance. Error bars represent standard deviation. Different subscripts represent the statistically differences using Tukey HSD comparisons ($p < 0.05$).

Particle size analysis were performed on a daily basis and they showed no big differences between flocculent reactors in which median particle size, D50, was 66.8 ± 61.1 µm and 78.5 ± 75.6 µm for F-AnMBR and F-UASB respectively while D90 was 280 ± 106 µm for F-AnMBR and 302 ± 107 for F-UASB. The granular AnMBR showed smaller particle size than flocculent reactors, with a D50 of 21.1 ± 11.1 µm and a D90 of 139 ± 78.8 µm. Despite the similar values in the flocculent reactors, the PSD curves (Figure 3.4) show F-AnMBR presented slightly smaller particles than F-UASB. As previously observed for the TSS values, the operation of the AnMBR did not allow the wash out of finer particles and this was reflected in the PSD. It is shown that the most important differences in PSD are related to the reactor biomass. Ozgun et al. (2015a) compared a flocculent UASB reactor before and after membrane addition, concluding that the membrane incorporation induced a decrease in PSD and a drop in sludge settleability while no decrease in permeate quality was observed, which is in agreement with the results obtained.

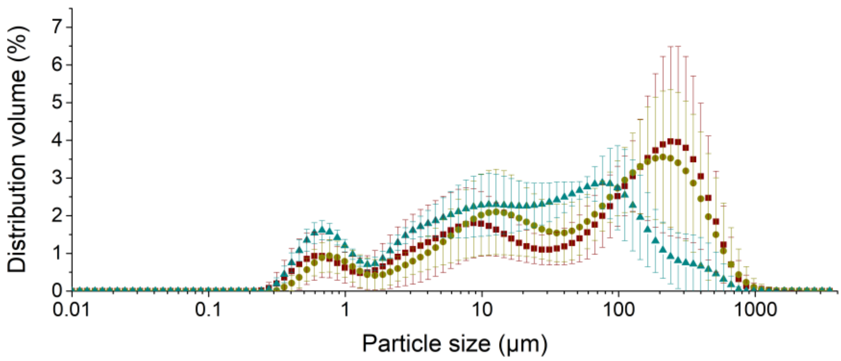


Figure 3.4. PSD in F-UASB (red ▪), F-AnMBR (green ○) and G-AnMBR (blue △).

Hydrolysis has been proved to be the limiting step in anaerobic digestion at low temperatures rather than methanogenesis, since methanogenesis is less temperature sensitive than hydrolysis (Lester et al., 2009; Petropoulos et al., 2017). From results shown in Figure 3.5, there was no big differences between hydrolysis and methanogenesis under the working conditions tested. After the acclimation period, hydrolysis was statistically similar for F-UASB (54 ± 12%) and F-AnMBR (38 ± 17%) while it was lower for G-AnMBR (23 ± 14%) (Figure 3.5). It can be stated that flocculent sludge seemed to perform better for hydrolysis step than granular sludge. On the other hand, no differences were observed in methanogenesis after the acclimation period since it was statistically similar for F-UASB (28 ± 3%), F-AnMBR (33 ± 7%) and G-AnMBR (32 ± 6%).

To further evaluate differences between the three configurations tested, the efficiency of solids hydrolysis in terms of mass solids hydrolysed per volume and time was calculated and it is shown in Figure 3.6. From this, it can be stated that, after the acclimation period, the higher solid hydrolysis was found in F-AnMBR configuration (38 ± 25%), given that it was lower in F-UASB (8 ± 6%) and G-AnMBR (23 ± 18%). Given that in UASB reactors settled biomass acts as a filter, it was hypothesized that granular sludge could act as a coarse filter while flocculent sludge as a fine filter. This differences in particle size in the biomass and thus its filtration performance, could explain the differences in the efficiency of solids hydrolysis in terms of mass solids hydrolysed per volume and time (Wang et al., n.d.). As can be shown in Table 3.1, TSS in the granular reactor effluent (173 ± 60.6 mg/L) were higher than for the flocculent one (121 ± 58.4 mg/L).

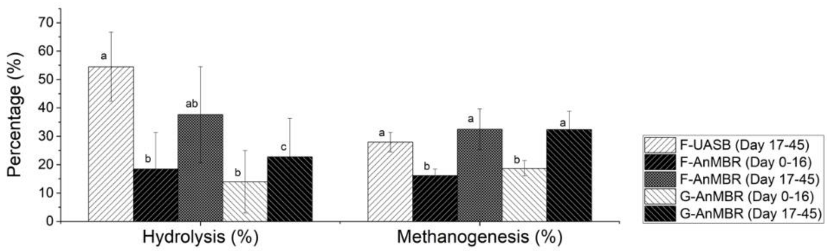


Figure 3.5. Percentage of hydrolysis and methanogenesis. Error bars represent standard deviation. Different subscripts represent the statistical differences using Tukey HSD comparisons ($p < 0.05$).

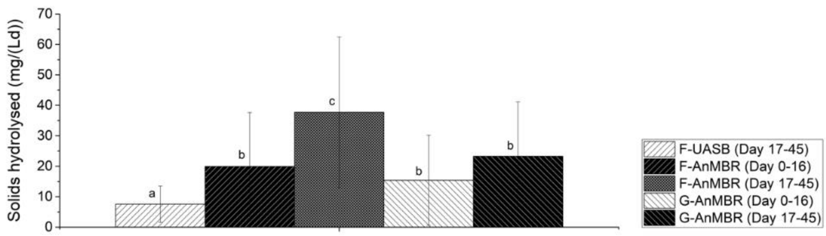


Figure 3.6. Solids hydrolysed (mg/(Ld)). Error bars represent standard deviation. Different subscripts represent the statistically differences using Tukey HSD comparisons ($p < 0.05$).

3.3.2. Microbial community structure

Microbial community analyses were performed. All the rarefaction curves showed gentle slopes under current sequencing depth. This fact indicates that the sequencing libraries could properly reflect the microbial communities. Alpha-diversity analysis revealed greater richness and diversity values in the bacterial community compared to the archaeal community, which is consistent with previous studies of microbial communities in AnMBR (Seib et al., 2016) (Table 3.2). Chao 1 and Shannon Indexes indicated that bacterial diversity was higher in F-AnMBR, while archaeal diversity was higher in F-AnMBR and G-AnMBR than in F-UASB when analysing 16S rRNA.

Table 3.2. Characteristics of sequencing libraries.

Target gene	Sample	Number of sequences	Number of OTUs	Chao 1 value	Shannon Index
Bacterial 16S rRNA (V3+V4)	F-UASB	239419	4187	4303	9.41
	F-AnMBR	294431	4181	4290	9.50
	G-AnMBR	153641	3345	3828	9.08
Archaeal 16S rRNA	F-UASB	161342	419	430	3.31
	F-AnMBR	151423	438	455	3.50
	G-AnMBR	209826	440	457	3.54

PCA analysis demonstrated that the three samples analysed were highly similar (Figure 3.7). The three samples were clustered near the same value for first principal component (PC 1), which explained >95% of the variance for both analyses (bacteria and archaea). Main differences between reactors were due to second principal component (PC 2) which explained less than 5% of the variance. As can be inferred from PCA results, higher similarities were found in flocculent reactors, regardless of the membrane presence in the system. Thus, reactor inoculum had higher influence in microbial community than reactor configuration. As the same wastewater was fed in the three reactors and the working temperature was the same in all cases, it could explain high similarity between samples. As commented before, this start-up was performed with a seed sludge which had been previously acclimated for 3 years treating the same wastewater although it had been left without feeding for five months before the operation commenced. This fact could also explain the low variability of microbial communities in the three reactors.

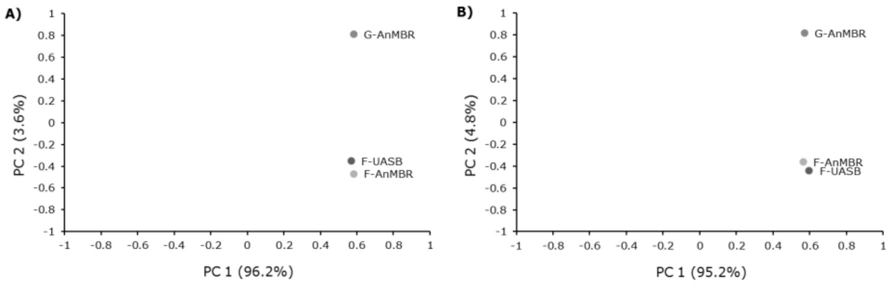


Figure 3.7. Principal component analysis (PCA) of (A) Bacteria and (B) Archaea 16S rRNA sequencing profiles for each reactor.

Proteobacteria, *Bacteroidetes* and *Firmicutes* phyla accounted for an abundance of around 70% in the three reactors although its distribution was slightly different. In the three reactors, *Proteobacteria* was the predominant phylum, followed by *Bacteroidetes* and *Firmicutes*. Flocculent reactors presented slightly higher percentages for *Proteobacteria* (F-UASB – 38.4%, F-AnMBR – 36.0%, G-AnMBR – 34.4%) and *Bacteroidetes* (F-UASB – 22.8%, F-AnMBR – 24.8%, G-AnMBR – 21.3%) than the granular reactor. On the other hand, *Firmicutes* abundance was higher in the granular reactor (F-UASB – 7.8%, F-AnMBR – 8.1%, G-AnMBR – 14.8%). The first step in the anaerobic digestion process is the hydrolysis of complex polymers to oligomers performed by hydrolytic fermentative bacteria. *Bacteroidetes* and *Firmicutes*, with a high level of metabolic diversity, are typically the predominant phyla of hydrolytic bacteria in anaerobic digestion as reviewed by Azman et al. (2015). In the three reactors, most of *Firmicutes* OTUs detected belonged to *Clostridiales* order (Figure 3.8**¡Error! No se encuentra el origen de la referencia.**), a group known for its capabilities in organic decomposition and fermentation (Desvaux, 2005). Also, microorganisms of the order *Bacteroidales*, belonging to the phylum *Bacteroidetes*, were the most predominant (F-UASB – 21.7%, F-AnMBR – 23.4%, G-AnMBR – 21.2%) (Figure 3.8). *Bacteroidales* are known for saccharolytic and proteolytic activity and are capable of producing propionate, acetate and succinate while *Clostridiales* are involved in hydrolysis and the fermentation of carbohydrates (Buettner and Noll, 2018; Ju et al., 2017). Ju et al. (2017) suggested the codominance of *Bacteroidales* and *Clostridiales* given their different ecological traits and rules, occupying different niches in anaerobic digestion communities.

Proteobacteria abundance in anaerobic digesters is usually lower than the amount detected in the reactors studied in this work. The main orders detected within *Proteobacteria* included syntrophic bacteria (i.e. *Syntrophobacterales*) and sulphate reducing bacteria (SRB) (i.e. *Desulfovibrionales* or *Desulfobacterales*), as can be observed in Figure 3.8. In anaerobic digestion, syntrophic organic acid degradation is crucial for stable wastewater treatment, given that acid accumulation is known to trigger acidification and process failure (Narihiro et al., 2015). Syntrophic bacteria, such as *Syntrophus* spp. or *vadinCA02* spp. (Figure 3.9), are capable of degrading organic matter to volatile fatty acids and hydrogen. Concretely, *Syntrophus* spp. produce H_2 through fermentation of organic compounds, being capable of maintaining syntrophic interactions with hydrogenotrophic methanogens (Botello Suárez et al., 2018). On the other hand, methanogens and SRB are hydrogen and acetate consuming organisms which contribute to the syntrophic relationship as consumers. However, despite the high relative abundance of SRB detected in the samples (as can be observed from Figure 3.9), methane production was not affected during operation, thus, high abundance of SRB did not hamper methanogenesis mediated by syntrophic bacteria. Fed wastewater had a low COD content with respect to SO_4^{2-} content, which led to a COD/SO_4^{2-}

ratio of the wastewater feed near 0.5. Lu et al. (2017) reported that methanogenic archaea could out-compete sulphate reducers even at a low COD/ SO_4^{2-} ratio of 0.5 and stated that low COD/SO_4^{2-} favoured the sulfidogenesis process and diversified the microbial community inside the reactor. Their research proved the beneficial effect of sulfidogenesis in favouring sludge re-granulation when treating high sulphate methanolic wastewater.

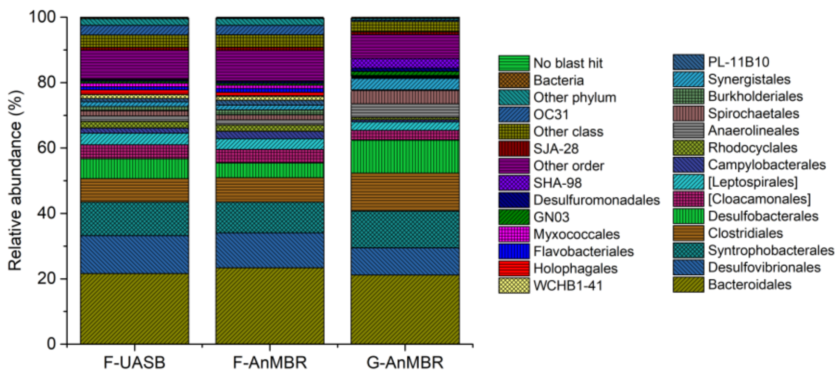


Figure 3.8. Taxonomic assignment of sequencing reads from bacterial community at 85% confidence level (order). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others" .

The potential enteric human pathogen *Arcobacter*, which was detected in all samples (Figure 3.9), has been previously reported as part of residue microbiota (Penfield, 2017). Residue populations associated with undigested feed wastewater have been commonly observed in anaerobic digesters, being more abundant in low-temperature digesters as reported by Mei et al. (2017).

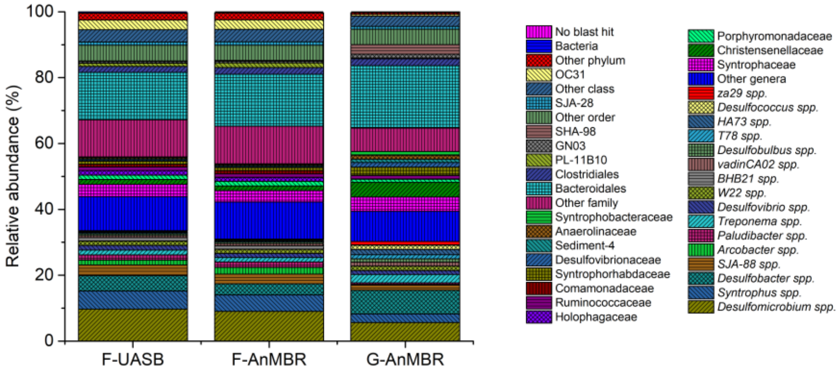


Figure 3.9. Taxonomic assignment of sequencing reads from bacterial community at 95% confidence level (genera). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others".

Archaea (Figure 3.10) are key organisms in anaerobic digestion processes, as they are responsible for methanogenesis step, in which CH_4 is produced. There are three main types of methanogens according to the substrates used: acetoclastic (acetate), hydrogenotrophic (H_2 and CO_2), and methylotrophic (methylated compounds) although most of the CH_4 is produced by the first two types. Only microorganisms of two genera are recognised as acetoclastic methanogens, *Methanosaeta spp.* and *Methanosarcina spp.* However, *Methanosarcina spp.* can use both acetate and H_2. The most common genera within hydrogenotrophic methanogens are *Methanobacterium*, *Methanothermobacter*, *Methanobrevibacter*, *Methanospirillum* and *Methanoculleus*. (P. Wang et al., 2018).

In the three reactors, *Methanosaeta* spp. was by far the most abundant genus (F-UASB – 53.7%, F-AnMBR – 50.1%, G-AnMBR – 50.0%) (Figure 3.10B). Thus, these results suggest acetoclastic pathway at low temperatures took place during operation. However, relatively high abundance of hydrogenotrophic archaea (*Methanospirillum* spp., candidatus *Methanoregula* spp., *Methanolinea* spp. and *Methanobacterium* spp.) was also detected (Figure 3.10B), meaning probably both pathways coexisted in the reactors. Reviewed literature presents opposing results when it comes to the methanogenic pathway favoured under psychrophilic conditions. On one hand, psychrophilic conditions have been described to favour hydrogenotrophic methanogenesis. *Methanomicrobiales* populations, and thus hydrogenotrophic methanogensis, have been reported to play an important role in low-temperature anaerobic granular sludge systems and digestion under psychrophilic conditions in a number of studies (Connaughton et al., 2006; Gunnigle et al., 2015; McHugh et al., 2004; Tian et al., 2018; Zhang et al., 2012). However, several authors also described an increase in acetoclastic methanogenesis and a high abundance of *Methanosarcinales* in anaerobic digestion under psycrophilic conditions (O' Reilly et al., 2009; Penfield, 2017; Zhang et al., 2018).

As can be observed in Figure 3.10A, *Methanomicrobiales* (F-UASB – 21.6%, F-AnMBR – 22.0%, G-AnMBR – 12.7%) and *Methanobacteriales* (F-UASB – 15.3%, F-AnMBR – 16.3%, G-AnMBR – 8.7%) were detected in higher abundance in the flocculent reactor while E2 (F-UASB – 8.2%, F-AnMBR – 10.0%, G-AnMBR – 26.4%) presented a higher abundance in the granular reactor Figure 3.10A. *Methanomicrobiales* detected included *Methanospirillum* spp., candidatus *Methanoregula* spp. and *Methanolinea* spp. While the first two were more abundant in the flocculent reactors, *Methanolinea* spp. was more abundant in the granular reactor. Zhang et al. (2012b) suggested *Methanomicrobiales* are likely to perform key roles in low-temperature anaerobic granular sludge systems and under psychrophilic conditions and also reported the detection of *Methanolinea* spp. at working temperatures of 5-18°C. Narihiro et al. (2015) reported *Methanolinea* spp. were specifically isolated by enrichment under syntrophic conditions. Group E2 (Figure 3.10A) was exclusively represented by candidatus *Methanomassiliicoccaceae* (Figure 3.10B) which has been reported to be an hydrogenotrophic methanogen (Iino et al., 2013).

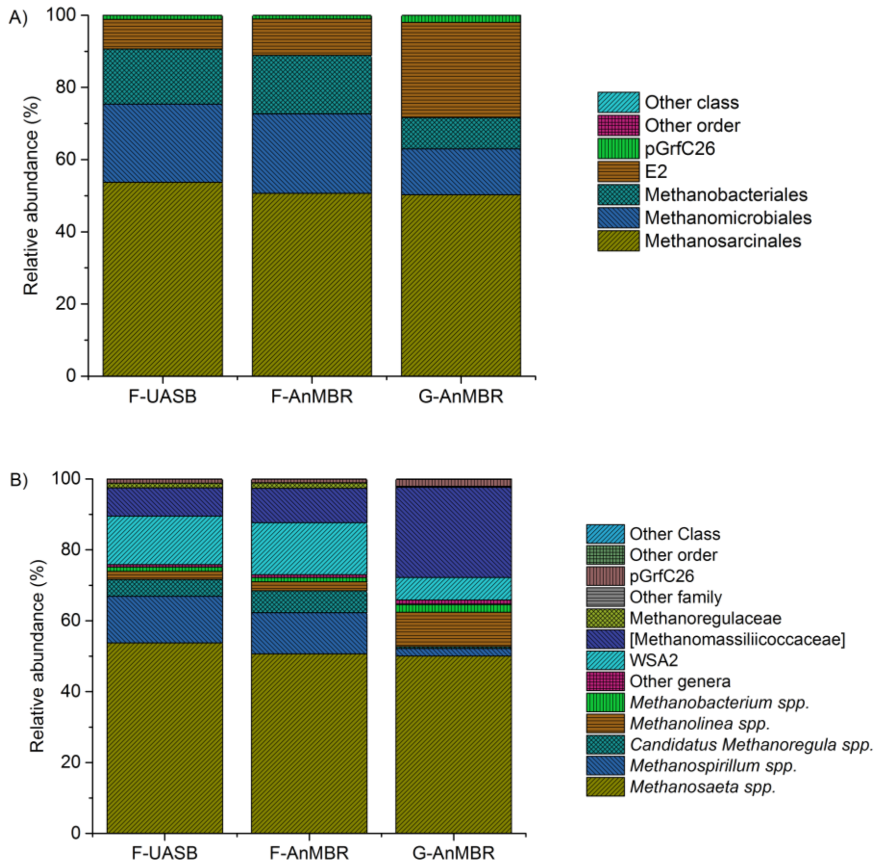


Figure 3.10. Taxonomic assignment of sequencing reads from archaeal community at 85% confidence level (order) (A) and at 95% confidence level (genera) (B). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others".

3.4. Conclusions

The quick start-up of the reactors can be attributed to the previous acclimation of the biomass to the temperate treatment conditions, even with a 4-months inoperative period. The results obtained for the first 45 days of operation of the three different reactor configurations showed that solids management is critical for the anaerobic treatment of domestic wastewater using UASB reactors. Solids, colloids and particles need to be retained in the reactor to increase solids hydrolysis efficiency and thus, membrane systems are necessary.

Flocculent biomass promoted slightly higher hydrolysis than granular biomass since flocculent sludge acts as a fine filter while granular acts as a coarse filter. From the results obtained, F-AnMBR shows a better performance for the treatment of domestic wastewater at 10°C.

PCA analysis demonstrated that the microbial communities from the three reactors analysed were highly similar. However, higher similarities were found in flocculent reactors, regardless of the membrane presence in the system. Thus, reactor inoculum had higher influence in microbial community than reactor configuration.

Proteobacteria, *Bacteroidetes* and *Firmicutes* phyla accounted for an abundance of around 70% in the three reactors although its distribution was slightly different. *Bacteroidales* and *Clostridiales* were the major bacterial fermenters orders detected and a relative high abundance of syntrophic bacteria, represented by *Syntrophobacterales,* was also detected. Additionally, an elevated abundance of SRB (i.e. *Desulfovibrionales* and *Desulfobacterales*) were identified and was attributed to the low COD/SO_4^{2-} ratio of the wastewater. A coexistence of acetoclastic and hydrogenotrophic methanogenesis in the reactors is suggested given the high abundance of *Methanosaeta* spp. as well as *Methanomicrobiales* and *Methanobacteriales*.

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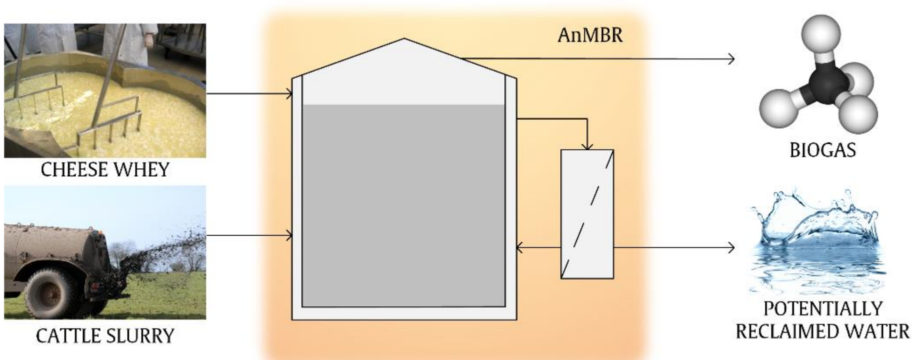
CHAPTER 4

Anaerobic Membrane Bioreactor (AnMBR) for the treatment of cheese whey for the potential recovery of water and energy



Abstract

A single-stage anaerobic membrane bioreactor (AnMBR) for the treatment of cheese whey and its co-digestion with cattle slurry was investigated with the aim of potentially recovering water and energy from the wastewater. A 9 L reactor coupled to an ultrafiltration flat sheet membrane module in an external configuration was employed. This configuration enabled the proper separation of solids from permeate. Cheese whey was stored at room temperature and its chemical oxygen demand (COD) varied between 51 and 80 g/L. The reactor was operated at an average hydraulic retention time (HRT) of 15 days and at an organic loading rate (OLR) of 1.2-8.4 kg COD/(m^3·day). During operation a COD removal average of 91% ± 7% was achieved. The biogas production ranged from 0.2 to 0.9 m^3 biogas/kg COD removed and its methane content was 51-73%. From these results, a potential energy recovery of 2.4 kWh/kg COD removed was calculated. Microbial community analysis showed that bacteria belonging to the orders *Bacteroidales* and *Clostridiales* became the most prevalent. The bioreactor was dominated by acetotrophic methanogenesis. The co-digestion of cheese whey with cow manure (3:1) did not decrease NaOH consumption for pH control. Water reuse for cleaning purposes is possible if permeate pH is maintained at 6. Prior to the scaling-up of the system, a pilot scale test would be necessary to optimise membrane performance. The use of AnMBR technology at a real scale would be appropriate since it is a compact technology which permits both energy and potential water recovery after permeate post-treatment, thus constituting a further step towards the establishment of a broader a circular economy approach.

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4.1. Introduction

The long-term situation whereby water demand exceeds available water resources has resulted in the issue of water scarcity being recognised as a major challenge in Europe and beyond. Consequently, water reuse strategies, identified as part of the global solution to this widespread problem (European Commission, 2012, 2007) have included the subject of water reclamation from industrial wastewater, an approach which is very much in line with the European Commission policy (European Commission, 2007).

The dairy industry is one of the principal sources of industrial effluent generation in Europe. The manufacturing of raw milk into elaborated products generates wastewater with a high organic content. Among such effluents is cheese whey, the liquid fraction obtained during cheese manufacturing, with a water content greater than 90% (Rivas et al., 2010).

Small-scale cheese manufacturers face wastewater disposal problems as they have low cheese whey production, and the existing technologies imply high investment costs (Prazeres et al., 2012). The valorisation of cheese whey for human food production is a common strategy but it requires large-scale production processes and fast, refrigerated transport. In the Catalonian region, most small-scale cheese manufacturers dispose of their residues in slurry ponds or it is transported to farms to be employed as pig feeding. These actions imply a lost opportunity for the possible valorisation of the residue whilst the expense of its transportation to farms constitutes an additional financial burden. A compact and decentralised technology, which would not require the refrigerated storage of the residue and which would also enable both energy and water recovery, would greatly benefit the aforementioned small-scale cheese manufacturers and continue to the creation of a more effective circular economy approach.

Cheese whey presents a biological oxygen demand (BOD) in the range of 27–60 g/L and a chemical oxygen demand (COD) in the range of 50–102 g/L (Carvalho et al., 2013). The BOD/COD ratio is usually above 0.5, which makes biological treatment suitable for this effluent. Nevertheless, it should be noted that its acidic properties make its treatment challenging from the biological perspective.

With regards to enhanced energy valorisation, the high COD content makes cheese whey suitable for energy recovery using anaerobic digestion as previously reported (Carvalho et al., 2013; Ergüder et al., 2001; Kalyuzhnyi et al., 1997; Prazeres et al., 2012). Anaerobic digestion of food waste is a complex process and, in some cases, it can become inefficient due to the accumulation of many inhibitors such as ammonia and volatile fatty acids (VFAs) (Wang et al., 2018). It is a multi-stage process, in which organic matter is degraded sequentially in several biological steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is carried out by hydrolytic bacteria. Acidifying bacteria then convert hydrolysis products into VFAs, alcohols, aldehydes, CO_2 and H_2. The next step is acetogenesis, in which all these products are transformed into acetate. Finally, methane and CO_2 are generated by acetotrophic methanogens. However, methane can also be produced directly from H_2 and CO_2 that are produced in the different steps of the anaerobic digestion process by the microorganisms called hydrogenotrophic methanogens. In addition, since hydrogen released by acetogenic bacteria is toxic, a syntrophy with hydrogenotrophic methanogens is necessary.

Each stage of the anaerobic process is executed by different microbial communities. The connections between microbial community structures and operational conditions are under study (Ali Shah et al., 2014; Park et al., 2017; Svojitka et al., 2017; Wang et al., 2018; Zhu et al., 2017). Microbial communities in anaerobic digesters have constituted, until recently, something of a mystery to investigators (Morris et al., 2014). The recent application of molecular technologies, such as next-generation sequencing, has increased the knowledge and understanding of the complex anaerobic digestion process (Fischer et al., 2016). While bacterial structures and functions are known, with elevated functional redundancy despite variable taxonomic composition, numerous methanogen groups remain unidentified or poorly understood, and changes between digesters have not been examined in detail (Wilkins et al., 2015). Given that methanogenesis is usually the rate-limiting step, diversity and abundance of methanogens is key to the successful operation of anaerobic digestion bioreactors. As is the case with the bacterial community, methanogen phylogeny can be determined by sequencing the 16S rRNA gene using archaea-specific primers. It can also be determined by sequencing the α subunit of the methyl coenzyme M reductase (*mcrA*) gene (Wilkins et al., 2015). This gene encodes the enzyme catalysing the terminal step in methanogenesis and is ubiquitous among known methanogens (Friedrich, 2005).

With regards to the improvement of anaerobic digestion performance, the co-digestion of different wastes has been reported as being a viable option (Rico et al., 2015). In the case of cheese whey, the low alkalinity content and its rapid acidification can reduce the reactor′s buffering capacity. If pH in the reactor is not controlled, the decrease of the buffering capacity can lead to pH decrease and the accumulation of VFA resulting in a failure of the process. Co-digestion of cheese whey with an alkaline residue, such as cattle slurry, could solve this problem.

Therefore, cheese whey (acidic) co-digestion with cattle slurry (alkaline) proves beneficial in that it involves treating two wastes that could be easily collected within the same facilities. Cattle manure is a valuable fertilizer providing nutrients for crops whilst improving soil structure. However, the emergence and growth of intensive livestock farming has led to an imbalance between livestock manure volumes generated and the surface of land where it can be applied as a fertilizer. In some areas, this has led to the overuse of cattle manure as a fertilizer, creating environmental problems such as the contamination of aquifers (especially with nitrates) or the eutrophication of some water bodies. Thus, the use of cattle slurry in the treatment of cheese whey proportions two benefits: the reduction of its overuse in fertilizing and its contribution to alkalinity.

Many properties of dairy manure and cheese whey are complementary. It is important to notice that the organic content of dairy manure is present mostly in the form of suspended solids and fibrous material, whilst only a minor part is soluble. Furthermore, dairy manure possesses sufficient alkalinity to undergo an anaerobic digestion process with an anaerobic biodegradability of approximately 45% (Rico et al., 2007). Therefore, in anaerobic digestion, hydrolysis is the limiting step for dairy manure, whilst for cheese whey, that step is methanogenesis. In a previous study, Rico et al. (Rico et al., 2015) showed that the co-digestion of cheese whey with cattle slurry, with a cheese whey fraction as much as 85% in the feed, was feasible.

Anaerobic bioreactors coupled to a membrane called anaerobic membrane bioreactors (AnMBRs) (Skouteris et al., 2012) have not been studied as much as anaerobic digestion itself. AnMBRs treat wastewater and enable its further reuse. AnMBRs permit the decoupling of sludge retention time (SRT) and hydraulic retention time (HRT), favouring the operation at high organic loading rates (OLR) in smaller reactors. Moreover, they produce a high quality effluent as well as resulting in the structuring of a compact technology. Membrane fouling is the mayor drawback of this technology (Lin et al., 2013). AnMBRs have been employed for the treatment of domestic wastewater (Gao et al., 2014; Lew et al., 2009; Shin and Bae, 2017) and industrial wastewater, such as that resulting from coking (Zhu et al., 2017), food processing (Galib et al., 2016) and winery (Basset et al., 2016) to name but a few. In the case of treating cheese whey, AnMBR technology has been approached from a phase separation process in which the acidic and methanogenic phases occur in separate reactors to improve process stability (Saddoud et al., 2007). This study showed good performances but it included the regulation of raw cheese whey pH at the beginning of the tests.

The potential use of co-digestion in AnMBR was studied by Moñino et al. (Moñino et al., 2016), specifically the co-digestion of the organic fraction of municipal solid waste with wastewater. However, research has not been reported on co-digestion in AnMBR for cheese whey together with cattle slurry.

This present study work aims to evaluate the feasibility of a one-stage AnMBR for the treatment of cheese whey stored at room temperature. This objective requires a simpler technology than the two-stage system already tested (Saddoud et al., 2007) as well as taking into account a possible higher chemical consumption for pH control. To assess the chemical consumption for pH control and the performance of the anaerobic digestion process, two different feeding systems have been examined: cheese whey digestion and cheese whey co-digestion with sieved cattle slurry. The final aim is to potentially recover energy and water, which could be reused in cheese whey manufacturing facilities, an extremely important aspect during droughts in many Mediterranean regions. Furthermore, microbial community characteristics at the beginning and the end of operation are assessed.

4.2. Materials and methods

4.2.1. Inoculum and substrates

The sludge used as inoculum was obtained from the anaerobic digestion sludge stabilisation stage from the urban wastewater treatment plant of Manresa (Barcelona, Spain).

The whey used in this study was obtained from the small cheese manufacturer "El Canadell" (Barcelona, Spain). Its collection was undertaken immediately after cheese manufacturing and it was stored at room temperature for up to six months.

Given that cheese whey was kept at room temperature, pH and organic content decreased in time. Table 4.1 shows the chemical composition of cheese whey including the variability of the determined parameters.

Cattle slurry was obtained from a local milk-producing farm located in Sagàs (Barcelona, Spain) and was collected from a storage tank in anaerobic conditions. Raw cattle slurry was kept at room temperature and sieved to 2 mm before use. The chemical composition of the sieved cattle slurry is shown in Table 4.1.

Table 4.1. Chemical characteristics of cheese whey and cattle slurry (mean ± standard deviation).

		Cheese whey	Cattle slurry (sieved 2 mm)
pH		3.7 ± 0.4	7.6 ± 0.7
EC	mS/cm	9.8 ± 1.8	13.5 ± 0.8
TSS	mg/L	10,700	17,600
VSS	mg/L	10,700	14,100
Alkalinity	mg $CaCO_3$/L	< 20	15,500 ± 4610
VFA	mg/L	2800	1700

		Cheese whey	Cattle slurry (sieved 2 mm)
COD	mg/L	65,200 ± 14,800	22,600 ± 7,600
TKN	mg/L	793 ± 659	1310 ± 337
NH_4^+	mg/L	35.9 ± 5	760 ± 117
NO_3^-	mg/L	3.1 ± 1.8	2 ± 0.6
NO_2^-	mg/L	88.4 ± 30.9	4.6 ± 3.8
SO_4^{2-}	mg/L	113 ± 12.7	5 ± 4.2
Cl^-	mg/L	2420 ± 453	1010 ± 132
TP	mg/L	257 ± 26.7	182 ± 95.2
PO_4^{3-}	mg/L	456 ± 208	80 ± 59
Ca^{2+}	mg/L	716 ± 168	194 ± 96.6
Mg^{2+}	mg/L	75.7 ± 12.8	144 ± 30.5
Na^+	mg/L	417 ± 87.4	483 ± 47.1
K^+	mg/L	1990 ± 177	1860 ± 202

4.2.2. Experimental setup

The configuration of the AnMBR system is presented in Figure 4.1. The bench-scale AnMBR consisted of a 9 L perfectly sealed glass reactor with a water jacket coupled to a thermostatic bath. Automated continuous feeding was maintained during the experimentation period using a programmed level device. Biogas produced was monitored by coupling a biogas pipe to an AER-208 respirometer (Challenge Technology, USA). This system counts bubbles of biogas generated passing through a calibrated cell; it computes both tabular and graphical for data interpretation.

A mesophilic range of 35°C ± 2°C was maintained in the reactor and the pH was automatically set at 7.0 ± 0.3 by dosing NaOH to avoid the acidification of the reactor. The reactor was operated at an average HRT of 15 days. The AnMBR feed consisted only of cheese whey during the first 79 days. From day 79 on, feed was changed to a mixture of cheese whey and cattle slurry to a ratio of 3:1, a conservative relation based on previous studies (Rico et al., 2015).

The bioreactor was coupled to an external polymeric ultrafiltration membrane module which was used to separate solids from permeate. Continuous recirculation between the bioreactor and the filtration module enabled the mixing of the reactor. The external module consisted of a 40 cm^2 PVDF flat sheet membrane (Martin Membrane Systems, Germany) with a nominal pore size of 35 nm. Crossflow velocity ranged from 0.6 to 0.8 m/s and flux was maintained at between 5-10 LMH, to prevent membrane fouling. Flux was controlled by permeate pumping rate, employing a Minipuls 3 pump (Gilson, USA), which created an under pressure that allowed permeate to pass through the membrane. The effluent was collected in a permeate tank.

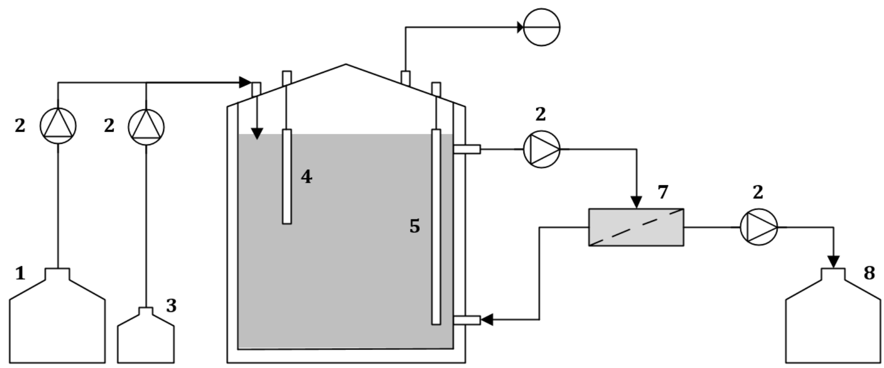

Figure 4.1. AnMBR setup scheme. 1- Feed tank; 2- Peristaltic pump; 3- NaOH tank; 4- pH probe; 5- Level sensor; 6- Biogas-meter; 7- Membrane module; 8-Permeate tank.

4.2.3. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), pH, electrical conductivity, alkalinity, chemical oxygen demand (COD), total nitrogen

(TN), total Kjeldhal nitrogen (TKN), ammonia (NH_4^+), total inorganic carbon (TIC), total phosphorous (TP), major anions, major cations, turbidity and metals were determined according to Standard Methods for the Examination of Water and Wastewater (APHA et al., 2012). Volatile fatty acids were determined by removal of CO_2(g) at pH 3 and back titration to pH 5.5 (Lahav and Morgan, 2004).

E. coli was determined as established in UNE-EN ISO 9308-1:2001. Intestinal nematode eggs were determined by microscopic observation.

Biogas quality was determined by CO_2 trapped within a KOH solution (Fuchs et al., 2003). Biogas samples were injected into a glass 10 mL meter filled with KOH solution. Remaining gas volume was registered as an estimation of biogas methane content.

4.2.4. Data analysis

Potential energy produced was calculated with the following equation, where Y-methane yield; ρ_{CH_4}-methane density at 20°C and 1 atm (0.668 kg/m³) and LCV_{CH_4}-lower calorific value for methane (13.89 kWh/kg).

$$\text{Potential energy in biogas} = Y \times \rho_{CH_4} \times LCV_{CH_4} \qquad (4.1)$$

The Langelier saturation indexes of the permeate were calculated using PhreeqC v3.4 software (Parkhurst and Appelo, 2013). A positive value of the saturation index suggests the water is oversaturated and that precipitation probably has occurred or will occur.

4.2.5. Microbial community analysis

4.2.5.1. Sludge sampling and DNA extraction

Biomass samples were taken at the beginning and at the end of the experiment (day 1 and day 278). Samples were frozen at −80 °C for further analysis. For DNA extraction, samples were centrifuged at 5000 x g for 10 min. DNA extraction from the obtained pellet was performed using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratory Inc., USA).

4.2.5.2. Library preparation

Library preparation was performed at the Centre for Omic Science, COS (Reus, Spain). Partial bacterial 16S rRNA gene sequences were amplified from extracted DNA using primer pair 341F-532R (5'-CCTACGGGRSGCAGCAG-3'; 5'-ATTACCGCGGCTGCT-3'), which targets the V3 region of the 16S rRNA gene sequence and primer pair 515F-806R (5'-GTGCCAGCMGCCGCGGTAA-3'; 5'-GGACTACHVGGGTWTCTAAT-3') which targets the V4 region. Partial archaeal 16s rRNA gene sequence was amplified using primer pair S-D-Arch-0787-a-S-20 and S-D-Arch-1043-a-A-16 (5'-ATTAGATACCCSBGTAGTCC-3'; 5'-GCCATGCACCWCCTCT-3') (Fischer et al., 2016). The primer pair used for the amplification of *mcrA* archaeal gene was designed by the Centre for Omic Science, COS (Reus, Spain); the reverse primer from Luton et al. pair (Luton et al., 2002) was employed and a forward primer for an amplification of 309 bp fragment was designed (5'-GGAAKMTCACTTCGGTGGTTC-3'; 5'-TTCATTGCRTAGTTWGGRTAGTT-3'). All the aforementioned primers were designed to include at their 5' end one of the two adaptor sequences used in the Ion Torrent sequencing library preparation protocol linking a unique Tag barcode of 10 bases to identify different samples.

PCR cycle parameters are described elsewhere (Ellis et al., 2012; Fischer et al., 2016; Milani et al., 2013; Tridico et al., 2014). To summarise, PCR products were confirmed by a 2% agarose gel and specific bands were excised and subsequently purified using Nucleospin Gel and PCR clean up kit (Macherey-Nagel). The concentration of the PCR amplicons was analysed by electrophoresis on a Bioanalyzer (Agilent). Equimolar pools of each fragment and sample were combined.

4.2.5.3. Ion Torrent PGM sequencing and sequenced-based microbiome analysis

Once libraries were created, they were diluted to 26pM DNA concentration prior to clonal amplification. Multiplexed samples were prepared for sequencing employing the Ion PGM™ Hi-Q™ View OT2 Kit and Ion PGM™ Hi-Q™ View Sequencing Kit according to the manufacturer′s instructions. Prepared samples were loaded on a 318 chip and then sequenced using the Ion PGM system (Life Technologies, USA).

After sequencing, individual sequence reads were filtered by the PGM software to remove low quality and polyclonal sequences. The readings were analysed using QIIME (Caporaso et al., 2011). The analysis included OTU clustering, Alpha-diversity analysis, OTU analysis and species annotation. The OTU assigning method was UCLUST and the taxonomy assigning method was BLAST. Sequence similarity threshold for both OTU and taxonomy assignments was 97%. The taxonomy database employed was GreenGenes for 16s rRNA gene sequences and a compilation of all *mcrA* published sequences for archaea taxonomy description.

4.3. Results and Discussion

4.3.1. Performance of the AnMBR system

Storage of cheese whey at room temperature did not hamper the performance of the anaerobic digestion. COD removal was one of the goals of the process since it signified the conversion of organic matter into energy as well as a higher quality water recovered. A highly efficient COD removal was to be observed during the length of the experiment, with an average value of 91% ± 7%, similar to Saddoud et al. (Saddoud et al., 2007) who obtained a 98.5% removal in their two-stage AnMBR for cheese whey treatment. During the experiment, OLR ranged from 1.2 to 8.4 kg

COD/(m³·day) (Figure 4.2), below the OLR range (3 to 19.78 kg COD/(m³·day)) used by Saddoud et al. (Saddoud et al., 2007).

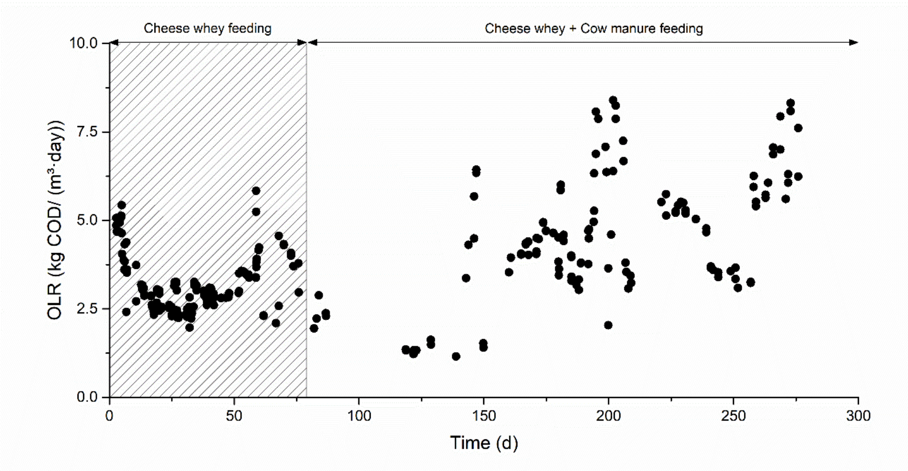

Figure 4.2. Organic Loading Rate (OLR) along operation. Grey shadow represents cheese whey feeding; white background represents co-digestion.

A lower OLR (an average of 3.1 kg COD/(m³·day)) was maintained when working with cheese whey digestion permitting biomass to adapt to feed. With the addition of cattle slurry, an increase in OLR (average of 5.0 kg COD/(m³·day)) was applied to increase the treatment capacity of the reactor with no significant decrease in COD removal efficiencies.

Biogas production ranged from very low values (<100 mL/h) to 1600 mL/h (Figure 4.3). There was a correlation between the increase in the OLR applied and the biogas produced. The biogas production related to COD removal ranged from 0.2 to 0.9 m³ biogas/kg COD removed. The produced biogas quality was in the range of 51-73% CH_4, representing a methane yield of 0.26 ± 0.12 m³ CH_4/kg COD removed. Similarly, Saddoud et al. (Saddoud et al., 2007) obtained a methane yield up to 0.3 m³ CH_4/kg COD removed. The potential average energy produced in this experiment was 2.4 kWh/kg COD removed, representing an average electrical and caloric potential of 156 kWh/m³ of cheese whey, slightly superior to that obtained by Escalante et al. (Escalante et al., 2018) when treating cheese whey employing cattle slurry as inoculum. At an average OLR of 4.8 kg COD/(m^3·day) the total energy produced was 4.35 x 10^5 J/day, which is lower than the value obtained by Nie et al. (2017) in their study.

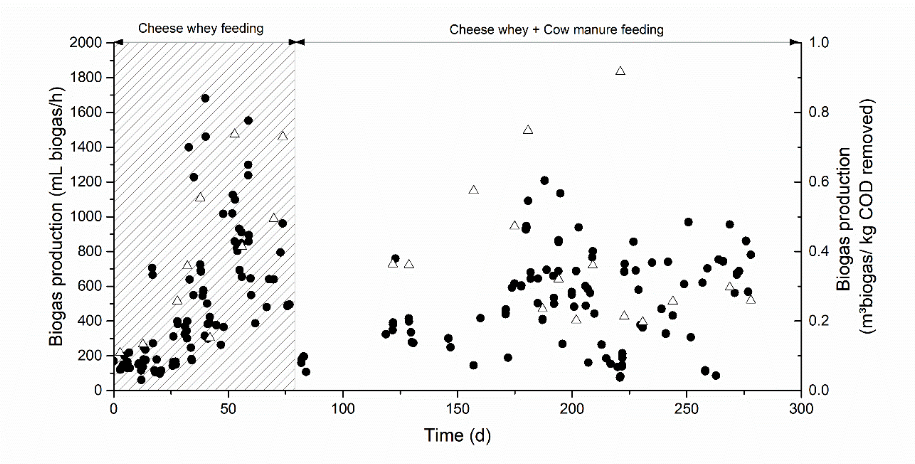

Figure 4.3. Biogas production (●) and biogas production per COD removed (△). Grey shadow represents cheese whey feeding; white background represents co-digestion.

Regarding NaOH consumption for pH control, a decrease in this consumption was observed throughout the operation; starting with 6.2 meq NaOH/g COD and reaching 0.8 meq NaOH/g COD before the addition of cattle slurry (Figure 4.4). This reduction in NaOH consumption was assumed to be due to an adaption of anaerobic biomass to cheese whey digestion. Bacteria performing the first steps of the anaerobic digestion process can rapidly adapt to changes in feed composition, since they have a high functional redundancy (Wilkins et al., 2015). This rapid acidogenesis can cause an accumulation of VFA, while slow-growing methanogenic communities adapt to the new substrate. Cattle slurry alkalinity (15.5 g/L) was presumed to contribute to the buffering capacity of the system; however, there was no noticeable decrease in NaOH consumption when co-digesting cheese whey and cattle slurry (3:1). The additional alkalinity of cattle slurry was not sufficient to affect the bioreactor's NaOH consumption. In the work of Rico et al. (Rico et al., 2015), the reactor feed pH ranged from 6.4 to 7.6 when co-digesting cheese whey with cattle slurry, provided that cheese whey was kept refrigerated at 4°C prior to digestion. The room temperature storage of cheese whey probably affected the buffering capacity of the co-digestion process, since the acidity of the cheese whey was most likely higher. A lower ratio of cheese whey:cattle slurry should be further tested to assess its buffering capacity and, thus, its chemical consumption for pH adjustment. However, given that cattle slurry presents a higher electrical conductivity than cheese whey, the permeate quality obtained would probably be lower if one considers its potential reuses.

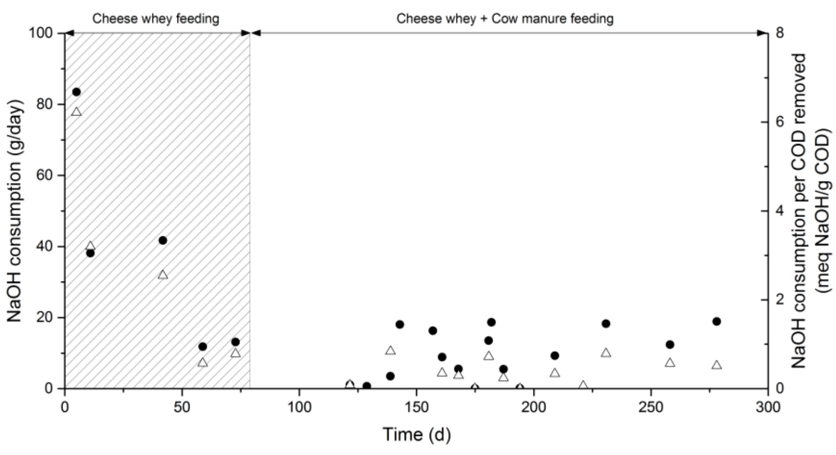

Figure 4.4. NaOH consumption (g/day) (●) and NaOH consumption per COD removed (meq NaOH/g COD) (△). Grey shadow represents cheese whey feeding; white background represents co-digestion.

The composition of the obtained permeate (Table 4.2) presented a decrease in total nitrogen and phosphorous content compared to feed (Table 4.1). Since anaerobic digestion does not remove N or P by biological transformations, their decrease might be due to salt precipitation. Moreover, calcium concentration also decreased, which could also be caused by salt precipitation. On the other hand, an increase in electrical conductivity and sodium concentration was also observed. This could be explained by pH adjustment with the addition of NaOH.

Although no major decrease in NaOH consumption was observed, co-digestion implied an enhancement of the permeate quality possibly due to an improved anaerobic digestion process. When comparing the permeate quality according to the feed characteristics (Table 4.2), there were no significant differences regarding permeate pH, electrical conductivity and alkalinity when feeding only cheese whey or co-digesting it with cattle slurry. However, the co-digestion period demonstrated a significant decrease of VFA and COD in the permeate concentration, which implied an enhanced and more efficient anaerobic digestion process.

Table 4.2. Permeate composition according to feed characteristics (mean ± standard deviation).

		Permeate composition according to feed characteristics		Spanish RD 1620/2007 limits for water reuse	
		Cheese whey	Cheese whey:cattle slurry (3:1)	For industrial cleaning purposes[1]	For irrigation purposes[2]
pH	upH	7.5 ± 0.4	7.5 ± 0.3	-	-
EC	mS/cm	14.8 ± 0.9	15.7 ± 3.5	-	3
TSS	mg/L	79.3 ± 59.4	131 ± 93.3	35	20
VSS	mg/L	69.1 ± 46.2	80.4 ± 58.9	-	-

[1] Legislation limits for water reuse in industry for cleaning purposes or process water, except for food industry.

[2] Irrigation of crops with a water irrigation system that allows direct contact of regenerated water with fresh edible parts for human consumption.

		Permeate composition according to feed characteristics		Spanish RD 1620/2007 limits for water reuse	
		Cheese whey	Cheese whey:cattle slurry (3:1)	For industrial cleaning purposes[1]	For irrigation purposes[2]
NH_4^+	mg/L	292 ± 387	72 ± 122	-	-
Alkalinity	mg $CaCO_3$/L	5220 ± 1020	4790 ± 994	-	-
VFA	mg/L	4110 ± 2390	1400 ± 376	-	-
COD	mg/L	3760 ± 1030	1810 ± 1160	-	-
TKN	mg/L	-	132 ± 86.6	-	-
NO_3^-	mg/L	< 5	2.8 ± 2.9	-	-
NO_2^-	mg/L	< 5	< 2	-	-
SO_4^{2-}	mg/L	12.3	3.6 ± 3.9	-	-
Cl^-	mg/L	2180	2200 ± 208	-	-
P total	mg/L	53.2	16.8 ± 2.5	-	-
PO_4^{3-}	mg/L	44.8	18.3 ± 7.8	-	-
Ca^{2+}	mg/L	429	307 ± 87	-	-
Mg^{2+}	mg/L	75	83.8 ± 23.1	-	-
Na^+	mg/L	2210	1830 ± 666	-	-
K^+	mg/L	1830	2040 ± 72.5	-	-
Turbidity	NTU	144 ± 113	63.7 ± 42.1	15	10
E. coli	cfu/100 mL	8.9E+04 ± 9.9E+04[a]	< 4	$1 \cdot 10^4$	100
Intestinal nematode eggs	eggs/L	< 1	< 1	1	1

[a] Damaged membrane.

Parameters regulated by Spanish legislation regarding water reuse (RD 1620/2007) (RD 1620/2007, 2007) were evaluated to determine the feasibility of the further direct use of the obtained permeate as water in farming or the dairy industry. Two of the identified possible uses for this permeate were irrigation and industrial cleaning. The obtained permeate demonstrated a high electrical conductivity making it unsuitable for irrigation purposes (>3 mS/cm) but, given that AnMBR technology does not remove dissolved salts and the high conductivity of cheese whey, an additional step in the treatment would be necessary for this purpose. Further salt removal could be performed by high-pressure filtration processes such as reverse osmosis or nanofiltration or by electrical-driven processes such as electrodialysis.

Another parameter regulated in the legislation is the concentration of intestinal nematode eggs. In the produced permeate the concentration was below the legal limit. High *E. coli* values (8.9E+04 ± 9.9E+04 cfu/100 mL) were found when feeding exclusively cheese whey due to membrane damage. After the membrane had been replaced, no *E. coli* was observed in the permeate. *E. coli* concentration in the different stages of the process was evaluated and it was not detected in permeate while it was observed in both the cattle slurry and the reactor mixed liquor (Table 4.3). Within this proves, *E. coli* reduction was successful and it complied with the limits established by the RD 1620/2007 legislation (RD 1620/2007, 2007).

The permeate obtained did not comply with limits of turbidity and total suspended solids (TSS) of RD 1620/2007 (RD 1620/2007, 2007). In fact, turbidity was below the legally-defined limit immediately after sampling. However, in the following 24 hours, turbidity increased to above 300 NTU. Furthermore, permeate pH also changed after sampling, increasing from 7 to 9. The hypothesis was that the increase in pH could be due to CO_2 degasification, which in turn could cause an increase in turbidity due to salt precipitation when increasing pH.

Table 4.3. *E. coli* concentration at different points of the treatment process.

Sample	*E. coli* (cfu/100 mL)
Cheese whey	< 4
Cattle slurry	8.0E+02 - 3.7E+03
Anaerobic sludge	1.2E+05 - 5.3E+06
Permeate	< 4

Using the composition of the obtained permeate, salt saturation indexes were determined by employing PhreeqC v3.4 software in order to simulate the saturated phases in the permeate before and after CO_2 degasification. Simulations were run by balancing the CO_2 inside and outside the reactor and using the phreeqc database. Permeate phases were balanced, first with the inner atmosphere of the AnMBR to simulate saturation indexes before degasification, and subsequently with the atmosphere to simulate saturation indexes after degasification. The results are shown in Table 4.4. It was observed that during some of the saturated phases in the reactor, the sudden increase in their saturation index, once permeate came into contact with the atmosphere, increased their supersaturation thus raising their precipitation rate and consequently increasing permeate turbidity. The saturated phases shown in Table 4.4, which probably precipitate after exiting the reactor, explain the decrease in calcium and phosphate concentrations in permeate compared to feed.

Turbidity (Table 4.5) could have been kept under acceptable values if the pH was lowered enough to avoid precipitation of salts (pH between 6 and 7). However, this resulted in a large amount of reagents consumption and significantly increased the final electrical conductivity of the effluent.

Table 4.4. Saturation indexes of saturated phases found in permeate.

	Phases balanced in the reactor	Phases balanced with the atmosphere
Temperature (°C)	35	35
Partial CO_2 pressure (atm)	0.4	0.04
pH	6.4	9.1
Saturated phases saturation indexes (SI)		
Aragonite ($CaCO_3$)	0	2.31
Calcite ($CaCO_3$)	0.1	2.45
Dolomite ($CaMg(CO_3)_2$)	0.1	4.79
Hydroxyapatite ($Ca_5(PO_4)_3OH$)	4	14.6

Table 4.5. Turbidity of the permeate after controlling pH and related acid (HCl) consumption.

Target pH	Conductivity (mS/cm)	Turbidity (NTU)	g HCl/m³
pH 5.5	16.7	3.8	3330
pH 6	17.3	4.1	3100
pH 7	13.7	231	1580

Instead of decreasing pH to decrease turbidity and TSS in the permeate, a possible valorisation route for salts should be considered given that saturated salts shown in Table 4.4 are rich in Ca, P and Mg and could potentially be used as a soil fertiliser or amendment. The removal of salts employing reverse osmosis would not be feasible as divalent salts are already saturated inside the reactor (Table 4.4) and would led to scaling on the membranes. A selective removal of monovalent ions (i.e. using electro-dialysis reversal with monovalent membranes (Güler et al., 2014)) could be an option but a very strict pH control should be established to avoid the precipitation of divalent salts. This would permit the recovery of a brine stream, which could be potentially used as a fertilizing agent and a product stream with less salt concentration. The potential result would be reclaimed water fulfilled the turbidity limits established by RD 1620/2007. Moreover, the brine stream could be used as a fertiliser or amendment given that all dissolved salts would be maintained in the said stream.

The procedure examined is potentially capable of producing a permeate of such quality that it could have further applications (e.g. industrial cleaning or irrigation) if salts were removed by electrodialysis processes.

4.3.2. Microbial community structure

Microbial community analysis was determined for the inoculum sample and for the sample at the end of experiment (day 278). All the rarefaction curves showed gentle slopes under current sequencing depth. This fact indicates that the sequencing libraries could correctly reflect the microbial communities. Alpha-diversity analysis showed that both richness and diversity of Bacteria and Archaea decreased with the acclimation, as expected (Table 4.6).

Table 4.6. Characteristics of sequencing libraries.

Target gene	Sample	Number of sequences	Number of OTUs	Chao 1 value	Shannon Index
Bacterial 16S rRNA	Inoculum	1367636	5595	5780	-[a]
	Day 278	951809	2595	2870	-[a]
Archaeal 16S rRNA	Inoculum	1187621	5140	5270	8.46
	Day 278	744093	2173	2500	5.58
mcrA	Inoculum	179196	646	672	4.17
	Day 278	206317	690	695	4.54

[a] Shannon index could not be calculated because the analytical results consist in the combination of two regions.

The changing of operational parameters normally results in changes within the microbial community structure of the reactor (Amha et al., 2017). Therefore, a change in microbial community structure from the inoculum was expected because of the acclimation of the microbial population to changes in feed composition. Figure 4.5, Figure 4.6 and Figure 4.7 show differences in bacterial and archaeal community structures in the two samples analysed.

Archaea (Figure 4.7) are key organisms in anaerobic digestion, as they are responsible for the methanogenesis in which CH_4 is produced. There are three main types of methanogens according to the substrates used: acetoclastic (acetate), hydrogenotrophic (H_2 and CO_2), and methylotrophic (methylated compounds) although most of the CH_4 is produced by the first two types. Only microorganisms of two genera are recognised as acetoclastic methanogens, *Methanosaeta spp.* and *Methanosarcina spp.* However, *Methanosarcina spp.* can use both acetate and H_2. The most common genera within hydrogenotrophic methanogens are *Methanobacterium*, *Methanothermobacter*, *Methanobrevibacter*, *Methanospirillum* and *Methanoculleus*. (Wang et al., 2018).

The analysis of sludge inoculum and digester sludge at the end of the experiment showed an increase in the *Methanosaeta*, *Metanosarcina* and *Methanobacterium* species while there was a decrease in *Methanospirillum spp* (Figure 4.7). *Methanosaeta spp.* was the most abundant identified genus in both samples, showing an increase from 37.5% to 62.4%. *Metanosarcina* and *Methanobacterium spp.* increased from 0.1% to 11.3% and from 0.2% to 3.9% respectively. *Methanospirillum spp.* decreased from 10.0% to <0.01%. In addition, the uncultured group WSA2 classified as a family of the *Methanobacteriales* order decreased in abundance from 30.5% to 0.03%. A decrease in unclassified *Methanomicrobiales* from 15.4% to 0.8%

and an increase in unclassified YC-E6 (both orders belonging to *Methanomicrobia* class), from 0.02 to 21.0% was observed.

Methanosaeta spp. have a lower maximum growth rate on acetate but a higher affinity for acetate than the *Methanosarcina* species (Conklin et al., 2006). It is apparent that higher acetate concentrations would favour the growth of *Methanosarcina*, while lower concentrations would favour the growth of the *Methanosaeta* (Conklin et al., 2006). Wang et al. (Wang et al., 2018) stated that *Methanosaeta* spp. increased with a stable mesophilic operation. In a stable reactor, low concentrations of acetate are expected. Therefore, the finding of an elevated quantity of *Methanosaeta* spp. compared to other archaea observed, is indicative of a stable acetoclastic process. Accordingly, an increase in both species would suggest the predominant existence of acetotrophic methanogenesis. However, hydrogenotrophic methanogenesis might also occur due to the increase in *Methanobacterium spp.*

The role of bacteria in the anaerobic digestion process occurs in the initial steps, performing hydrolysis, acidogenesis and acetogenesis. As bacteria play a wider and more diverse role, a higher variability than in archaea was detected in the analysis.

Bacteroidales and *Clostridiales* were the two major groups identified in both samples (Figure 4.5). The sum of the two orders represented a proportion of 38.3% in the inoculum and 73.2% in the final sample. While *Bacteroidales* showed a slight increase in relative abundance (from 25.8% to 28.9%), the relative abundance of *Clostridiales* increased more than 3-fold (from 12.5% to 44.2%). *Bacteroidales* are suggested to mediate the methane producing rate and methane content in biogas while *Clostridiales* are involved in hydrolysis and the fermentation of carbohydrates (Ju et al., 2017). In fact, Wu et al. (Wu et al., 2016) identified *Clostridiales*, known for its capacity with regards to organic decomposition and fermentation, as a key population with an important role in the maintaining of the stability of the anaerobic digestion process. The wide range of metabolic capabilities of *Clostridiales* should permit them to fulfil multiple functions, explaining its increase in the reactor (Ju et al., 2017). Therefore, since *Bacteroidales* and *Clostridiales* play different roles, they do not compete between each other, and both become dominant in anaerobic digesters.

According to Chao value (Table 4.6), bacterial diversity decreased in the digester in comparison with the inoculum. For example, *Sedimentibacter spp.*, the major bacteria genus detected in the inoculum, showed a decrease from 7.9% to 0.1% (Figure 4.6). *Sedimentibacter* spp. is an amino acids-utilizing bacterium, the feeding with cheese whey, which is rich in sugars and lipids but poor in proteins may explain the decrease in the major bacteria genera found in the inoculum sample. It is reported that *Sedimentibacter spp.* increases in cases of anaerobic digestion failure (Wang et al., 2018).

Syntrophus spp. was the second major bacteria genera found in the inoculum sample (3.1%) but it also decreased to 0.03% at the end of experiment (Figure 4.6). Syntrophy is a form of symbiosis of two metabolically different groups of microorganisms, which enables the degradation of various substrates. The genera *Syntrophus* and *Syntrophomonas* are responsible for the oxidation of butyrate and other fatty acids. Syntrophic acetogenesis is critical and plays an important role in the rate-limiting stage to maintain a rapid and stable anaerobic digestion operation. This is due to the fact that some of the VFAs, especially propionate, inhibit methanogenesis at high concentrations even at neutral pH (Wang et al., 2018). The decrease of hydrogenotrophic methanogens explains the decrease of *Syntrophus* spp. since the syntrophy of acetogenic bacteria and hydrogenotrophic methanogens diminished to the detriment of the acetotrophic pathway.

On the other hand, *Kosmotoga spp.* experienced an increase from 0.4% to 4.8%. *Kosmotoga olearia* has been characterized as an anaerobic heterotroph capable of fermenting sugars into hydrogen, carbon dioxide and acetic acid (DiPippo et al., 2009). As a hydrogen producer, it should function as syntrophic bacteria in the presence of methanogenic microorganisms. It could also act in syntrophy with *Methanobacterium spp.*, which also experienced an increase in its abundance.

In conclusion, several families decreased their abundance as was the case with *Comamonadaceae*, *Chitinophagaceae*, *Anaerolinaceae*, *Xantomonadaceae*, *Syntrophaceae* while the presence of others such as *Porphyromonadaceae*, *Ruminococcaceae*, *Propionibacteriaceae* and uncultured groups SB-1 and ML635J-40 classified as a family of the order *Bacteroidales* increased.

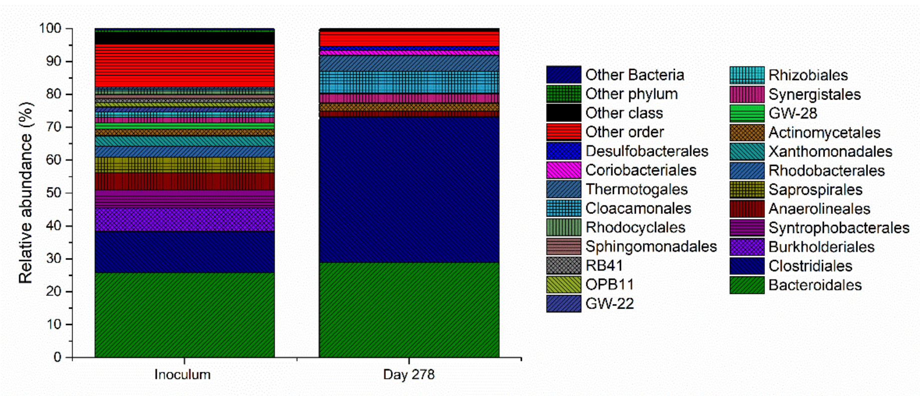

Figure 4.5. Taxonomic assignment of sequencing reads from bacterial community at 85% confidence level (order). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorised as "others".

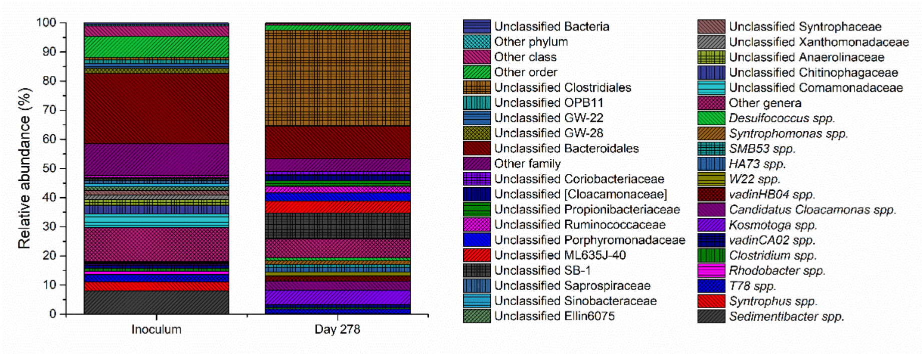

Figure 4.6. Taxonomic assignment of sequencing reads from bacterial community at 95% confidence level (genera). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorised as "others".

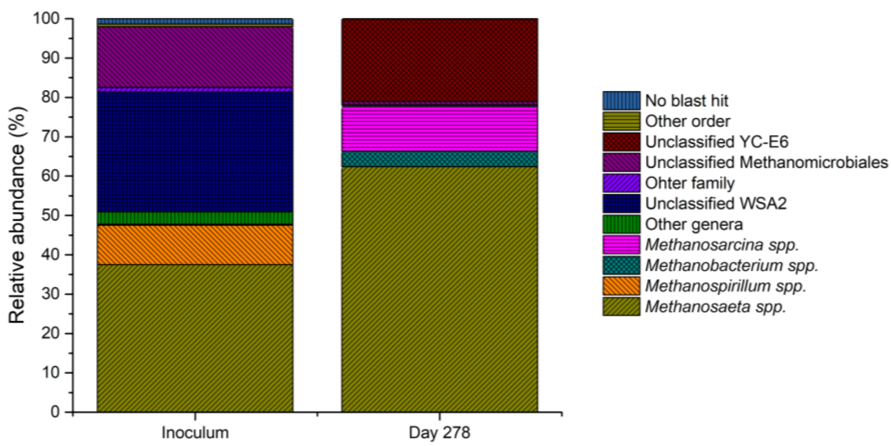

Figure 4.7. Taxonomic assignment of sequencing reads from archaeal community at 95% confidence level (genera). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorised as "others".

Methanogens can also be determined by sequencing the α subunit of the *mcrA* gene (Wilkins et al., 2015). This gene encodes the enzyme catalysing the terminal step in methanogenesis and it is ubiquitous among known methanogens (Friedrich, 2005). Thus, sequencing of the *mcrA* gene provides specific information concerning archaea with a methanogenic capacity. Taxonomic classification of the *mcrA* genes (Figure 4.8) suggests that the bioreactor was predominantly populated by acetoclastic methanogens, particularly *Methanosarcina* spp., with an increase of 25% in the reactor, in accordance with results from 16S archaeal analysis. The high percentages of unclassified sequences in *mcrA* analysis, leading to important differences between results from 16S and *mcrA*, could be explained by the much lower amount of annotated *mcrA* sequences in the available databases compared to 16S rDNA.

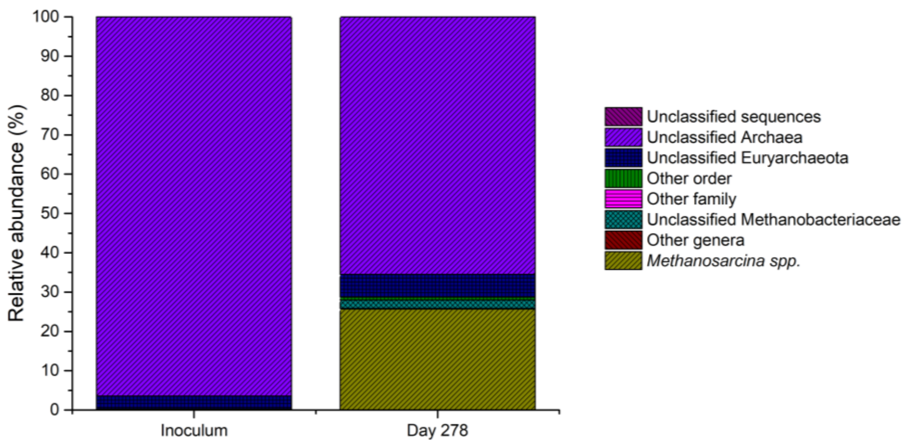

Figure 4.8. Taxonomic assignment of sequencing reads from *mcrA* gene sequences at 95% confidence level. Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorised as "others".

4.4. Conclusions

The use of a one-stage AnMBR with cheese whey stored at room temperature provided positive results which demonstrated that said treatment would not require the cheese whey to be refrigerated in storage or treated immediately after its production. However, the acidification of cheese whey prior to anaerobic digestion could be the cause of a NaOH demand for pH adjustment. High COD removals of up to 99% were observed with a COD removal average of 91% ± 7%. The biogas production ranged from 0.2 to 0.9 m^3 biogas/kg COD removed and the methane content ranged from 51 to 73%. Therefore, one can state that high energy recovery can potentially be obtained within this process with a mean value of 2.4 kWh/kg COD removed. Employing the examined methodology, a maximum OLR of 8.4 kg COD/(m^3·day) was achieved with no decrease in AnMBR performance. The OLR could be further increased to assess the maximum treatment capacity of the technology.

A microbial community analysis revealed the acclimation of the microbial community after the experimentation had been completed. As expected, a decrease in bacterial richness was detected. Bacteria belonging to the orders *Bacteroidales* (29%) and *Clostridiales* (44%) were the most predominant. It was further observed that the bioreactor was populated by acetotrophic methanogenesis, with a prevalence of the archaea species *Methanosaeta spp.* and *Methanosarcina spp.*

AnMBR for co-digestion of cheese whey and cattle slurry could prove to be a suitable technology for water recovery although a further stage whereby electro-dialysis reversal with monovalent membranes were employed for permeate treatment, would be necessary in order to satisfy the demands of existing Spanish water reuse legislation (RD 1620/2007). In addition, the authors suggest that a study of the composition of the brine be undertaken in order to assess its potential use as a fertiliser given that all dissolved salts would remain in said brine.

The application of the described AnMBR technology at a larger scale, constituting a compact and decentralised treatment of cheese whey, is feasible given that it does not require the residue to be refrigerated in storage and permits the recovery of both energy and water after a permeate post-treatment, thus signifying a further step towards a process compatible with the circular economy approach.

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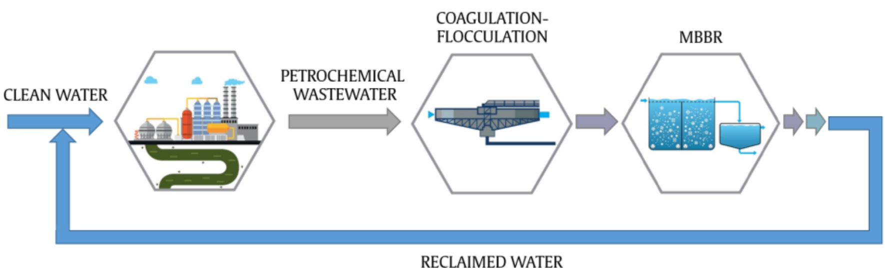
CHAPTER 5

Coagulation-flocculation and Moving Bed Biofilm Reactor (MBBR) as pre-treatment for water recycling in the petrochemical industry



Abstract

Water recycling and reuse is of important value in water using sectors like petrochemical industry. The aim of this research was to optimise the pre-treatment of petrochemical wastewater to undergo a further membrane treatment, with the final objective of water recycling within the same industry. Different mixtures of three wastewater streams from an ethylene cracking facility were tested in the present study.

Laboratory coagulation-flocculation tests prior to biological treatment were performed using Actiflo® Veolia commercial technology and an optimal coagulant dose of 30 mg/L ferric chloride was obtained. A bench-scale Moving Bed Biofilm Reactor (MBBR) system with two sequential reactors with working volumes of 5L was filled with Z-carriers at 35% of their working volume. Organic loading rate (OLR) was varied from 0.2 to 3.25 kg/(m^3 d) and the hydraulic retention time (HRT) ranged from 23.4 h to 4.5 h. High soluble chemical oxygen demand (sCOD) removals were obtained in stationary states (80-90%) and the calculated maximum sCOD that the system could degrade was 4.96 ± 0.01 kg/(m^3 d) at 23 ± 2°C. Changes in feed composition did not decrease sCOD removals showing that MBBR is a robust technology and coagulation-flocculation step could be by-passed. Further removal of total suspended solids (TSS) and turbidity from the MBBR effluent will be required before a reverse osmosis (RO) step could be performed. A biofilm-forming genus, *Haliscomenobacter* spp., and an oil degrading genus *Flavobacterium* spp. were found in all the attached biomass sampled. *Acinetobacter* spp. was the major bacterial genera found in suspended biomass. *Proteobacteria* and *Bacteroidetes* were the major phyla detected in the carrier samples while *Proteobacteria* the main one detected in the suspended biomass. The lack of fungal annotated sequences in databases led to a major proportion of fungal sequences being categorised as unclassified Fungi. However, known petroleum hydrocarbon

degraders such as *Acremonium* spp., *Trichosporon* spp. or *Hypocreaceae* were detected. The obtained results indicate that MBBR is an appropriate technology for hydrocarbon degrading microorganisms acclimation and, thus, for petrochemical wastewater pre-treatment for water regeneration.

5.1. Introduction

Freshwater resources are increasingly under stress conditions and there exists a huge mismatch between available water resources and water demand in many parts of the world. In Europe, water scarcity affects at least 11% of the population and 17% of the territory (EC, 2012, 2007). Regions with low rainfall and high population densities are prone to water stress same as areas with intense agricultural, industrial or tourism activities. Therefore, protection of water resources is one of the main milestones of environmental protection in Europe (EEA, 2017; EC, 2012).

Industry sector is one of the main water users in Europe, accounting for about 40% of total water abstractions. Within this sector, the industry of refined petroleum products is, in most European countries, the manufacturing industry with the highest water demand. Based on data from 2010, the petrochemical industry uses annually up to 2,725 hm^3 in Germany, 797 hm^3 in Belgium, 511 hm^3 in Norway, or 201 hm^3 in Spain (Förster, 2014). Among the main industrial sectors, the manufacturing industry, in which refined petroleum industry is included, has also the highest wastewater production.

Most petrochemical companies in Europe have crackers by which complex organic molecules are broken down into simpler molecules such as ethylene and propylene. The estimated cracker capacity in Europe in 2015 was 23,303,000 Kt ethylene/year (Petrochemicals Europe, 2016). Significant large volumes of freshwater, primarily for processing and cooling, are needed for the cracking process, requiring an average amount of freshwater of 5.7 hm^3/year (Barthe et al., 2015).

The increasing water scarcity and water pollution control efforts in many countries have made water reuse a suitable economic means of maintaining and increasing the existing water supply, especially when compared to expensive alternatives such as desalination or the development of new water sources involving dams and reservoirs. Treated wastewater (also referred to as reclaimed wastewater or recycled water) can be used for various purposes. The dominant applications for the use of treated wastewater include agricultural irrigation, landscape irrigation, industrial reuse, and groundwater recharge (Alcalde Sanz and Gawlik, 2014). Within the industrial sector, the most popular applications are for cleaning purposes and cooling towers make-up. The use of reclaimed water within the industrial sector provides economic, social and environmental benefits (Wintgens et al., 2013).

Since 2012, petrochemical plants in Tarragona (Spain) are using reclaimed water from municipal wastewater treatment plants (WWTP) (AEQT, 2014). The water reclamation plant in Tarragona supplies from 6.8 to 20 hm^3/year of reclaimed water to the industrial park where petrochemical industry is located, as a way to save equivalent flows of surface water, being used for increasing the reliability of supply of urban users. Currently, reused water is blended with Ebro River water in order to provide make-up cooling water for the Tarragona petrochemical complex plants.

Although this option can decrease fresh water usage and, therefore, increase the water efficiency, the access to secondary treated effluents from urban WWTP is not obvious, so the industry must be near a municipal WWTP in order to meet both cost-efficiency and sustainable criteria. Furthermore, tertiary treatment in centralized urban WWTP plants usually has a larger environmental impact in terms of energy consumption due to water transport than in-situ water recycling and reuse schemes. In this sense, in situ water recycling within the petrochemical plant is a fundamental strategy to a more efficient water use in the sector. The benefits of petrochemical wastewater recycling and reuse will derive not only from savings in the freshwater supply but also a reduction in wastewater generation and discharge, including related energy consumption and treatment costs.

In petrochemical industry, different wastewater treatment approaches have been considered depending on wastewater composition. Flotation, coagulation, biological treatment and membrane separation processes as well as advanced oxidation processes for treating oily wastewater were reviewed by Yu et al. (2017). Regarding biological treatment, Cao and Zhao (2012a) proved Moving Bed Biofilm Reactors (MBBR) were better capable of organic removal in petrochemical wastewater compared to activated sludge processes (Cao and Zhao, 2012). Hansen et al. (2016) showed the feasibility for the reuse of the petrochemical internal streams as makeup water in cooling towers, in a cascade based system. Venzke et al. (2017) used an RO system for water reclamation in the petrochemical industry. Although it showed promising results, calcium concentrations of reclaimed water were too high for the water being used in high-pressure boilers. Additionally, different pre-treatment methods such as coagulation-filtration and ultrafiltration (UF), and two final membrane treatment technologies, nanofiltration (NF) and RO, for desalination of a cooling tower blowdown (CTBD) were investigated by Davood Abadi Farahani et al. (2016). MBR systems have also been studied for the treatment and reuse of petrochemical wastewater (Bayat et al., 2015; Lin et al., 2011).

In the project from which this work is derived (Rewatch, 2018), an innovative scheme is proposed with the aim to treat, recycle and reuse the water exiting the petrochemical process into the same process, obtaining reclaimed water for boiler feed water and for cooling tower make up water. The on-site water-recycling scheme proposed includes five different technologies to constitute a completely new treatment scheme never used before in the petrochemical industry: Actiflo®-based physicochemical pre-treatment (coagulation-flocculation (CF)), MBBR, DOW™ Ultrafiltration

(UF), FILMTEC™ Reverse Osmosis (RO) and DOWEX™ Ion Exchange Resins (IER) (Figure 5.1). The process is designed in such a way that the treated water may exit after any treatment step depending on the desired water quality. In this work, the two first steps (shadowed in Figure 5.1) were studied at lab and bench scale.

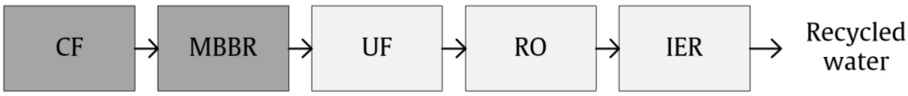


Figure 5.1. Proposed scheme for wastewater treatment and reuse.

By adopting the proposed innovative on-site recycling technology, petrochemical companies could, not only increase the control of the release of emerging pollutants and other contaminants generated in the petrochemical process into the generic wastewater treatment systems, but also avoid the loss of water and increase the energy efficiency related to water management when compared to currently adopted water management strategies.

Since MBBR has been proved a good option for petrochemical wastewater treatment (Cao and Zhao, 2012), it was chosen as the option to pretreat petrochemical wastewater prior to membrane steps. MBBR is a completely mixed continuously operated biofilm reactor in which biomass grows on small suspended polymeric carriers that move in the reactor (Ødegaard et al., 1994). It is reported that MBBR technology is more favourable than conventional activated sludge considering industrial wastewater thanks to the accumulation of active biomass due to microorganism immobilization (Cao and Zhao, 2012). The reactor may be used for aerobic, anoxic or anaerobic processes and, thus, for BOD/COD removal, phosphorous removal and nitrification-denitrification (NDN) process.

MBBR has many advantages when compared with conventional biological treatment systems. The biomass growing in biofilm instead of suspended flocs enables the decrease of reactor volume creating a very compact technology. Moreover, biofilm formation allows different microbial groups compete and co-exist in different niches, even those microorganisms with lower growth rates (Piculell, 2016). Since petrochemical wastewater usually contains hydrocarbons and a wide range of toxic and non-easily biodegradable compounds, the long sludge age in biofilms permits the growth of slow-growing bacteria and the removal of recalcitrant compounds such as micropollutans (Jiang et al., 2018). In general, fixed-film processes are less sensitive to environmental variations and, thus, to toxic compounds (Renou et al., 2008; Schneider et al., 2011).

MBBR systems need an increased dissolved oxygen (DO) concentration and a higher mixing intensity due to diffusion limitation compared to activated sludge processes. The enhanced mixing both prevent carrier clogging and increases substrate availability. On the other hand, the oxygen diffusion limitation creates a concentration gradient in the biofilm that, instead of being considered a drawback, permits the growth of different microbial niches in the different depths of the biofilm (Piculell, 2016).

The biofilm formation process itself compresses four steps, namely attachment, accumulation, re-generation and maturation (Zhu et al., 2015). Once the steady state is reached, the detachment of biomass causes that part of it remains in suspension. This suspended biomass can contribute to the overall performance of the reactor but may vary due to the growth rate, the specific activity, the loading rate or the hydraulic retention time (HRT) (Piculell, 2016).

Hydrocarbons have been in Earth for millions of years, and a diverse group of organisms has evolved to use them as a source of carbon and energy (Prince et al., 2010). In recent years, some studies have been published which aim to treat oilfield wastewater using microorganisms (Dong et al., 2011). However, little is known about the microbial community of MBBRs treating petrochemical wastewater. Analysis of bacterial community based on 16S rDNA sequence would help improve this knowledge. Likewise, fungal community is well known for being capable of degrading organic polymers of high molecular mass. Hence, a microbial community capable of degrading petroleum hydrocarbons would probably include fungi. Sequencing of an MBBR treating petrochemical wastewater should include fungal analysis. In this case, the internal transcribed spacer (ITS) sequencing would help determine the molecular ecology of fungi in the reactor (Schoch et al., 2012).

Jia et al. (2018), in their petrochemical wastewater characterisation study, concluded that the main pollutants found in the wastewater were settable particles, which contributed to over 54.8% of the total COD. According to those results, the present work includes a pre-treatment step of clarification of the raw wastewater using coagulation-flocculation technology to help improve MBBR performance. This would both help decrease solids concentration as well as possible toxic inhibitor compounds or refractory COD. The technology used for coagulation-flocculation of the wastewater is Veolia′s commercially available Actiflo® process. Actiflo® technology consists in a high-rate and easing their settling velocities. Testing of commercial Actiflo® and Actiflo® Carb (when activated carbon is also added) technologies for wastewater pre-treatment was performed to assess if this pre-treatment could help enhance the biological performance of the MBBR. Although Actiflo® and Actiflo® Carb processes are well established commercial technologies, a previous step of laboratory optimization is mandatory before pilot test operation due to the different characteristics of each industrial wastewater, particularly in the petrochemical sector.

This work, therefore, shows the optimization at bench scale of the physicochemical and biological pre-treatment to membrane technology.

Concretely, lab tests are performed to assess possible biological inhibitions by optimising coagulation-flocculation pre-treatment and performing respirometry assays. Finally, bench scale MBBR is tested for the degradation of organic matter from a challenging wastewater with low BOD/COD values. Moreover, a study of microbiological population is included to detect and understand changes in its composition during MBBR operational changes.

5.2. Materials and methods

5.2.1. Wastewater characteristics

Wastewater (WW) used in this study was a mixture of three different effluents from the olefins cracker plant from Dow Chemical Iberia′s (DCI) petrochemical complex located in La Pobla de Mafumet (Tarragona, Spain). The olefins cracker plant consumes an 88% of the total water fed to DCI′s petrochemical complex, and it also generates an 88% of the total wastewater produced. Different mixtures of those three wastewater streams from DCI′s Tarragona ethylene cracking facility were tested in the present study. The three effluents composing the wastewater used in this work are described below and its composition is shown in Table 5.1.

- *Cooling towers wastewater (tWW).* Cooling tower blowdown and water from the drains from the refrigeration system of the different equipment in the olefins cracker (i.e. instruments, pumps, etc.).
- *Cleaning wastewater (cWW).* Water collected in the trench system that is present all along the olefins cracker plant. This water mainly comes from the rain, safety auxiliary equipment (i.e. showers and eye washers), fire protection systems, water used to clean plant′s floor and open drains from different equipment.
- *Process wastewater (pWW).* Water involved within the process and in contact with the different chemicals (such as hydrocarbons) present in the different sections of olefins cracker plant.

Specifically, two wastewater mixtures were tested in this work. MIX A consisted of 77.2% of tWW, 20.3% of cWW and 2.5% of pWW (Table 5.1) while MIX B consisted of 58% tWW, 37.4% cWW and 4.6% pWW. The relative amounts of wastewater streams were defined after a study of water flows in the oleofins cracker plant (data not shown).

Table 5.1. Historical data of wastewater effluents and composition of raw wastewater (MIX A) and MBBR inlet (MIX A). Average ± standard deviation.

Parameter	Cooling towers WW (tWW)	Cleaning WW (cWW)	Process WW (pWW)	Raw WW (MIX A)	MBBR inlet (MIX A)	MBBR inlet (MIX B)
pH	7.6 ± 0.3	7.3 ± 1.4	8.9 ± 1.2	6.5 ± 0.7	6.8 ± 0.3	7.1 ± 0.1
Alkalinity *(mg $CaCO_3$/L)*	62 ± 22	112 ± 110	212 ± 182	125 ± 57.8	87.2 ± 29.7	115 ± 95.8
EC *(µS/cm)*	4190 ± 356	1140 ± 563	541 ± 486	3630 ± 260	4140 ± 90	3540 ± 550
TDS *(mg/L)*	3360 ± 321	674 ± 400	325 ± 289	-	-	-
TSS *(mg/L)*	6.3 ± 8.1	25 ± 19	45 ± 120	16.6	20.2 ± 25.2	-
VSS *(mg/L)*	2.9 ± 1.7	20 ± 12	25 ± 64	14.2	20.1 ± 25.4	-
Turbidity *(NTU)*	4.6 ± 3.8	23 ± 17	29 ± 73	20.9 ± 18.7	2.54 ± 1.24	2.0 ± 0.1
TOC *(mg/L)*	42 ± 104	211 ± 362	709 ± 575	-	-	-
DOC *(mg/L)*	-	-	-	95.6 ± 50.0	69.7 ± 59.2	84.0 ± 29.8
TIC *(mg/L)*	9.4 ± 4.5	22 ± 24	16 ± 97	-	19.3 ± 4.7	19.3 ± 8.1
COD *(mg/L)*	31 ± 9.4	742 ± 523	1890 ± 388	-	-	-
sCOD *(mg/L)*	-	-	-	567 ± 238	357 ± 233	301 ± 47.0
BOD_5 *(mg/L)*	11 ± 15	163 ± 99	699 ± 613	30.0	35.0	129 ± 153
Ammonium *(mg/L)*	0.0 ± 0.1	3.2 ± 2.1	2.8 ± 2.0	<0.5	1.0 ± 1.6	<0.5
Nitrate *(mg/L)*	31 ± 9.5	0.04 ± 0.2	0.8 ± 1.9	25.1	3.7 ± 2.1	1.1 ± 1.2
Nitrite *(mg/L)*	0.5 ± 1.8	BDL	BDL	1.95	1.2 ± 2.1	0.5 ± 0.4

Parameter	Cooling towers WW (tWW)	Cleaning WW (cWW)	Process WW (pWW)	Raw WW (MIX A)	MBBR inlet (MIX A)	MBBR inlet (MIX B)
Phosphate *(mg/L)*	1.9 ± 0.8	0.2 ± 0.3	0.04 ± 0.1	2.6	0.3 ± 0.1	1.0 ± 0.4
TN *(mg/L)*	-	-	-	3.7 ± 3.8	2.7 ± 2.8	3.5 ± 2.2
TPH *(mg/L)*	<0.96	<0.96	181 ± 43	1.40	-	-
Methanol *(mg/L)*	-	47 ± 258	381 ± 765	-	-	-
Sulphide *(mg/L)*	-	468 ± 621	57 ± 112	-	-	-
O&G *(mg/L)*	6.4 ± 2.2	23 ± 29	321 ± 244	-	-	-
Ca *(mg/L)*	-	-	-	382	314 ± 53.0	297
Fe *(µg/L)*	-	-	-	506	513	-

5.2.2. Experimental procedure

5.2.2.1. Coagulation-flocculation

Coagulation-flocculation tests were performed in batches using a Flocumatic laboratory bench jar test (J.P. Selecta, Spain) provided with six positions for glass beakers. For Actiflo® testing the steps followed were: (1) stirring at 160 rpm for 2 minutes for coagulation, (2) stirring at 160 rpm for 1 minute for flocculation, and (3) settling for 3 minutes. After the settling period, samples were taken from the clarified liquid to analyse turbidity and dissolved organic carbon (DOC).

The two previously described wastewater mixtures (MIX A and MIX B) were tested in Actiflo® jar test experiments. Aluminium polychloride and ferric chloride were tested as coagulants with doses from 5 to 100 mg/L. The influences of the coagulant type and dose were evaluated in terms of turbidity and DOC removals.

Additional coagulation-flocculation tests were performed using Actiflo® Carb technology. Actiflo® Carb uses powdered activated carbon (PAC) for the adsorption of non-flocculable organic matter and toxic compounds. These tests were performed in the clarified water from Actiflo® treatment after the optimal coagulant type and dose were obtained with the aim to assess if this step would increase turbidity and DOC removal efficiencies. After the Actiflo® testing in optimal conditions, the clarified water underwent the following steps: (1) stirring at 180 rpm for 8 minutes for PAC contact with wastewater, (2) stirring at 180 rpm for 2 minutes for coagulation, (3) stirring at 120 rpm for 3 minutes for flocculation, and (4) settling for 5 minutes. After the settling period, samples were taken from the clarified liquid to analyse turbidity and DOC. The influence of PAC dose was evaluated in terms of turbidity and DOC removals.

5.2.2.2. Respirometry assay

Biological respirometry assays were performed in a BM-T+ respirometer (Surcis S.L., Spain). Vessel was filled with 1 L of sludge in endogenous conditions (basal respiration rate of heterotrophic microorganisms present in the sludge). Respirometry experiments were performed using two different sludges as inoculum. Industrial acclimated sludge was obtained from the biological treatment of Repsol WWTP (Tarragona petrochemical complex, Spain) and urban WWTP sludge was obtained from the biological reactor of Manresa WWTP (Spain).

Temperature was kept at 20°C throughout the assays. Samples of DCI wastewater were mixed in the MIX A proportions and assays were performed on raw mixture (raw), mixture pre-treated with Actiflo® (Actiflo®) and mixture pre-treated with Actiflo®-PAC (Actiflo®-PAC). Between 7 and 50 mL of sample were added at each assay in order to have an initial chemical oxygen demand (COD) concentration of 8 mg/L in the respirometer.

The biodegradable fraction of the sample (bCOD) was calculated using total oxygen consumption measured in the respirometer and the calculated value of normal biomass yield growth ratio (0.67 $gCOD_{biomass}/gCOD_{degraded}$, according to the manufacturer) for activated sludge from conventional municipal wastewater treatment plants. The ratio bCOD/COD is an indicative of the sample biodegradability, being considered biodegradable values above 0.3 and non-biodegradable values lower than 0.05 (Ballesteros Martín et al., 2010).

Specific oxygen uptake rates (SOUR) were calculated for each experiment. Acetate was used as biodegradable control compound at the same COD concentration than the sample (8 mg/L). Inhibition percentages were calculated according to the following equation:

$$Inhibition\ (\%) = \frac{(SOUR_{control} - SOUR_{sample})}{SOUR_{control}} \cdot 100 \qquad (5.1)$$

5.2.2.3. MBBR system

A bench-scale MBBR system with two reactors with working volumes of 5L was used (Figure 5.2). Reactor 1 was designed to be able to remove easily biodegradable organic matter while reactor 2 would remove refractory organic matter. Each reactor was filled with carriers at 35% of their working volume. Each reactor was provided with mechanical stirring in order to achieve a proper mixing and maintain the carriers in suspension. The carriers used were AnoxKaldnes′ Z-carriers (Figure 5.3). These are an innovative range of carriers, which enable the predefinition of biofilm thickness together with specific tolerance to Ca^{+2} and scaling (Piculell, 2016). Their exposed biofilm area was 1280 mm^2/carrier.

pH and conductivity were daily monitored in order to detect any possible failure in the reactor. Conductivity was constant during all the operation at 4.1 ± 0.3 mS/cm. pH was maintained at around neutral in all biological reactors: 7.5 ± 0.3 at R1 and 7.6 ± 0.3 at R2. DO concentration was maintained between 4.5 and 6.5 mg O_2/L by means of a DO probe and an on/off controller. Temperature was maintained at 23 ± 2°C using a submersible heater. DO and temperature data were constantly and automatically recorded through a data acquisition system.

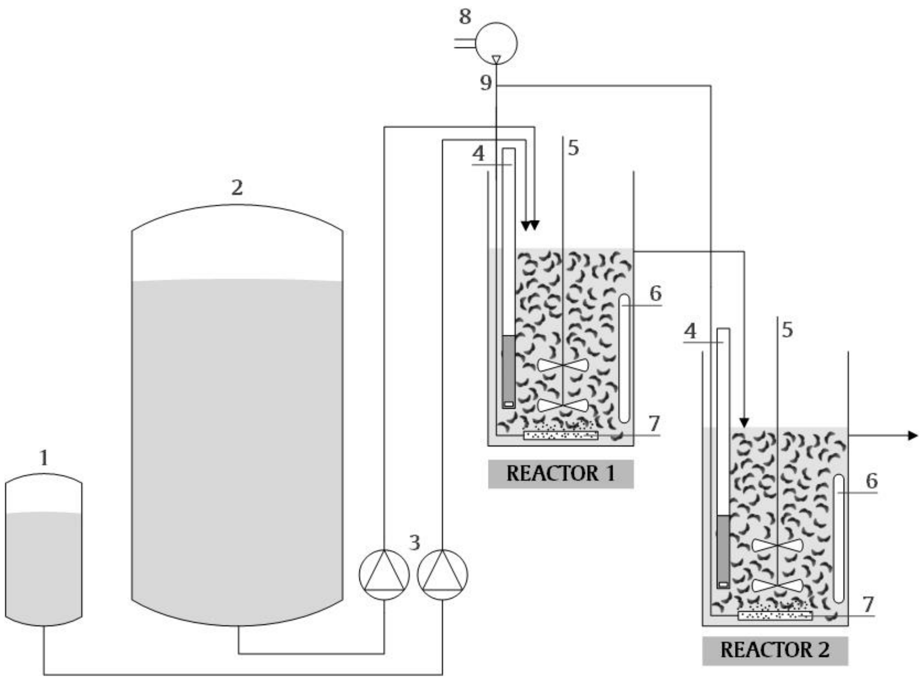


Figure 5.2. MBBR setup scheme. 1- Nutrients mixture; 2- Feed tank; 3- Peristaltic pumps; 4- DO probe; 5- Stirrer; 6- Heating system; 7- Air diffuser; 8- Compressor; 9- Air pipes.

Figure 5.3. Z-carriers picture.

Raw wastewater was stored in a 1000 L tank before the coagulation-flocculation process. Coagulation and flocculation pre-treatment was performed in batches of 300 L every time that feed wastewater for the MBBR plant was needed. Actiflo® conditions were those determined in Jar Test. pH after coagulation-flocculation was readjusted to 7 for the proper operation of the biological system. When necessary, nutrients in form of phosphate and urea were added at C:N:P proportions of 200:5:1 mg/L. Therefore, total nitrogen (TN), ammonium, nitrate, nitrite and phosphate were monitored in the MBBR effluent to check that they were always in excess (around 1 ppm). Initially, feed water was inoculated with acclimated sludge from Repsol WWTP (from day 3 to day 9).

Different operational conditions were studied and grouped in different feeding periods, which are summarized in Table 5.2.

Table 5.2. Reactors operating, flow, HRT, OLR and wastewater characteristics at each experimental period of the MBBR plant.

Period	Time (d)	Flow (L/h)	HRT (h)	OLR (kg sCOD/ (m^3 d))	Wastewater	Purpose
A	0-76	0.5-2	9.4-2.4	0.2-3.25	Mix A, pre-treated Actiflo®	Start-up and optimisation of OLR
B	77-85	1	4.7	1.62	Mix A, raw wastewater	Test wastewater without pre-treatment
C	86-97	1.7	2.8	1.37	Mix B, pre-treated Actiflo®	Change wastewater mix proportions

5.2.3. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), pH, electrical conductivity (EC), alkalinity, COD, biological oxygen demand (BOD_5), turbidity and metals were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Major anions and cations were analysed using ICS 2100 (Dionex). Metals analysis was performed using ICP MS 7500CX (Agilent Technologies) equipment. Total organic carbon (TOC), DOC, total inorganic carbon (TIC) and TN concentrations were measured using analyser C/N 3100 (Analytik Jena). Total petroleum hydrocarbons (TPHs) were analysed following method EPA 418. Alcohols, chlorinated compounds, organochlorines, aromatic hydrocarbons, polycyclic hydrocarbons, and PCBs were determined by HRGC-MS and HRGC-FID.

5.2.4. Microbial community analysis

5.2.4.1. Sampling and DNA extraction

Microbial community analysis was performed for the inoculum sample, for the attached and suspended biomass in reactors 1 and 2 at day 76 of operation (end of period A, maximum OLR) and for the biomass attached to carriers in reactor 1 at days 55 and 97 of experimentation as well (medium OLR and end of MBBR operation, respectively). Samples were frozen at −80 °C for further analysis.

Carriers′ biomass was recovered by rinsing the carriers with buffer solution Ringer ¼. After the rinse, the solution was discarded and 20 carriers were put in 150 mL of clean Ringer ¼ and were gently mixed and slightly sonicated to recover all the attached biomass present.

For DNA extraction, samples were centrifuged at 5000 x g for 10 min. DNA extraction from the obtained pellet was performed using PowerSoil® DNA Isolation Kit (Mo Bio Laboratory Inc., USA).

5.2.4.2. Next Generation Sequencing (NGS) and data analysis

DNA sequence library preparation and sequencing was performed at Research and Testing Laboratory (Lubbock, TX, USA) using Illumina MiSeq (San Diego, CA, USA) and 2x300 bp technology. Partial bacterial 16S rDNA gene sequences were amplified from extracted DNA using primer pair 357wF-785R (CCTACGGGNGGCWGCAG; GACTACHVGGGTATCTAATCC) (Klindworth et al., 2013). For fungal analysis, the internal transcribed space (ITS) DNA region was targeted. The primer pair used to amplify this region was ITS3F-ITS4R (GCATCGATGAAGAACGCAGC; TCCTCCGCTTATTGATATGC) (White et al., 1990).

Initial processing of the raw data obtained from NGS was performed through the Pipeline Initial Process tool provided by the Ribosomal Database Project (RDP) webpage (Cole et al., 2014). Paired-End reads were Assembled using PANDAseq program. Chimeras were removed using USEARCH 6.0 tool, based in UCHIME algorithm (Edgar et al., 2011). Finally, the sequences obtained from the previous steps were taxonomically assigned using the Bayesian Classifier tool of the RDP for a taxonomy based analysis of the data (Wang et al., 2007).

On the other hand, chimera checked sequences were aligned using the secondary-structure aware Infernal aligner version 1.1rc4 and operational Taxonomic Units (OTUs) for taxonomy independent analysis were obtained through Complete Linkage Clustering tool, both provided by the RDPipeline webpage (Cole et al., 2014). Representative sequence was obtained for each OTU, rarefaction tool was used to assess the sequencing depth and, finally, ecological metrics such as Shannon and Chao1 alpha diversity were estimated using the pertinent RDPipeline tools (Cole et al., 2014). Sequence similarity threshold for both OTU and taxonomy assignments was 95%.

5.3. Results and Discussion

5.3.1. Laboratory scale pre-treatment optimization

5.3.1.1. Coagulation-flocculation tests

Coagulation-flocculation tests were performed to remove particulate matter present in the wastewater with the aim of decreasing its possible toxic effect in the biological treatment. Actiflo® jar test experiments with wastewater mixtures MIX A and MIX B achieved turbidity removals of 70-85% and >85% respectively, with an initial turbidity between 3.0 and 7.3 NTU. In both cases, final turbidity of the clarified water was <1 NTU. Although a coagulant dose of 30 mg/L was found to be appropriate (Figure 5.4), for further experimentation a dose of 50 mg/L was selected to avoid disturbances if wastewater composition changed along operation of the pilot plant. Taking into account that both coagulants performed well for the mixture of wastewaters, ferric chloride was chosen as, in equality of performance; iron was preferred over aluminium for a better formation and settling velocity of flocs. No significant removal of DOC was observed in Actiflo® jar tests (data not shown).

After the optimization of coagulant dose, further tests were performed in order to assess if an extra step of PAC addition (Actiflo® Carb) would improve the quality of the effluent in terms of decrease possible toxic compounds. It was assumed that a decrease in toxic compounds could be indicated by a reduction of DOC. Initial DOC concentration of the wastewater mixture was 94.4 mg/L (MIX B). When Actiflo® Carb tests were performed, DOC removal observed was proportional to PAC concentration, ranging from 6 to 12% removal at PAC doses between 10 and 50 mg/L.

Turbidity was no further removed with Actiflo® Carb compared to the conventional Actiflo® system.

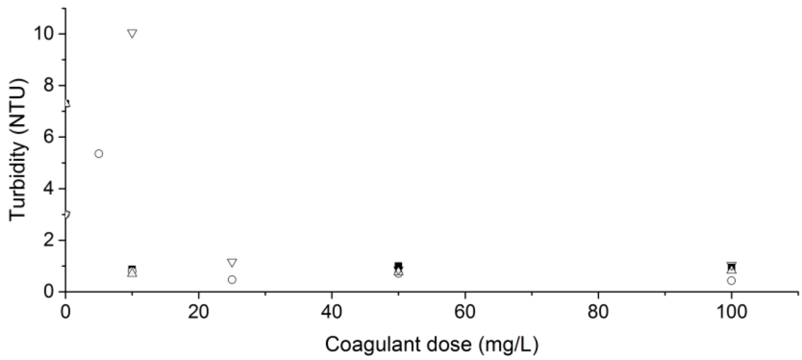


Figure 5.4. Turbidity after Jar tests with different doses of coagulant. Polyaluminium chloride (MIX A) (■); Polyaluminium chloride (MIX B) (○); Ferric chloride (MIX A) (△); Ferric chloride (MIX B) (▽).

5.3.1.2. Respirometry assays

Respirometry assays were performed prior to MBBR design and operation to determine biodegradability and/or possible inhibitions of the wastewater with and without pre-treatment. Accordingly, respirometry assays were performed on raw mixture (raw) and mixture pre-treated with Actiflo®-PAC (Actiflo®-PAC) in MIX A proportions.

No short-term inhibitions were observed in any of the respirometry assays. As shown in Table 5.3, no inhibitions were observed in any case and bCOD/COD ratio in all cases was greater than 0.3. Industrial sludge showed higher maximum SOUR values and bCOD/COD ratio values than urban sludge in raw and Actiflo®PAC tests.

From SOUR values shown in Table 5.3, it can be inferred that removal of biodegradable compounds when using industrial sludge was faster than urban sludge tests, lasting the tests 15-20 min when industrial sludge was used and 50-70 min when urban sludge was used. Thus, using acclimated biomass helped achieving shorter times in the removal of biodegradable compounds, as expected, showing the importance to keep specific hydrocarbon-degrading microorganisms in the system. Accordingly, Perrota et al. (2017) concluded in their work that the composition of the original inoculum predictably contributes to bioreactor community structure and function. In line with this and to the respirometry results obtained, it was decided to perform the latter inoculation of the MBBR pilot system using the acclimated sludge from Repsol WWTP. Since no short term inhibition was observed in any case, Actiflo® pre-treatment was chosen prior to biological process to prevent possible toxic effects along operation since it offered good turbidity removals and the use of reagents was lower than Actiflo®PAC treatment.

Table 5.3. SOUR, bCOD/COD ratios and inhibition (%) of respirometry assays. n.d.: not detected.

		$SOUR_{max}$ (mg O_2/(g VSS h))	bCOD/COD ratio	Inhibition (%)
Industrial sludge	Raw	6.95	0.67	n.d.
	Actiflo®PAC	6.96	0.74	n.d.
Urban sludge	Raw	6.0	0.46	0.1
	Actiflo®PAC	6.8	0.67	n.d.

5.3.2. MBBR operation

5.3.2.1. Organic removal capacity

The organic loading rate (OLR) of the MBBR system was progressively increased during period A of treatment (Table 5.2) from 0.20 kg sCOD/(m^3 d) –during the start-up– to 3.25 kg sCOD/(m^3 d). Feed wastewater flow had to be adjusted to obtain the expected OLR due to the changes in sCOD concentration among wastewater lots. This OLR increase was linked to a decrease in HRT from 23.4 to 4.5 h. MBBR removal of phenolic compounds in coastal wastewater performed by Li et al. (2011) decreased the HRT from 48 to 32 h, observing a decrease in their performance. Dong et al. (2011) reported minimum HRT of 10 h in an MBBR treating oilfield wastewater while Ahmadi et al. (2016) used an MBBR for phenol removal working at an HRT of 6 h. Hence, the HRT obtained in this work is lower than previously reported in similar studies.

High sCOD degradation percentages (>50%) were already achieved during the start-up period – from day 0 to 21– (Figure 5.5), probably helped by the use of acclimated biomass in the inoculation of the bioreactors. After OLR increases in period A, a first decay in the removal capacity was observed when changing the operational parameters. Nonetheless, degradation percentages were quickly recovered and maintained between 80 and 90% in the stationary states (Figure 5.5). This means that only 10-20% of organic matter present in the wastewater mixture was refractory to biological treatment. It has to be pointed out that all removal was achieved in the R1, which implies that the higher residence time given by the R2 did not help in the removal of those recalcitrant compounds. Accordingly, DOC removal showed a similar trend to sCOD removal along operation (Figure 5.5). This results are in accordance with Dong et al. (2011) which concluded that MBBRs had a strong capacity to resist shock loading caused by the change in influent flow rate.

The increase in OLR during period A was performed to assess the maximum treatment capacity of the system. As can be noted in Figure 5.5, maximum sCOD removal in the stationary state was mostly achieved in the first reactor (R1) (>75%) except for the period with higher OLR (3.25 kg sCOD/(m^3 d)) when R1 was not able to totally degrade the organic matter entering the system. Therefore, and according to these results, the calculated maximum sCOD that the system could degrade was 4.96 ± 0.01

kg sCOD/(m^3 d) at 23 ± 2°C, as to the organic load removal data from the first reactor indicated. The obtained OLR of the MBBR system is higher than those found in literature for MBBR treating petrochemical-derived wastewaters. Bina et al. (2018) obtained the best performance of their MBBR treating AlkylPhenol-containing synthetic wastewater at an OLR of 0.53 kg/(m^3 d) and a HRT of 16h. Li et al. (2011) worked at a loading rate of 1 kg/(m^3 d) in their MBBR treating coal gasification wastewater while Ahmadi et al. (2016) worked at a maximum OLR of 2 kg/(m^3 d) in a MBBR treating synthetic wastewater containing phenolic compounds. Also, OLR was higher than that reported for conventional activated sludge systems treating petrochemical wastewater, as is the case of Behnami et al. (2018) which worked at an organic loading rate (OLR) of 0.09–0.11 kg BOD/(m^3 d). The high OLR achieved could be due to the very specific microbial community developed thanks to the inoculum used and to the nature of the MBBR systems.

During period B, feed wastewater was switched to raw wastewater without Actiflo® pre-treatment (Table 5.2). In the course of this period, the sCOD degradation percentages were maintained at 84.5 ± 4.7 %. Therefore, since sCOD removal did not vary from the period in which pre-treated wastewater was used, this results imply that no short-term inhibition was derived from treatment of raw wastewater without pre-treatment. Also, during period C, in which feed wastewater effluent proportions were changed to MIX B with Actiflo® pre-treatment (Table 5.2), the sCOD removal percentages were kept at 81.4 ± 3.1 % (Figure 5.5). Those results showed that biomass presented no short-term inhibition when process and oily effluent percentages were increased in the feed wastewater mixture. Consequently, results obtained from operation during periods B and C indicate that the Actiflo® pre-treatment could be removed from the wastewater treatment scheme. However, careful monitoring should be performed if this configuration was evaluated in order to determine possible long-term inhibitions.

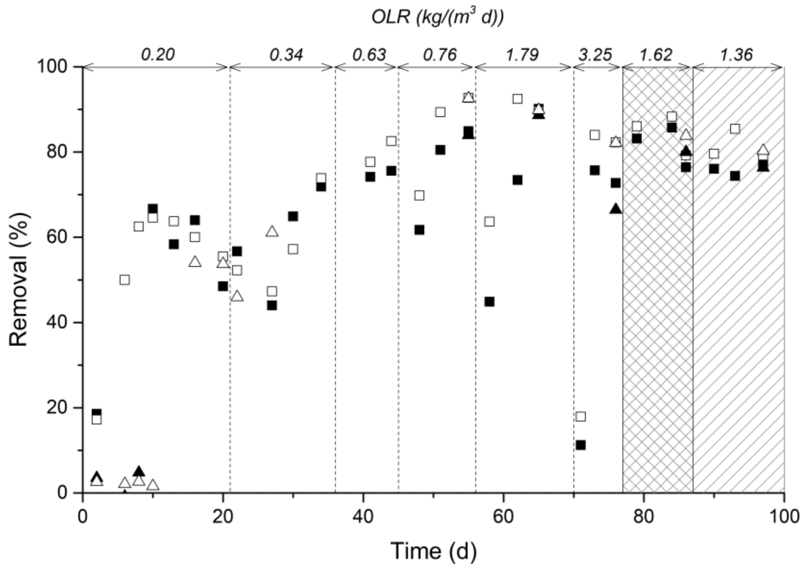


Figure 5.5. Accumulated sCOD removals in each reactor R1 (■) and R1+R2 (□) and accumulated DOC removals in each reactor R1 (▲) and R1+R2 (△) of the MBBR system. OLR (kg/(m^3 d)) for each period are indicated above. White background corresponds to period A, crossed pattern background corresponds to period B, lined background corresponds to period C.

Average effluent sCOD value from steady state operation was 64.5 ± 20.3 mg/L while the recommended COD value for water use in cooling towers is 20 mg/L. Since the MBBR is intended to be a pre-treatment to a membrane process in a whole water reuse system, further decrease in COD concentration could easily be obtained when including a membrane treatment step.

5.3.2.2. Degradation of volatile and semi-volatile compounds

Petrochemical wastewater complexity implies the presence of toxic compounds such BTEX (Benzene, Toluene, Ethylbenzene, Xylene) and PAHs (polycyclic aromatic hydrocarbons) among other volatile and semi-volatile compounds which could potentially hamper biological treatment of the wastewater and must be removed prior to wastewater release or reuse (Jia et al., 2018). Analysis performed on day 55 showed the detailed compound concentration and removal percentages in raw water and at each step of the process, showing the degradation extent of the examined compounds (Table 5.4). Analysis performed included alcohols, chlorinated compounds, organochlorines, aromatic hydrocarbons, polycyclic hydrocarbons, and PCBs. While a total of 100 compounds were analysed, Table 5.4 includes only those compounds found at an initial concentration of >100 µg/L.

Alcoholic compounds were expected in raw wastewater since they are produced within the petrochemical process. Contrarily, octanol was found in all samples in a concentration below 0.5 mg/L. Still, methanol and ethanol were the compounds detected at higher concentration in the raw wastewater mixture (1910 mg/L and 101 mg/L respectively). After the Actiflo® process, both methanol and ethanol were below the quantification limit. According to Jia et al. (2018), the organic substances that may be contained in the settable particles of petrochemical wastewater include alcohols, acids, ethers, phenols, alkanes, proteins, and aromatic compounds. In this study, when coagulation-flocculation was performed, it was observed a substantial decrease in alcohols, polycyclic hydrocarbons and PAHs concentration and a mild decrease in some aromatic compounds. No concentration decrease was observed for smaller compounds such as phenol, acetone, cresol or benzene.

After MBBR treatment, all analysed compounds were removed >90%, being most of them at concentrations below the detection limit. This results show that, with the wastewater mixture used, the concentrations of volatile and semi-volatile organic compounds were not only not toxic to the bioreactor but could properly be degraded. This results are in accordance with previous studies in which volatile and semi-volatile organic compounds are degraded with a biological treatment after an acid hydrolysis pre-treatment (Wu et al., 2016). The nearly complete removal of those compounds in the biological reactor rules out the possible addition of PAC in the Actiflo® pre-treatment as no additional benefits would be obtained. In addition, sCOD removals obtained in MBBR period B of operation, suggest the operation of the MBBR using raw wastewater could be feasible but long-term inhibitions should be further studied.

Additionally, on day 86 of operation TPHs were also analysed, showing an initial concentration of 1.4 mg/L in raw wastewater. The effluent of reactor 1 already achieved a concentration below the quantification limit (<0.5 mg/L).

Table 5.4. Organic volatile and semi-volatile concentration and degradation percentages in raw wastewater, after Actiflo® process and after each of the MBBRs on day 55 of operation. Data provided only for those compounds detected at initial concentration >100 µg/L.

Compound	Concentration (µg/L)				Removal (%)		
	Raw wastewater	Actiflo® pre-treated wastewater	MBBR effluents		Actiflo®	MBBR	
			R1	R2		R1	R2
Methanol	$1.91 \cdot 10^6$	$< 10^3$	$< 10^3$	$< 10^3$	> 99.9	-	-
Ethanol	$1.01 \cdot 10^5$	$< 10^3$	$< 10^3$	$< 10^3$	> 99.0	-	-
Aromatics >C8-C10	3050	1240	98.5	6.4	59.5	92.0	99.5
1H-Indene	867	48	n.d.	n.d.	94.5	100	100
Phenol	745	737	< 0.1	< 0.1	1.1	100	100
m,p-Xylenes	479	237	5.5	< 0.5	50.6	97.7	> 99.8
Styrene	452	140	< 5	< 5	69.0	> 96.4	> 96.4
o-Xylene	359	210	8.3	< 0.5	41.7	96.0	> 99.8

Compound	Concentration (µg/L)				Removal (%)		
	Raw wastewater	Actiflo® pre-treated wastewater	MBBR effluents		Actiflo®	MBBR	
			R1	R2		R1	R2
Acetone	266	219	< 100	< 100	17.5	> 95.0	> 95.0
Toluene	233	126	< 0.5	< 0.5	45.9	> 99.6	> 99.6
Naphthalene	222	45.2	0.06	< 0.05	79.6	99.9	> 99.9
P-Cresol	151	108	0.35	< 0.1	28.7	99.7	> 99.9
Benzene	139	99.8	< 0.5	< 0.5	28.2	> 99.5	> 99.5
O-Cresol	138	145	0.19	< 0.1	< 1	99.9	> 99.9

5.3.2.3. Suitability of MBBR effluent for further membrane treatment processes

Turbidity and TSS are key parameters that have to be monitored since they have an influence on the performance in the next membrane steps of the process. MBBR suspended solids come from the excess sludge that detaches from the carriers, thus, some TSS were expected in the effluent although in much less concentration than in conventional activated sludge treatments. Figure 5.6 shows that turbidity and TSS were slightly dependent on the feeding flow, reaching values of > 100 NTU of turbidity and > 150 mg TSS/L in some periods.

These results suggest that a previous step is needed before reverse osmosis can be carried on. Ultrafiltration could help decrease TSS and turbidity to values acceptable for RO feeding (i.e. < 1 mg/L and < 1 NTU). If UF could not achieve the required values for TSS and turbidity, Actiflo® coagulation-flocculation system could be placed after MBBR treatment instead of prior to it.

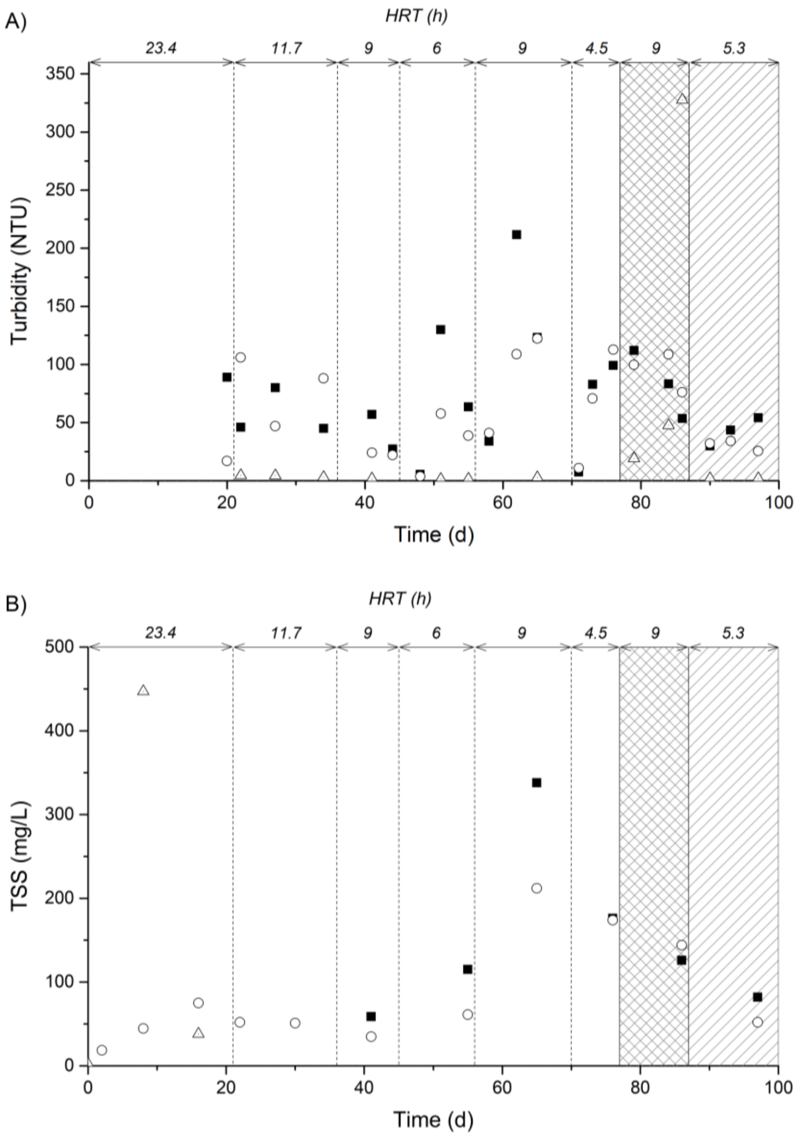


Figure 5.6. Turbidity (A) and TSS (B) in feed (△), R1 (■) and R2 (○) of the MBBR system. HRT (h) for each period are indicated above. White background corresponds to period A, crossed pattern background corresponds to period B, lined background corresponds to period C.

5.3.2.4. Microbial community and biodiversity

Rarefaction curves for bacterial analysis presented mild slopes under current sequencing depth, meaning sequencing libraries could properly reflect the microbial communities. Alpha-diversity analysis showed that both richness and diversity of bacteria and fungi were lower in suspended biomass than in attached biomass (Table 5.5). Bacteria richness and diversity was higher than fungi. Nevertheless, in both cases, richness and diversity were higher in attached biomass than in the suspended phase. According to these results, MBBR technology seems adequate for this wastewater treatment since it accumulates highly diverse microbial population in its attached biomass to carriers.

Table 5.5. Characteristics of sequencing libraries.

Target gene	Sample*	Number of sequences	Number of OTUs	Chao 1 value	Shannon Index
Bacterial 16S rDNA	Inoculum	30318	2985	6055.8	5.3
	R1 C 55	25792	2438	4946.7	4.6
	R1 C 76	22862	3089	6606.1	5.5
	R1 C 97	25454	2189	4450.7	4.7
	R1 S 76	28681	271	484.9	1.5
	R2 C 76	20454	2642	6144.5	5.1
	R2 S 76	16626	244	427.4	1.3
Fungal ITS	Inoculum	80514	319	581.6	0.8
	R1 C 55	27160	330	504.7	2.2
	R1 C 76	25506	150	229.6	1.2
	R1 C 97	75164	561	859.5	2.2
	R1 S 76	61628	126	211.0	1.2
	R2 C 76	13832	181	280.2	1.5
	R2 S 76	61938	190	255.6	1.1

* Samples are referred according to: reactor where they were taken (R1 or R2), its origin whether is from attached biomass to carriers (C) or from suspended biomass (S) and the operation day of sampling.

Changes in microbial community are usually linked to changes in operational parameters of bioreactors as reported by several authors (Amha et al., 2017; Li et al., 2010; Watanabe et al., 2016). Hence, changes in microbial community structure were expected along operation of the MBBRs. PCA analysis, performed both at genera and at class levels, demonstrated that the samples analysed presented higher differences between attached and suspended biomass rather than between samples of attached biomass along operation (Figure 5.7). The inoculum and the attached biomass samples were clustered near the same value for the first principal component (PC1), which explained 83.4% of variance in the analysis at genera level and 67.4% at class level. Main differences between reactors were due to second principal component (PC2), which explained 10.9% and 24.2% of variance for genera and class analysis respectively. Thus, although PC2 also presents mild differences between attached samples from different operation conditions; the attached or suspended nature of the biomass had higher influence in the differences of the microbial community structure than the changes in the operational conditions, which can also be observed in Figure 5.8 and Figure 5.9 showing the bacterial community structure of the samples analysed.

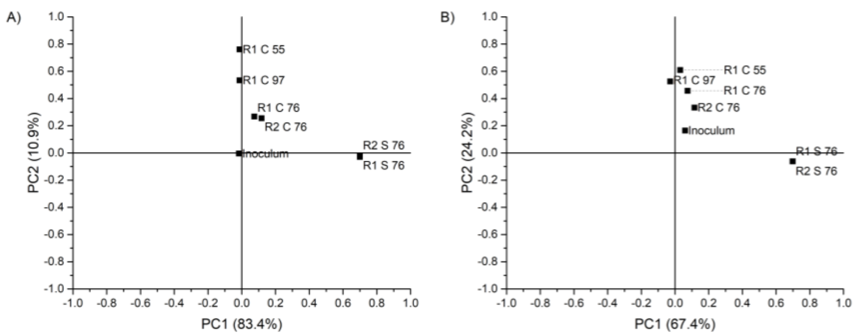


Figure 5.7. Principal component analysis (PCA) of bacteria at (A) genera and (B) class levels. Samples are referred according to: reactor where they were taken (R1 or R2),

its origin whether is from attached biomass to carriers (C) or from suspended biomass (S) and the operation day of sampling.

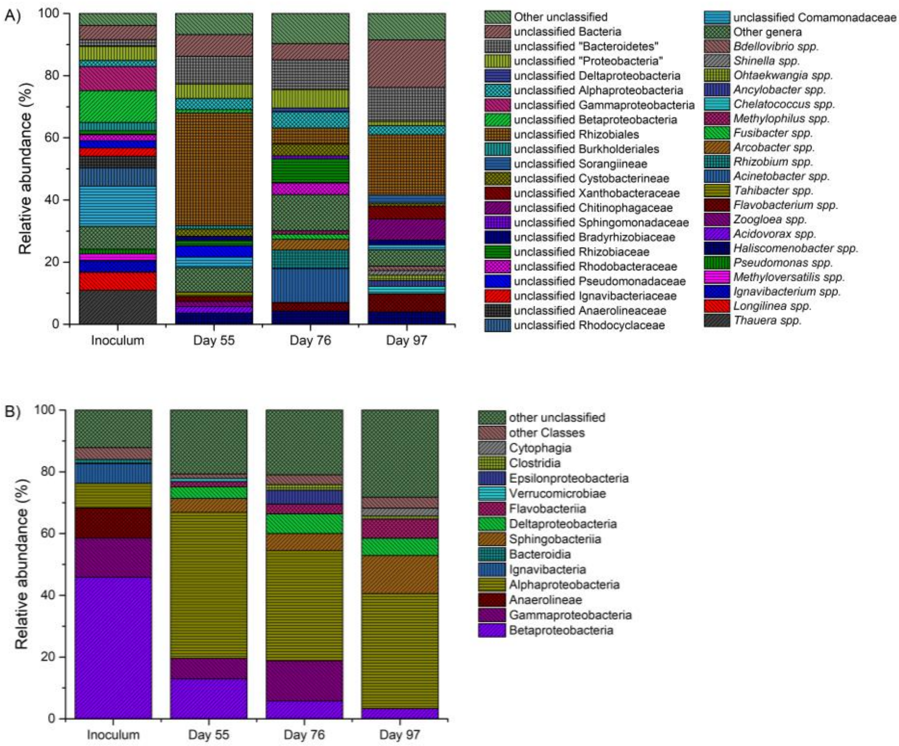


Figure 5.8. Taxonomic assignment of sequencing reads from bacterial community in the biomass in the inoculum and attached to carriers in reactor 1 (R1) at days 55, 76 and 97 at 95% confidence level (genera (A)) and at 80% confidence level (class (B)). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others".

Comparison of attached biomass community structure in reactor 1 along operation showed differences as it adapted to feed and operational conditions (Figure 5.8A). Inoculum contained several genera that disappeared after 55 days of operation. Major genera found in inoculum was *Thauera* spp. (10.9%) which is an important nitrate-reducing bacterium in wastewater treatment systems, significant for its metabolic versatility which includes the use of aromatic hydrocarbons under anoxic conditions (Li et al., 2018). *Thauera* spp., was not further detected in the rest of the samples. Also, *Methyloversatilis* spp. (2.1%), which is a known benzene degrader (Rochman et al., 2017), was only found in the inoculum sample. Both *Thauera* spp. and *Methyloversatilis* spp. have been reported to be two significant genera in denitrifying systems (Li et al., 2018). The lack of nitrogen species in the feed, would have led to its abundance decrease in the bioreactors.

The aerobic nature of the MBBR operation used in this study, decreased anaerobic species abundance found in the inoculum. *Longininea spp.* which is a strictly anaerobic genus (5.8%) and *Ignavibacterium spp.* (3.7%) which is a moderately thermophilic anaerobic genus, appeared in the inoculum but did not appear in the rest of the samples. Also *Pseudomonas spp.* (1.6%) was only detected in the inoculum sample.

Proteobacteria was the major phylum found in inoculum as well as in the attached biomass (Figure 5.8B) since the majority of the described genera of hydrocarbon-degrading bacteria belong to this huge phylum of gram-negative organisms (Prince et al., 2010). At family level, the inoculum showed a great abundance of *Comamonadaceae* (12.9%) followed by *Rhodocyclaceae* (5.9%) and by *Anaerolineaceae* (3.8%), *Ignavibacteriaceae* (2.7%), *Pseudomonadaceae* (2.3%), *Rhodobacteraceae* (2.0%) and *Rhizobiaceae* (1.2%). From those, only *Comamonadaceae*, *Pseudomonadaceae*, *Rhodobacteraceae* and *Rhizobiaceae* families were further detected in R1 attached biomass.

Haliscomenobacter spp. (Day 55 – 3.6%; Day 76 – 4.2% and Day 97 – 4.0%) and *Flavobacterium* spp. (Day 55 – 1.6%; Day 76 – 2.8% and Day 97 – 5.9%) were the two only genera found along the whole operation of reactor 1 (Figure 5.8A). *Haliscomenobacter* spp. cells grow as "needle-like" filaments and have been reported to be important components in biofilm formation and development (Zhu et al., 2015). On the other hand, *Flavobacterium* spp. are known for its potential for aerobic hydrocarbon degradation (Rochman et al., 2017) and they have been reported to be part of a biofilm composition (Montpart et al., 2018). Therefore, as it would be expected from an MBBR treating petrochemical wastewater, it is likely to have a biofilm-forming genus and an oil-degrading genus found in all the carriers sampled (Figure 5.8 and Figure 5.9).

Microbial community composition of carriers on day 55 also showed the presence of polycyclic aromatic hydrocarbons degraders such as *Acidovorax* (2.1%) (Jiang et al., 2019) and *Zooglea* spp., which have been reported to increase its abundance in a reactor treating phenol-containing wastewater (Papadimitriou et al., 2018).

Microbial abundance analysis of R1 carriers on day 76 showed high abundance of genus *Acinetobacter* (10.9%) (Figure 5.8A). This sample also contained genera such as *Rhizobium* (5.9%), *Arcobacter* (3.5%), *Fusibacter* (1.7%) and *Methylophilus* (1.1%). *Acinetobacter* spp. have been widely described as petroleum-degrading bacteria for their hydrocarbon utilization and biosurfactant production (Varjani, 2017). Their biosurfactant production permits the solubilization of water-insoluble compounds such as oil and its derivatives (Sanchez-Salas et al., 2016). *Rhizobium* spp. have also been described with ability to decompose PAHs (Zhang et al., 2012).

Genera found in R1 carriers on day 97 include *Chelatococcus* (2.3%), *Ancylobacter* (1.9%), *Othaekwangia* (1.7%), *Shinella* (1.6%) and *Bdellovibrio* (1.4%) (Figure 5.8A). *Chelatococcus* spp. can grow in a wide range of thiophenic compounds (Bordoloi et al., 2016) and *Shinella* spp. are PAH degrading bacteria (Subhash and Lee, 2016; Wu et al., 2014).

As commented before, all samples taken from attached biomass to carriers in reactor 1 have in common the presence of *Haliscomenobacter* spp. and *Flavobacterium* spp. The differences in genera composition between samples are probably due to a sum of factors, including: i) biofilm formation and acclimation of microorganisms, ii) changes in the organic load to the reactors and iii) variations in the feed composition, which probably slightly varied among batches, as well as changes in the mixture percentages and in the coagulation-flocculation pre-treatment.

While *Proteobacteria* was the major phylum detected in all samples (Figure 5.8B), its distribution at class level changed between samples. *Betaproteobacteria* was the major class detected in the inoculum, while in the attached biomass to carriers it was *Alphaproteobacteria*. Furthermore, in the attached biomass an increase in *Sphingobacteria* and *Flavobacteria* classes, both belonging to *Bacteroidetes* phylum, was observed. Since *Proteobacteria* and *Bacteroidetes* are gram-negative, the major components of their outer membrane are bacterial lipopolysaccharides. This characteristic facilitates its attachment to the surface of the carriers (Zhu et al., 2015).

When comparing microbial community structure of suspended and attached biomass in both reactors (Figure 5.9A), it can be observed that *Acinetobacter* spp. was the major genera found in all samples, especially in suspended biomass samples. Analysis was performed on day 76, during the stationary phase of the maximum OLR of the reactor. *Acinetobacter* spp. was detected in the attached biomass to carriers in reactor 1 at a relative abundance of 10.9% and in the attached biomass in reactor 2 at an abundance of 16.5%. In suspended biomass, its percentage was 92.8% and 92.7% in reactors 1 and 2 respectively. Biswas et al. (2014) also detected *Acinetobacter* spp. both attached and in suspension when studying two full-scale MBBR systems treating municipal wastewater. Its high abundance during this period, could be related to the high organic load of the reactor, since its biosurfactant production would help degrade the hydrocarbons contained in the feed. Some species of *Acinetobacter* spp. have been reported to have μ_{max} as high as 0.562 h^{-1} (Cutter and Stroot, 2008) which would avoid its washout from the reactor. The high grow rate of *Acinetobacter* spp. could explain its high abundance in the suspended biomass indicating the extreme importance of the MBBR system, which increases the attached biomass retention time to allow the growth of slow rate growing microorganisms and increase microbial diversity.

Rhizobium spp. (R1 – 5.9%; R2 – 3.4%), *Haliscomenobacter* spp. (R1 – 4.2%; R2 – 5.9%) and *Flavobacterium* spp (R1 – 2.8%; R2 – 7.8%) were detected in attached biomass of both reactors. *Arcobacter* spp. (3.5%), *Fusibacter* spp. (1.7%) and *Methylophilus* spp. (1.1%) were only detected in attached biomass of reactor 1 while *Zooglea* spp. (3.0%), *Bdellovibrio* spp. (2.0%) and *Shinella* spp. (1.1%) were only detected attached biomass of in reactor 2. Since they all have been reported to be able to degrade hydrocarbons, as stated above, it was assumed that genera found in reactor 1 utilised the more easily-degrading hydrocarbons, while genera found in reactor 2 would specifically degrade more recalcitrant compounds. In general, it can be said that more similitudes can be found between reactors 1 and 2 at the same operation time than between samples of the same reactor at different times (Figure 5.7).

Again, *Proteobacteria* was the major phylum detected in all samples but its distribution at class level varied between suspended and attached biomass samples (Figure 5.9B). *Alphaproteobacteria* was the most abundant class within attached biomass while *Gammaproteobacteria* was, by far, the most abundant class. *Bacteroidetes* were also found in both attached biomass samples (R1 and R2, Figure 5.9B) whilst *Verrucomicrobia* was only detected in reactor 2 attached biomass.

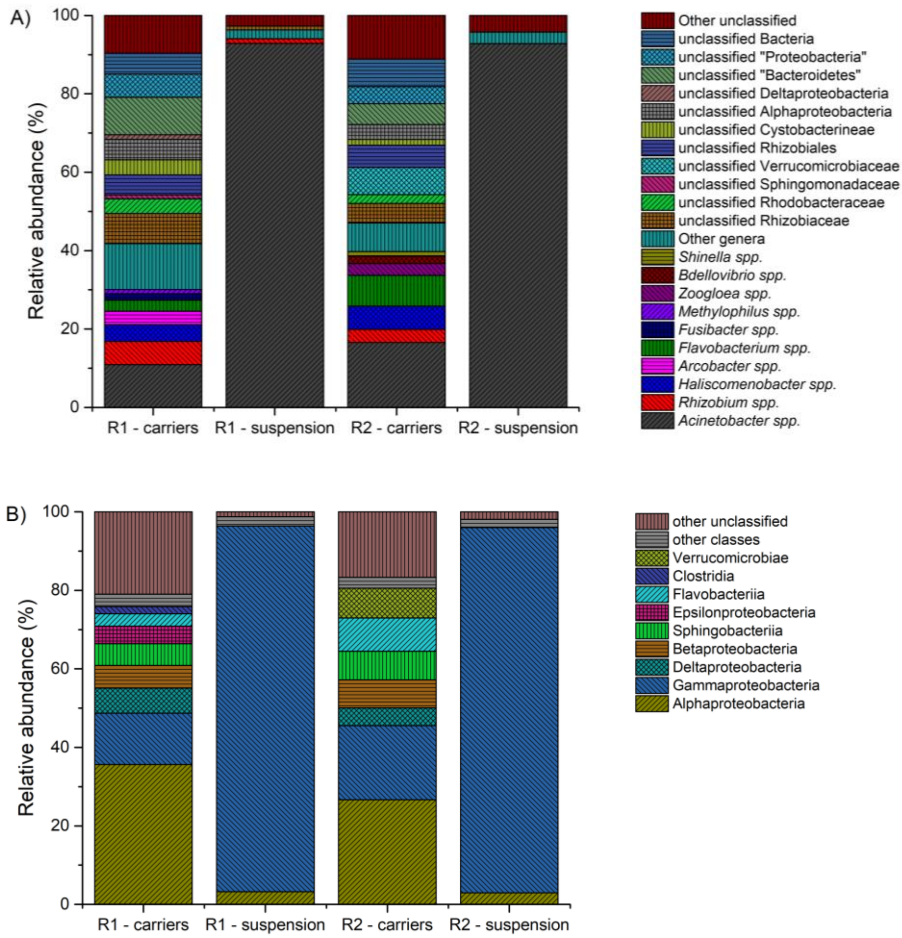


Figure 5.9. Taxonomic assignment of sequencing reads from bacterial community in reactors 1 (R1) and 2 (R2) in the biomass both in suspension and attached to carriers at day 76 at 95% confidence level (genera (A)) and at 80% confidence level (class (B)). Relative abundance was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others".

Fungal community is known for its versatile degrading capabilities and its presence in a reactor degrading petrochemical wastewater was expected. In this work the major proportion of fungal sequences were categorised as unclassified Fungi (Table 5.6), probably due to a lack of annotated sequences in databases. In spite of that, *Hypocreaceae* unidentified spp. (7.2%) and unidentified *Hypocreaceae* (3.9%) were detected in the inoculum. Its presence could be explained by reported phenantrene utilisation by mitosporic *Hypocreaceae* (Banjoko and Eslamian, 2016). *Trichosporon* spp. are capable of degrading TPHs (Gargouri et al., 2015) and were found both in carriers and suspended biomass of some of the samples. Moreover, *Acremonium* spp. which is capable of degrading petroleum hydrocarbons was detected (Sanchez-Salas et al., 2016). Due to the high number of unclassified sequences, no further discussion can be completed from this data. Therefore, this confirms the great research gap that needs to be covered to be able to identify fungal species implied in the degradation of industrial wastewaters containing hydrocarbons.

Table 5.6. Taxonomic assignment of sequencing reads from fungal community at 95% confidence level (genera). Relative abundance (%) was defined as the number of reads (sequences) affiliated with that taxon divided by the total number of reads per sample. Phylogenetic groups with relative abundance lower than 1% were categorized as "others".

	Inoculum	**R1 C 55**	**R1 C 76**	**R1 C 97**	**R1 S 76**	**R2 C 76**	**R2 S 76**
Hypocreaceae unidentified spp.	7.2	-	-	-	-	-	-
Trichosporon spp.	-	14.9	5.0	-	3.9	-	7.7
Acremonium spp.	-	-	2.0	-	1.3	-	-
Other genera	1.7	-	0.9	0.1	0.9	1.0	1.5
unclassified *Hypocreaceae*	3.9	-	-	-	-	-	-
unclassified *Chaetothyriales*	-	-	-	-	-	2.9	-
unclassified *Ascomycota*	-	-	2.7	-	2.2	-	1.4
unclassified Fungi	86.9	85.0	89.2	99.5	91.5	95.4	89.1
Other unclassified	0.4	0.2	0.2	0.4	0.2	0.7	0.3

5.4. Conclusions

Coagulation-flocculation tests were performed to remove particulate matter present in the wastewater with the aim of decreasing its possible toxic effect in the biological treatment. Actiflo® jar test experiments achieved a final turbidity of the clarified water was <1 NTU but no significant DOC removal was observed. Ferric chloride was chosen as coagulant and although a dose of 30 mg/L was found to be appropriate, for further experimentation a dose of 50 mg/L was used to avoid disturbances if wastewater composition changed along operation of the pilot plant. Actiflo® Carb tests achieved a DOC removal proportional to PAC concentration, ranging from 6 to 12%.

Respirometry assays were performed prior to MBBR design and operation to determine biodegradability and/or possible inhibitions of the wastewater with and without pre-treatment and the suitability of MBBR inoculation with urban or industrial acclimated sludge. No short term inhibitions were observed in any of the respirometry assays and Actiflo® pre-treatment was chosen prior to biological process since it offered good turbidity removals and lower use of reagents than Actiflo®Carb for the same respirometric results. Respirometries performed using industrial biomass helped achieving shorter times in the removal of biodegradable compounds. Thus, inoculation of the MBBR pilot system was performed using the acclimated sludge from Repsol WWTP.

The OLR of the MBBR system was progressively increased when using MIX A pre-treated wastewater from 0.20 kg/(m^3 d) –during the start-up– to 3.25 kg/(m^3 d) and HRT from 23.4 to 4.5 h. In steady states, sCOD degradation percentages were maintained between 80 and 90% which implies that only 10-20% of organic matter present in the wastewater mixture was refractory to biological treatment. The calculated maximum sCOD that the system could degrade was 4.96 ± 0.01 kg/(m^3 d) at 23 ± 2°C. When feed wastewater was changed to raw wastewater the sCOD degradation percentages were maintained at 84.5 ± 4.7 % and those percentages were also maintained when feed wastewater effluent proportions were changed to MIX B (81.4 ± 3.1 %). This results indicate that MBBR system is a robust technology able to cope with changes in the wastewater composition. Moreover, Actiflo® pre-treatment could be removed from the wastewater treatment scheme. However, careful monitoring should be performed if this configuration was evaluated in order to determine possible long-term inhibitions.

The analysis of alcohols, chlorinated compounds, organochlorines, aromatic hydrocarbons, polycyclic hydrocarbons, and PCBs performed on day 55 showed that Actiflo® removed some of the compounds while, after the MBBR treatment all analysed compounds were removed >90%, being most of them at concentrations below the detection limit.

Turbidity and TSS in MBBR effluent were slightly dependent on the feeding flow, reaching values of > 100 NTU of turbidity and > 150 mg TSS/L in some periods. These results prove that a further step is needed before reverse osmosis. Ultrafiltration could help decrease TSS and turbidity to values acceptable for RO feeding (i.e. < 1 mg/L and < 1 NTU), likewise, Actiflo® coagulation-flocculation system could be placed after MBBR treatment instead of prior to it. In the design of the future pilot plant, an adaptable system should be installed and Actiflo® should be tested before and after MBBR in a long-term operation.

Higher differences in microbial community composition were detected between attached and suspended biomass rather than between samples of attached biomass along operation; thus, the attached or suspended nature of the biomass had higher influence in the differences of the microbial community structure than the changes in the operational conditions. Moreover, sequencing of microbial species of the system led to the observation of two common genera in all the attached biomass samples. A known biofilm-forming genus, *Haliscomenobacter* spp., and an oil degrading genus *Flavobacterium* spp. were commonly found in the attached biomass analysed. Further differences in microbial community composition were attributed to changes in feed and in operational characteristics although all the genera found were hydrocarbon degraders. *Acinetobacter* spp. was the major bacterial genera found in suspended biomass of the samples taken when maximum OLR was applied. *Proteobacteria* and *Bacteroidetes* were the major phyla detected in the carrier samples while *Proteobacteria* (*Acinetobacter* spp.) the main one in the suspended biomass. Regarding fungal community composition, probably due to a lack of annotated sequences in databases, the major proportion of sequences were categorised as unclassified Fungi. However, known petroleum hydrocarbon degraders such as *Acremonium* spp., *Trichosporon* spp. or *Hypocreaceae* were detected.

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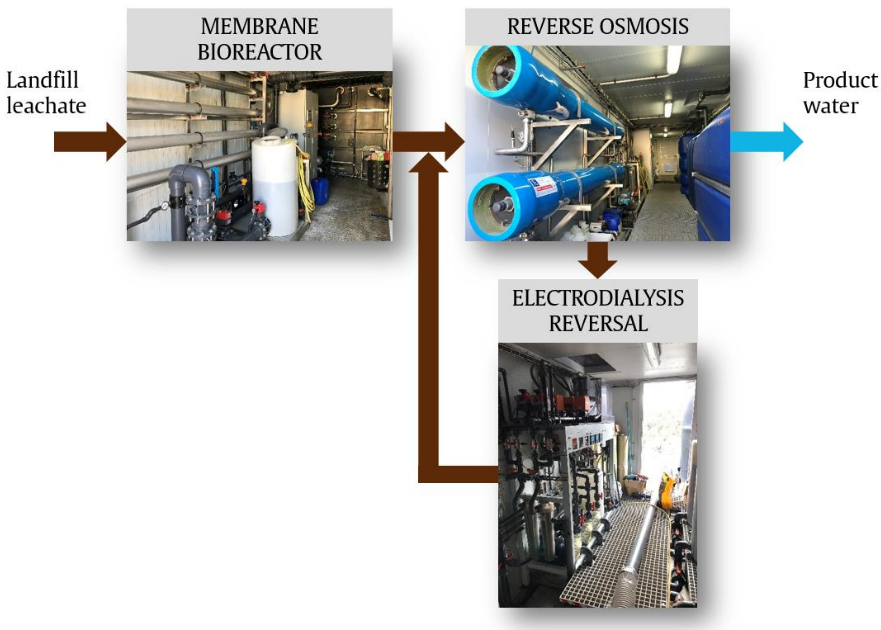
CHAPTER 6

Decreasing environmental impact of landfill leachate treatment by an innovative treatment using end-of-life membranes



Abstract

A prototype pilot plant testing for a novel complete treatment strategy for landfill leachate aimed to decrease its environmental impact was studied. Pre-treatment of leachate was performed by means of a membrane bioreactor (MBR) decreasing inorganic carbon concentration by 92 ± 8% and achieving N removals of 85%. Suspended solids removal in the MBR >99.9% conditioned leachate for the next membrane step. Spiral-would reverse osmosis (RO) regenerated membranes were used to treat the MBR effluent. This RO unit achieved a global recovery of 84% along operation and rejections of >95% for most of the analysed compounds. Since RO permeate did not meet discharge standards, promising results were obtained after a second RO pass was applied. The RO brine produced was further concentrated by an electrodialysis reversal (EDR) unit, achieving an averaged recovery of 67% along operation. Average recovery of the whole pilot plant system was >90%. The reduction of global brine volume together with the use of regenerated membranes are key to the environmental impact of the process and contribute to closing the loop of the circular economy. From Life Cycle Assessment (LCA) analysis performed, it was demonstrated that proposed new treatment had lower environmental impact than conventional treatments currently used in landfill facilities. Concretely, for the nine impact categories evaluated, the proposed treatment presented an average impact reduction of 93% compared to an advanced oxidation system and an average reduction of 26% when compared to a conventional RO treatment.

6.1. Introduction

Current global production of municipal solid waste (MSW) approaches roughly 1.3 billion tonnes per year, and is expected to double to about 2.2 billion tonnes per year by 2025 (Hoornweg and Bhada-Tata, 2012). In 2016, European Union generated $2.5 \cdot 10^{11}$ kg of MSW, 24% of which was managed through landfilling. In southern European countries this percentage was increased up to double or three-fold as is the case of Spain (57%), Croatia (77%) or Greece (82%) (Eurostat, 2018). Landfilling is a widely used process for disposing industrial and municipal solid waste thanks to its low exploitation and capital costs (Renou et al., 2008a). Landfilling inevitably implies the generation of leachate, which is a strongly polluted wastewater. Leachate is produced because of rainwater percolation through the landfill, biochemical processes and water content of wastes themselves. Landfill leachates typically contain high amounts of organic matter, ammonium, heavy metals, chlorinated organic and inorganic salts, although its composition may vary according to the origin and age of the landfill (Alvarez-Vazquez et al., 2004).

Leachates must be treated prior to its discharge into receiving waters and the removal of organic matter and ammonium is always a pre-requisite. Given the complexity of the leachate composition, a combination of technologies is usually required. Nitrogen removal via biological treatment of nitrification/denitrification is a cost-efficient option. However, the toxic nature of the leachate and the presence of refractory compounds can hamper this type of treatment. Besides, the efficiency of denitrification depends on the biodegradability levels of the organic matter contained in the leachate, particularly refractory in mature landfills. Thus, biological treatment of landfill leachate is preferable when treating young leachate since it can grant acceptable performances in terms of chemical oxygen demand (COD) and ammonium removals (Renou et al., 2008a). On the other hand, when treating old leachate or previously biologically treated leachate, physicochemical conventional treatments have been considered adequate. These technologies include air stripping, coagulation, flocculation and settling, which are usually costly regarding initial investment in equipment, energy expenses or chemical consumption (Wiszniowski et al., 2006). Advanced oxidation processes (AOPs) have been proposed and used in the recent years since they offer an effective alternative for mineralization of recalcitrant organics. Nevertheless, high chemical consumption and elevated economic costs hamper their application in full-scale landfill leachate treatment (Renou et al., 2008a). Alternatively, reverse osmosis (RO) has been recently used in landfill leachate treatment since its high rejection performances allow to obtain a high quality treated water effluent that can easily fulfil legal limits for discharge into water bodies (Renou et al., 2008a). Recently, the use of RO and evaporation for on-site treatment of leachate proved to be an efficient solution for pollutant removal and for reducing global environmental impacts (Di Maria et al., 2018). However, RO feasibility depends on the influence of concentrate treatment costs and the feed pre-treatment chosen to decrease membrane fouling (Renou et al., 2008a). On one hand, brine produced in RO is usually unsuitable to be further treated, discharged, or landfilled. The management of this brine, either by incineration or inertization by solidification, represents the greatest economic burden of the process. On the other hand, to avoid membrane fouling, disc tube RO (DT-RO) technology has been proposed for on-site landfill leachate. Compared to the conventional spiral wound modules, the plate-and-frame configuration of the DT module entail a wider feed channel that makes them more resistant to fouling and scaling (Cingolani et al., 2018). Yet, the initial investment required when using DT- RO membranes is still great.

On the other side, conventional spiral-wound RO membranes have been abundantly applied and established in the municipal and industrial sector for freshwater production in desalination of seawater and brackish water. Membranes replaced at the end-of-life stage, together with the continuous growth of RO technologies, derives to a vast accumulation of end-of-life modules that are disposed in landfills (Goh et al., 2016), classified as inert solid waste. It has been reported that in brackish water treatment facilities there is an average replacement of modules between 10-20% per year, depending on the pre-treatment. Additionally, in industrial and tertiary wastewater treatment facilities, the replacement is around 30% per year (Burn et al., 2015). An alternative to landfill management is recycling end-of life membranes by conditioning them for their reuse which can be either indirect or direct. On one hand, indirect reuse implies deconstructing the membrane element and reusing its parts for the assembly of other membranes. On the other hand, direct reuse consists of cleaning the membranes and recovering their commercial properties (Coutinho de Paula and Santos Amaral, 2017). Among other options, it is possible to chemically modify with an oxidative agent the membrane polymeric active layer leading to a membrane with new properties and uses, which is also known as membrane regeneration (Coutinho De Paula et al., 2017; Lawler et al., 2013).

García-Pacheco et al. (2018) used regenerated RO membranes for long-term filtration of brackish water at pilot scale with no performance decline after 4 months. Coutinho de Paula et al. (2018) tested NF regenerated membranes for water river treatment. The authors reported a cost of chemically recycling end-of-line nanofiltration (NF) membranes for a river water treatment of approximately 1.1% of the cost of using a new ultrafiltration (UF) membranes. However, there is no report of RO regenerated membranes being used in landfill leachate treatment.

Therefore, the proposed treatment tested in this work is the combination of a membrane bioreactor (MBR) as a pre-treatment of the landfill leachate, followed by a RO step performed using regenerated RO membranes, which will produce the final treated water of the system. Additionally, an electrodialysis reversal (EDR) unit will be used to treat RO brine stream, to further concentrate it and decrease the final volume of waste produced in the process (Figure 6.1).

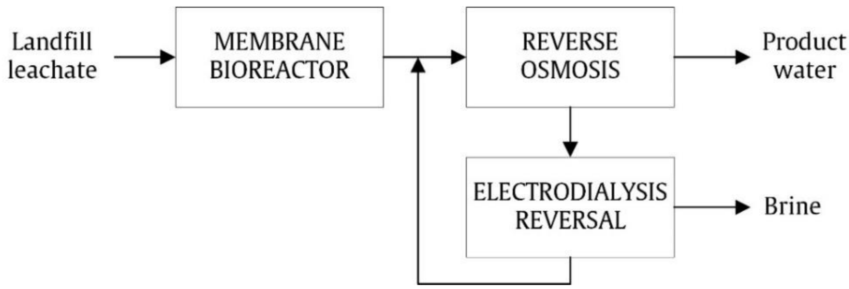


Figure 6.1. Treatment scheme.

Accordingly, the main objective of the present work is to minimise the environmental impact in landfill leachate treatment. Concretely, it will be achieved by increasing the recovery of current membrane-based systems and using tailor-made regenerated membranes. This environmental impact will be determined by means of Life Cycle Analysis (LCA).

6.2. Materials and methods

6.2.1. Leachate composition and inoculum

Leachate used was provided by a municipal solid waste landfill located in Orís (Spain). Its composition is given in Table 6.1. Leachate presented a low BOD_5/sCOD ratio and a relative high nitrogen content, thus, it can be classified as old or mature leachate according to Alvarez-Vazquez et al. (2004). The high variability in nitrate content is explained by the fact that the treated leachate was recycled back to the pond during experimentation.

During first 33 days of operation nitrate content was <1 mg/L, afterwards its concentration increased to 115 ± 91 mg/L due to the nitrification performed in the MBR.

Table 6.1. Landfill leachate characterization.

Parameter	Units	Mean	SD	n
pH	upH	8.4	0.1	10
EC	mS/cm	15.3	1.0	10
TSS	mg/L	918	746	2
VSS	mg/L	672	535	2
sCOD	mg/L	3350	409	3
DOC	mg/L	1240	147	10
TIC	mg/L	661	74.0	9
BOD_5	mg/L	182	110	3
BOD_5/sCOD		0.05	0.03	3
Alkalinity	mg $CaCO_3$/L	3710	440	10
TN	mg/L	555	68.2	9
NH_4^+	mg/L	693	92.7	10
NO_3^-	mg/L	77.0	91.8	9
NO_2^-	mg/L	2.2	1.3	9
PO_4^{3-}	mg/L	38.6	9.1	9
SO_4^{2-}	mg/L	437	51.3	9
Br^-	mg/L	5.5	1.1	9
Cl^-	mg/L	2800	488	8
Ca^{2+}	mg/L	127	29.5	10
Mg^{2+}	mg/L	139	157	10
Na^+	mg/L	1970	267	10
K^+	mg/L	1040	162	10

Inoculum used for MBR consisted in biomass from a partial nitrification bioreactor treating landfill leachate from another location. Main characteristics of inoculum were an electrical conductivity (EC) of 30.7 mS/cm, pH of 6.8, ammonium concentration of 553 mg/L, nitrate concentration of 9.7 mg/L and nitrite concentration of 3480 mg/L.

6.2.2. Experimental setup

The pilot plant scheme is detailed in Figure 6.2. The side-stream MBR consisted of a 23 m³ bioreactor equipped with air diffusers and fed directly from the landfill leachate pond using a feeding pump. The steady state feeding flowrate was set at HRT of 1.92 d, corresponding to the design flowrate of the system (12 m³/d). Dissolved oxygen (DO) in the bioreactor was kept between 2 and 4 mg/L. pH ranged from 6 to 8 without the need of chemical adjustment. The MBR system was completed with two tubular UF membranes ME-C100-12-2995-4.0 (Memos, Germany) connected in series. UF membranes were operated at crossflow mode at a crossflow velocity of 4.8 m/s and flux of 30-50 LMH. Transmembrane pressure (TMP) was kept at <2 bar along operation. Periodical chemical cleanings of the UF membranes were automatically performed every 15 days. The cleanings consisted in the recirculation of a pH 10 NaOH-NaClO solution through the membranes for a 3h period. Membrane rejection was recirculated back to the bioreactor and permeate produced was stored in a 2 m³ tank. Chemical conditioning of the ultrafiltrated leachate prior to RO unit was provided by the dosage of sulphuric acid (50% w/w). RO was operated using two regenerated membranes in series in a continuous mode. RO main operating parameters are detailed in Table 6.2. Periodical acidic and alkaline membrane cleanings were performed to avoid membrane fouling. Permeate produced constituted the final clean water of the system while the concentrate stream, was accumulated in the RO concentrate tank. RO brine was then fed to EDR feeding tanks. EDR stack (PC Cell ED 1000 H)

(PCCell GmbH, Germany) included 50 membranes (300 x 500 mm), 25 cation-exchange (PC SK) and 25 anion-exchange (PC SA) (PCA GmbH, Germany). Each membrane presented an active area of 1050 cm^2 (5.25 m^2 of total membrane area). Platinum-coated titanium electrodes were used. EDR dilute and concentrate streams flow was 250-500 L/h. Every 60 min polarities of electrodes were reversed, switching dilute and concentrated channels. EDR was performed at a current density varying from 14 mA/cm^2 to 17 mA/cm^2 and a potential per cell of 0.5-0.6 V/cell. EDR was operated in a semi-continuous mode by replacing 15% of feeding tanks volume with RO brine when either dilute or concentrate streams reached their target value (45-65 mS/cm for dilute stream and 100-135 mS/cm for concentrate stream). While MBR and RO were operated in a continuous and sequential mode, EDR was not dimensioned to treat the total volume of brine produced in the RO. As described below, global recovery of the system was calculated taking into account the recovery of each element.

Table 6.2. RO operation conditions.

Parameter	Units	Value
Permeate flowrate	L/h	400-500
Rejection flowrate	L/h	120-140
Recovery	%	80
Maximum operating pressure	bar	65
Maximum operating temperature	°C	40

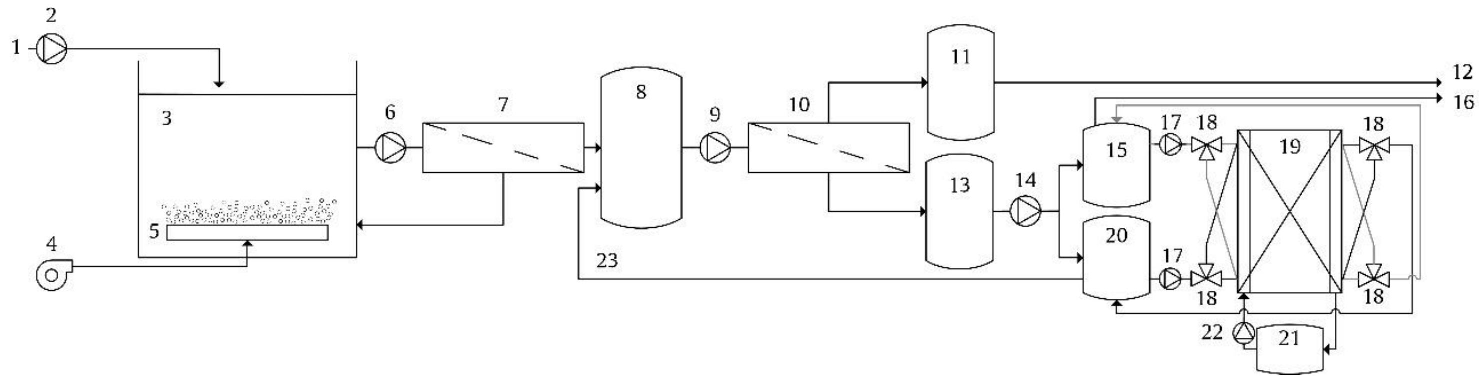


Figure 6.2. Simplified treatment scheme. 1- landfill leachate from storage pond, 2- feed pump, 3- bioreactor, 4- blower, 5- air diffusers, 6- UF feed pump, 7- UF membranes, 8- UF permeate tank, 9- RO feed pump, 10- RO membranes, 11- RO permeate tank, 12- produced water, 13- RO brine tank, 14- pump, 15- EDR concentrate tank, 16- Brine, 17- EDR feed pump, 18- solenoid valves, 19- EDR stack, 20- EDR Dilute tank, 21- Electrolyte tank, 22- electrolyte pump.

6.2.3. RO regenerated membranes

Seawater membranes regenerated were end-of-life DOW FILMTEC™ SW30HR–380 provided by desalination plants located in Spain. RO membranes used in this work were regenerated within Remembrane project (LIFE11 ENV/ES/00626) and regeneration procedure is described elsewhere (Muñoz et al., 2014). Briefly, the process of regeneration was conducted in two steps: hydration and oxidation. The effect of each of the regenerating steps on the membranes was measured with NaCl standard test until a product water flowrate ≥ 800 L/h and a salt rejection ≥ 98% were reached.

6.2.4. Permeate quality improvement tests

6.2.4.1. Ion exchange resins (IER)

The IER capacity of selectively removing nitrate and nitrite from pilot plant permeate was evaluated at lab scale. A single column connected to a peristaltic pump was used. The ion exchange resin used was a strong anionic resin (AMBERLITE IRA458 Cl, DOW) and experimental conditions are detailed in Table 6.3.

Table 6.3. IER experimental conditions.

Parameter	Units	Value
Column diameter	cm	2.5
Column length	cm	20.4
Bed Volume (BV)	mL	100
Flowrate	mL/h	800
Flowrate	BV/h	8
Experimental time	h	6.3

6.2.4.2. Double pass RO

Removal of conductivity, nitrate, nitrite, ammonium and boron from pilot plant permeate was evaluated by testing a double pass RO. That is, permeate produced in the prototype was further filtered by another RO membrane. A regenerated membrane from the same regeneration batch used in the pilot plant was used. Two fluxes (32 and 26 LMH) and two recoveries (68.5% and 62.5%)) were tested in a single 8″ module RO pilot plant.

6.2.5. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), pH, electrical conductivity (EC), alkalinity, soluble chemical oxygen demand (sCOD), biological oxygen demand (BOD_5), total phosphorus (TP), total kjeldahl nitrogen (TKN), oil and grease (O&G), anionic surfactants, total cyanide (CN_t), phenolic compounds and turbidity were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Major anions and cations were analysed using ICS 2100 (Dionex). Metals analysis were performed using ICP MS 7500CX (Agilent Technologies) equipment. Dissolved organic carbon (DOC), total inorganic carbon (TIC) and total nitrogen (TN) concentrations were measured using analyser C/N 3100 (Analytik Jena). Aldehydes were measured by HPLC-UV and chlorinated pesticides were analysed by HRGC/MS. Adsorbable organic halides (AOX) were measured according to ISO 9562:2004. Toxicity was analysed according to UNE EN ISO 11348-3:2009. *E. coli* was determined as established in UNE-EN ISO 9308-1:2001. *Legionella* spp. were determined as established in UNE-EN ISO 11731:2007. Intestinal nematode eggs were determined by microscopic observation.

6.2.6. Data analysis

Recovery rate (%) for each of the treatment steps was calculated as follows:

$$Recovery\ (\%) = \frac{V_p}{V_i} \times 100 \tag{6.1}$$

where V_p and V_i are the volumes of the permeate and the corresponding feeding streams. Recovery of the whole system was calculated taking into account the recoveries of each of the treatment steps. RO performances were also evaluated by the removal of salt content. RO rejection was calculated according to:

$$Salt\ rejection\ (\%) = \left(1 - \frac{C_p}{C_i}\right) \times 100 \tag{6.2}$$

where C_p and C_i are the ion concentrations (mg/L) in the permeate and the corresponding feeding stream. RO flow normalization followed the equation extracted from DOW FILMTEC™ Reverse Osmosis Membranes Technical Manual (Dow Water & Process Solutions, 2016):

$$Q_s = \frac{P_{f_s} - \frac{\Delta P_s}{2} - P_{p_s} - \pi_{fc_s}}{P_{f_o} - \frac{\Delta P_o}{2} - P_{p_o} - \pi_{fc_o}} \cdot \frac{TCF_s}{TCF_o} \cdot Q_o \tag{6.3}$$

where P_f is the feed pressure, ΔP is device pressure drop, P_p is the product pressure, π_{fc} is the osmotic pressure of the feed-concentrate mixture, TCF is the temperature correction factor, Q is the product flow and subscripts s and o correspond to standard and operation conditions respectively. EDR performance was evaluated in terms of concentration factor, by the following equation:

$$Concentration\ factor = \frac{C_c}{C_i} \tag{6.4}$$

where C_c and C_i are the ion concentrations (mg/L) in the concentrate and the corresponding feeding stream.

6.2.7. Life cycle assessment (LCA)

LCA model developed analysed the environmental sustainability of the proposed process, including different scenarios. In addition, the comparison of the proposed treatment in terms of environmental impact against the current existing competing technologies was also evaluated. LCA was performed according to ILCD Handbook guidelines (EC et al., 2010) as well as UNE-EN ISO 14040:2006 (AENOR, 2006a), UNE-EN ISO 14044:2006 (AENOR, 2006b) and ISO 15686-5:2017 (ISO, 2017) standards. Calculations were carried out using SimaPro Developer v8.5.2.0. through ReCIPE 2016 Midpoint (H) method. The model was constructed using the information compiled from the technical work performed; hence, primary data were collected directly from the source.

The functional unit (FU) was defined as the treatment of 1 m^3 of leachate that met the quality standards to be discharged into water bodies or either sent to a WWTP. Processes examined under the LCA scope included the treatment of the leachate itself (pilot plant) as well as all the necessary additional processes to discharge the treated effluent to natural water bodies. The LCA study comprised the three main treatment stages: MBR, RO and EDR.

The environmental impact categories were selected based on the ReCIPE 2016 Midpoint (H) method and, for the total energy demand quantification, the CED method (2014, v1.10) was also used. Impact categories selected included: global warming (GW), stratospheric ozone depletion (OD), human non-carcinogenic toxicity (HT-NC), human carcinogenic toxicity (HT-C), fine particulate matter formation (PM), freshwater eutrophication (FE), freshwater ecotoxicity (FET), fossil resource scarcity (FS) and cumulative energy demand (CED).

The proposed treatment (PT) scheme was evaluated and compared to current landfill treatment strategies in two landfill facilities. AOPs were used for leachate treatment in the existing MSW landfill located in Consorci del Bages per la Gestió de Residus (CBGR) (Manresa, Spain) which is operating since 1999. RO was used for leachate treatment in the MSW landfill located in Orís (Spain) which is operating since 1995.

- **Proposed treatment** (PT). This configuration represented the impact of treating landfill leachate at the pilot plant described before (Figure 6.2) with the addition of a second RO pass to improve permeate quality (Figure 6.3). In this scheme, as can be seen in Figure 6.2, processes considered were MBR, double pass RO and EDR.
- **Advanced oxidation processes** (AOP). Steps involved in this configuration included an oxidation reactor, decantation and dehydration of sludge (Figure 6.3). The analysis included the use of chemicals in the oxidation reactor stage as well as energy and oil consumptions. Process outputs comprised ammonium sulphate, which was treated externally, treated water, which was discharged to a WWTP pipeline, and sludge that was sent to external management.
- **Reverse osmosis** (RO). In this configuration treatment was based on plate-and-frame RO membrane filtration system (Figure 6.3). Leachate pumped from leachate pond entered the filtration system where solids, suspended matter and sedimentary particles, up to a certain grain size, were separated in a pre-treatment. Pre-treated leachate underwent a RO step to produce the treated water (permeate) and a brine stream which needed to be further managed at other facilities. Chemicals for membrane cleaning and energy consumption were taken into account for the analysis.

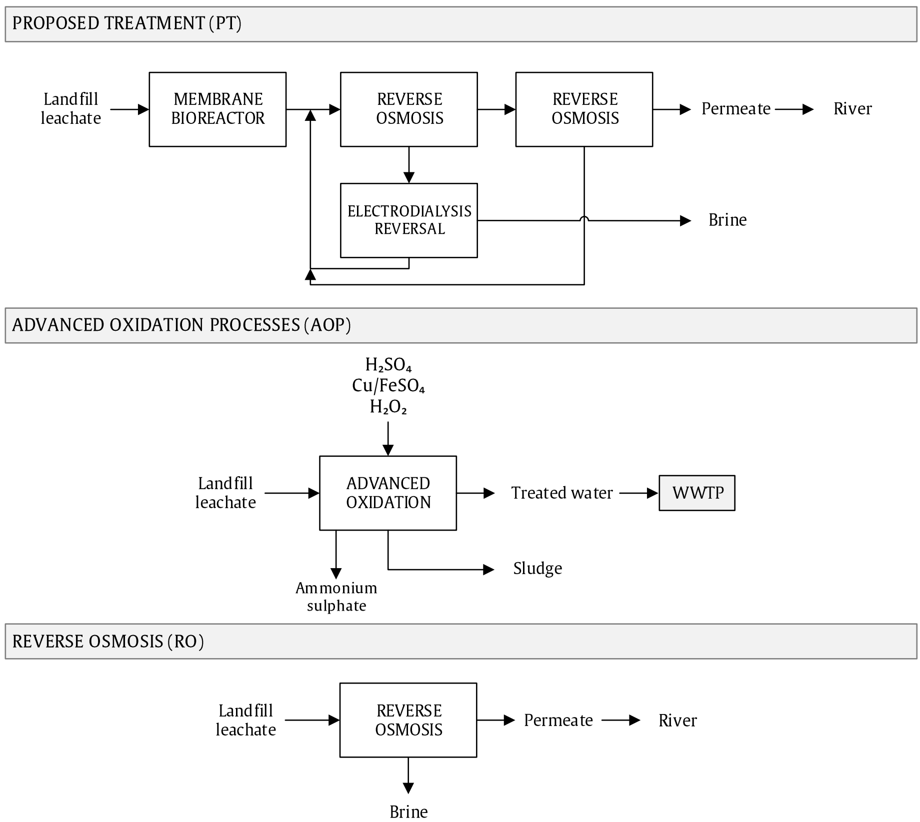


Figure 6.3. Scheme of the on-site treatment system for the proposed treatment (PT), advanced oxidation processes (AOP) and reverse osmosis (RO)

6.3. Results and discussion

6.3.1. MBR pre-treatment

MBR treated a total volume of 1300 m³ landfill leachate along the 146 days of operation, obtaining 1250 m³ of permeate. Average organic loading rate (OLR) of the operation was 1.3 ± 0.2 kg COD/(m³ d), starting with a period of 0.7 ± 0.1 kg COD/(m³ d) and reaching a maximum value of 1.7 ± 0.2 kg COD/(m³ d) corresponding to a HRT of 1.92 d. The OLR range was close to that used by Hashisho et al. (2015), who operated a MBR treating stabilized leachate at OLR fluctuating between 0.94 and 1.87 kg COD/(m³ d). Hashisho et al. (2015) reviewed MBR technology for leachate treatment reporting HRT of 0.5 to 9 d, thus, the present work was conducted at a relatively low HRT compared to literature.

Removal of ammonium and TIC through nitrification process in the MBR was achieved from the beginning of the operation, reaching removal percentages of 83 ± 13% for NH_4^+ and 92 ± 8% for TIC (Figure 6.4). Thus, the limiting factor for the complete removal of ammonium was the lack TIC concentration. From day 80 of operation, nitrogen removal was detected reaching a maximum value of 85% by the end of operation. Nitrogen removal was probably due to denitrification and nitrification taking place in the reactor. This period corresponded to a phase in which DO probe was not working properly and DO was not correctly measured. This fact, together with the possibility of not having a perfect mixture in the reactor, could have led to anoxic zones in the reactor in which denitrification could have taken place.

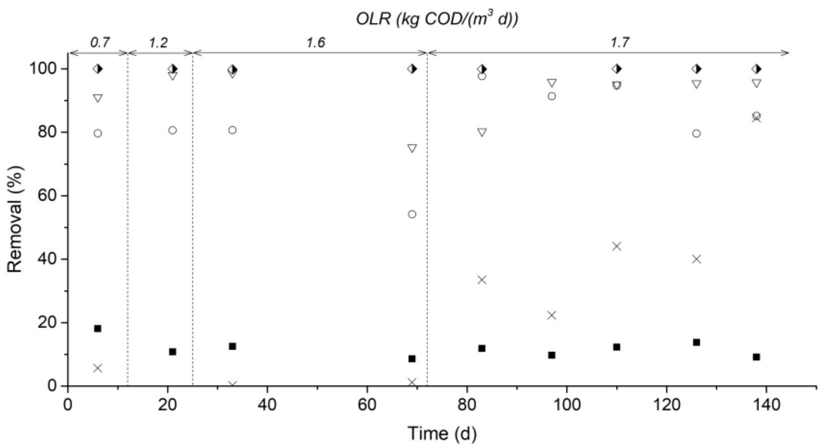


Figure 6.4. Leachate pre-treatment performance in MBR along operation of the pilot plant: decrease of electrical conductivity (■), removals of NH_4^+ (○), N (x), TIC (▽) and TSS (◆). OLR (kg/(m³ d)) are indicated above.

pH and EC decreased after the MBR as a result of alkalinity consumption during nitrification (Levlin, 2010), with effluent values of 6.5 ± 0.7 upH and 14.0 ± 0.8 mS/cm. Recovery of the MBR process was up to 94% and TSS removal through the membrane step was >99.9% and permeate produced presented turbidity values of 1.0 ± 0.8 NTU which was a suitable value to feed RO membranes. Acidic pH achieved after MBR decreased the amount of acid needed to condition the effluent prior to RO step in order to avoid membrane scaling. Moreover, complete removal of TIC prior to membranes was crucial to avoid further scaling issues in the RO membrane due to carbonate salts.

Membrane flux was kept between 30 and 50 LMH along operation, preserving it at a flux of 35-40 LMH most of the time (Figure 6.5). Although Figure 6.5 shows an increasing tendency of TMP, it was maintained at <2 bar thanks to the periodical cleanings performed. Thus, ultrafiltration stage of the MBR was held under appropriate filtration conditions and no major issues were detected.

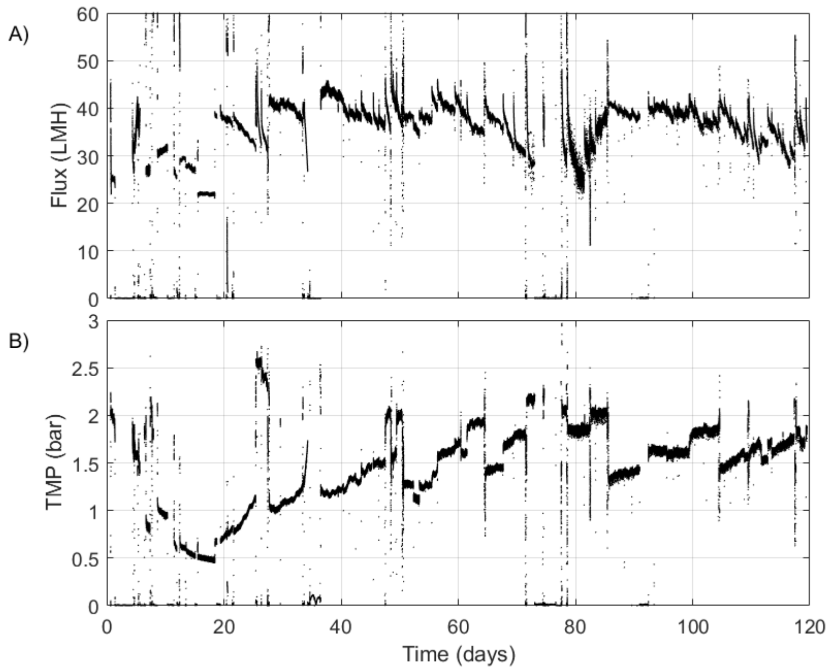


Figure 6.5. Flux (A) and transmembrane pressure (B) of the UF membrane of the MBR along operation.

6.3.2. Reverse osmosis

RO filtration was performed at a normalized flux of 3.4–4.7 LMH and at a pressure varying from 20 to 55 bar. Global recovery of the RO step was around 84% generating a total volume of brine stream of 165.5 m^3 from the 1020 m^3 leachate treated in the RO step. The averaged brine stream flowrate was 1.2 m^3/d. Normalized permeate flow (Figure 6.6) showed a steady tendency although start-stop cycles hinder to see the trend. Pressure drop was kept constant until approximately day 80 (Figure 6.6). However, from there on, pressure drop slightly increased mainly due to increased TDS in RO feed. This increased TDS in RO feed was due to the contribution of EDR dilute stream. Thus, increased TDS increased membrane fouling tendency as observed from increased pressure drop, although it did not hamper the preservation of the normalized permeate flow.

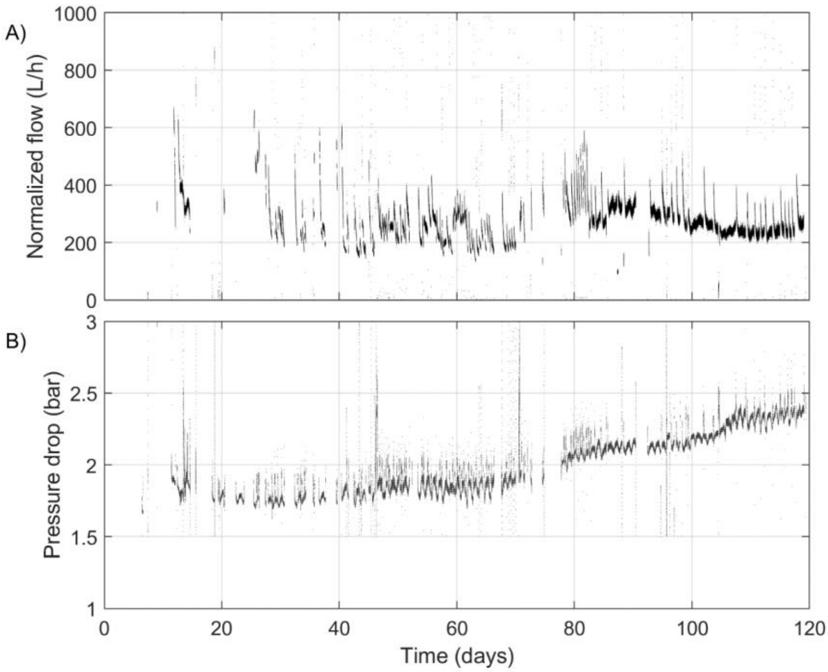


Figure 6.6. RO normalized permeate flow (A) and pressure drop (B).

Most compounds presented rejections >95% during operation (Figure 6.7). Several compounds presented lower rejection percentages. Ammonium was rejected in a 93 ± 5%, due to its low molecular weight. Nitrate and nitrite presented rejections of 94 ± 3% and 89 ± 5% respectively, which could be due to its low degree of hydration (Dow Water & Process Solutions, 2016). Cingolani et al. (2018) also reported nitrate and nitrite values above discharge standards when treating landfill leachate using RO plate and frame membranes. TIC (65 ± 20%) presented lower rejections along with boron (67 ± 13%) given that at working pH of 6, boron is in its not ionized form (boric acid) and RO efficiency is much better at removing ionized ions (Öztürk et al., 2008).

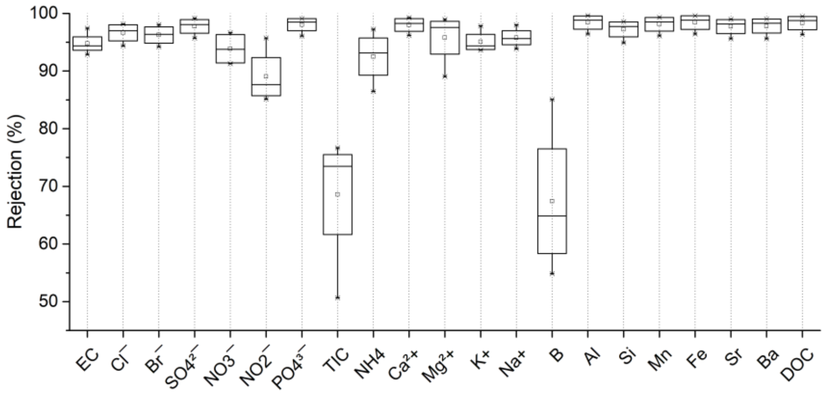


Figure 6.7. BoxPlot of compound rejection percentages of RO treatment.

Main characteristics of RO permeate obtained during pilot plant operation as well as surface water bodies discharge limits for the site are detailed in Table 6.4. It can be seen that permeate quality did not meet standards in terms of electrical conductivity, nitrate, nitrite, ammonium and boron concentrations in some cases. As previously shown in Figure 6.7, membrane rejection for these compounds was low and it was reflected in permeate quality. Nitrate and nitrite high concentration in RO feed along with their lower membrane rejection led to values over discharge limits in the permeate. Several strategies could be applied to meet standard water quality for its discharge. Optimisation of the pre-treatment step with a complete nitrification-denitrification process could improve nitrogen removal and avoid those compounds in the final product water. Given that TIC was the limiting factor for complete nitrification, dosing stoichiometric amount of carbonates into the MBR would favour the complete nitrification of ammonium. Also, the addition of easily biodegradable organic matter into the bioreactor would favour the denitrification process and thus, the nitrogen removal. In their work, Peng et al. (2018) proved the increased nitrogen removal efficiency when using solid digestates in landfill bioreactors as organic matter for the denitrification phase. Instead, partial nitrification could also be employed, thus decreasing the amount of inorganic carbon and organic matter required for the biological process. Alternatively, Sharon-Anammox process could be used for nitrogen removal, avoiding the need of organic matter.

Table 6.4. Pilot plant effluent characteristics, limit values for its disposal and characteristics of post-treatment of the pilot plant effluent by IER and RO. In bold, values above discharge limits.

Parameter	Site discharge limits [a]	Pilot plant RO permeate (av., min.-max.)	IER effluent treating RO permeate	2nd stage RO treating pilot plant RO permeate	
				2nd stage RO permeate	2nd stage RO rejection (%)
pH (upH)	5.5-9.5	6.0 (3.6-6.5)	11.9	6.3	-
EC (mS/cm)	2	1.8 (0.9-2.6)	2.36	0.14	90%
DOC (mg/L)	-	49.6 (10.3-99.3)	-	-	-
Cl^- (mg/L)	750	272 (114-436)	-	22.6	90%
Br^- (mg/L)	-	0.7 (<0.2-1.0)	-	-	-
SO_4^{2-} (mg/L)	750	40.9 (19.1-92.3)	-	1.2	94%
NO_3^- (mg/L)	10	297 (<0.2-527)	<0.2	29.9	88%
NO_2^- (mg/L)	0.5	94.2 (1.1-367)	<0.2	1.2	86%
TKN (mg/L)	20	-	-	< 3	-
F^- (mg/L)	6	-	-	< 0.2	-
PO_4^{3-} (mg/L)	10	2.5 (0.8-4.8)	-	< 0.2	>87%
TP (mg/L)	10	-	-	< 0.15	-
TIC (mg/L)	-	2.2 (<1-2.7)	-	-	-
NH_4^+ (mg/L)	15	15.7 (5.5-36.4)	-	1.2	85%
Ca^{2+} (mg/L)	-	4.7 (1.7-8.4)	-	-	-
Mg^{2+} (mg/L)	-	8.5 (1.3-18.4)	-	0.5	87%

Parameter	Site discharge limits [a]	Pilot plant RO permeate (av., min.-max.)	IER effluent treating RO permeate	2nd stage RO treating pilot plant RO permeate	
				2nd stage RO permeate	2nd stage RO rejection (%)
K^+ (mg/L)	-	132 (54-159)	-	9.1	90%
Na^+ (mg/L)	-	231 (107-367)	-	19.3	90%
B (µg/L)	2000	1530 (730-2100)	980	580	61%
Al (µg/L)	-	40.8 (8-93)	-	-	-
Si (µg/L)	-	1380 (690-2400)	-	-	-
Mn (µg/L)	2000	14.9 (3.5-30)	< 1.0	0.22	97%
Fe (µg/L)	2000	122 (38-250)	< 5.0	3.7	95%
Sr (µg/L)	-	83.5 (46-150)	-	-	-
Ba (µg/L)	20000	17.1 (5-33)	11.5	1.3	91%
Sb (µg/L)	30	-	< 1.0	0.17	94%
As (µg/L)	500	-	< 2.0	0.28	96%
Cd (µg/L)	2	-	< 1.0	< 0.1	-
Cu (µg/L)	50	-	< 5.0	< 0.5	>72%
Cr (µg/L)	500	-	< 1.0	0.9	96%
Cr (VI) (µg/L)	50	-	-	< 50	-
Sn (µg/L)	500	-	< 1.0	0.22	95%
Hg (µg/L)	0.35	-	< 2.0	< 0.2	-
Ni (µg/L)	340	-	< 1.0	0.46	95%
Pb (µg/L)	140	-	< 1.0	< 0.1	>80%
Se (µg/L)	10	-	< 5.0	< 5	-
Zn (µg/L)	300	-	< 10.0	< 0.5	96%
TSS (mg/L)	35	-	-	< 0.3	-
BOD_5 (mg/L)	40	-	-	5	-

Parameter	Site discharge limits [a]	Pilot plant RO permeate (av., min.-max.)	IER effluent treating RO permeate	2nd stage RO treating pilot plant RO permeate	
				2nd stage RO permeate	2nd stage RO rejection (%)
COD (mg/L)	125	-	-	< 50	-
O&G (mg/L)	10	-	-	< 10	-
Anionic surfactants (mg/L)	2	-	-	< 0.06	-
Toxicity (equitox)	5	-	-	< 4	-
CN_{total} (mg/L)	0.4	-	-	< 0.015	-
Phenolic compounds (µg/L)	500	-	-	< 0.03	-
AOX (mg/L)	0.2	-	-	<0.15	-
Aldehydes (µg/L)	1000	-	-	< 10	-
Pesticides [b] (µg/L)	50	-	-	< 0.65	-

[a] According to the environmental authorisation of the landfill.

[b] Including: alachlor, atrazine, chlorfenvinphos, chlorpyrifos, aldrin, dieldrin, endrin, isodrin, DDT total, p,p-DDT, DCMU, endosulfan, hexachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane, isoproturon, simazine, trifluralin.

In order to achieve the discharge limits, IER and a second pass for the RO were considered. IER was considered a viable option since, given their high retention capacity (≥ 1 eq/L), the amount of IER required would be minor. Thus, an IER test was performed with the permeate obtained from the pilot plant. IER effluent composition is detailed in Table 6.4. IER was capable of retaining nitrate and nitrite below discharge limits. Trace metal concentration was also slightly reduced, with a boron concentration decrease of 35%. However, it came at an increase of pH (11.9) and conductivity (2.36 mS/cm) above discharge limits. Thus, IER post-treatment of the permeate was discarded.

Alternatively, a second RO step was considered adequate to improve permeate quality. Four tests were performed on pilot plant RO permeate, main working conditions and conductivity rejection are described in Table 6.5. Major salt rejection was obtained in Test 4 (25.7 LMH and 62.5% recovery) and permeate obtained in this test was characterised concerning parameters included in discharge standards (Table 6.4). Second stage RO permeate met all discharge standards but nitrate and nitrite, whose rejections were 88% and 86% respectively. As detected during pilot plant operation, regenerated membranes presented low rejections for nitrate, nitrite and boron, and this was also found in second stage RO. Although boron concentration was kept below limits, its rejection was 61%. Thus, a second RO stage was a promising solution to meet discharge standards if the biological system was improved.

Table 6.5. Second RO pass working conditions and main result.

	Flux (LMH)	**Recovery (%)**	**TMP (bar)**	**Temp. (°C)**	**Feed EC (µS/cm)**	**Permeate EC (µS/cm)**	**EC rejection (%)**
Test 1	31.7	68.5%	49	23.3	1390	185	86.7%
Test 2	31.7	62.7%	47	24.9	1390	134	90.4%
Test 3	25.7	68.2%	36	26.3	1390	150	89.2%
Test 4	25.7	62.5%	35	26.4	1390	119	91.5%

Second stage RO permeate composition was also compared to the Spanish standards for water reuse (RD 1620/2007, 2007) considering the following industrial reclaimed water uses: process and cleaning water, and cooling towers and condensers (Table 6.6). Water obtained with the pilot plant and a second RO stage could be used within landfill facilities decreasing water consumption of the landfill and contributing to close the water circular economy loop.

Table 6.6. Limits of RD 1620/2007 and 2nd stage RO permeate composition.

Parameter	RD 1620/2007 limit values according to reclaimed water use		2nd stage RO permeate
	Process and cleaning water	**Cooling towers and condensers**	
Nematode eggs (eggs/10L)	-	1	< 1
E. coli **(cfu/100mL)**	10	absence	absence
Legionella **ssp.** (cfu/L)	100	absence	< 70
Turbidity (NTU)	15	1	1.2

6.3.3. Electrodialysis reversal

EDR produced 18.2 m^3 of concentrate stream, with an average flowrate of 0.15 m^3/d, and 0.3 m^3/d for dilute stream, resulting in an average recovery of 67%. EDR working conditions permitted a smooth operation of the stack showing no increase in impedance, that is no increase in resistance was detected during operation (Figure 6.8). Thus, the EDR step of the process was operated under stable conditions throughout the experimentation.

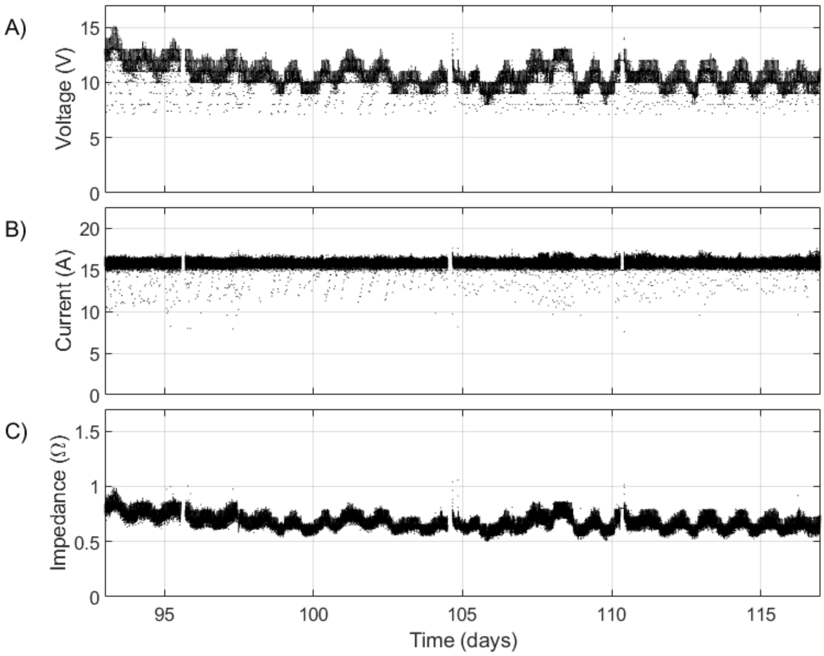


Figure 6.8. Voltage (A), Current (B) and Impedance (C) from a representative period of EDR operation in the pilot plant.

The global brine volume decrease achieved, with the EDR recovery of 67%, reduced the final amount of leachate to be further managed by incineration or inertization. An EDR dilute stream with a conductivity less than or equal to the conductivity of the RO feed was achieved and could be returned to RO feed tank to increase the global recovery of the system. Dilute stream conductivity ranged from 30 up to 65 mS/cm and concentrate stream conductivity ranged from 100 to 135 mS/cm. The concentration factor was determined for main compounds during continuous operation. Compounds whose concentration factor was >1 are represented in Figure 6.9. Concentration factor average between 1.1 and 1.9 was achieved for Mn, Mg^{2+}, Na^+, K^+, Cl^-, Br^- and NH_4^+ (from highest to lowest) while concentration factor >2 was achieved for Ca^{2+}, Sr, Ba, NO_3^- and NO_2^- (from highest to lowest). Concentration factor of SO_4^{2-}, PO_4^{3-}, B, Al, Si and Fe was <1.1, meaning those compounds were not efficiently removed from dilute stream by EDR process. High variability in the concentration factor for nitrite was due to high variability in RO brine stream, which in turn was due to the performance of the bioreactor. Main characteristics of concentrate stream produced are detailed in Table 6.7.

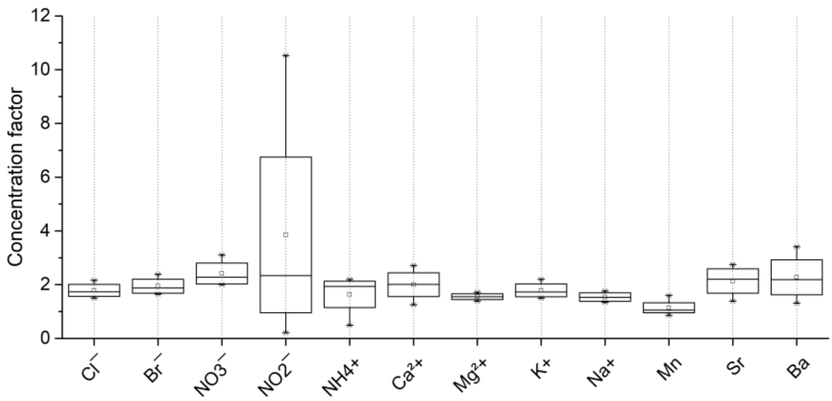


Figure 6.9. BoxPlot of concentration factor >1 of main compounds in EDR.

Table 6.7. EDR concentrate stream characteristics.

Parameter	Units	Value (av. ± SD)
pH		5.9 ± 0.4
Cond	mS/cm	103 ± 22.6
Cl^-	mg/L	29100 ± 7610
Br^-	mg/L	58.6 ± 23.4
SO_4^{2-}	mg/L	3160 ± 731
NO_3^-	mg/L	13200 ± 8930
NO_2^-	mg/L	2210 ± 3930
PO_4^{3-}	mg/L	223 ± 90.5
TIC	mg/L	6.1 ± 3.2
NH_4^+	mg/L	595 ± 294

Parameter	Units	Value (av. ± SD)
Ca^{2+}	mg/L	924 ± 101
Mg^{2+}	mg/L	689 ± 177
K^{+}	mg/L	9970 ± 2590
Na^{+}	mg/L	17900 ± 4890
B	µg/L	7500 ± 1890
Al	µg/L	3450 ± 1570
Si	µg/L	79800 ± 24500
Mn	µg/L	1740 ± 247
Fe	µg/L	12000 ± 3960
Sr	µg/L	16000 ± 3480
Ba	µg/L	3440 ± 1030
DOC	mg/l	5490 ± 2570

6.3.4. Treatment overview

Recovery obtained in MBR stage was around 94%, mainly due to operational challenges faced during operation. As discussed before, RO presented an average recovery of 84% while EDR recovery was 67%. According to these results, average recovery of the whole pilot plant system could be as high as >93% (Figure 6.10). A similar recovery was obtained by Cingolani et al. (2018) who studied a triple-stage DT-RO as tertiary treatment for landfill leachate observing a recovery rate higher than 90% in the first two stages. However, a third stage had to be added to meet permeate nitrate and boron discharge standards decreasing the final recovery. In their work, Renou et al. (2008b) tested a leachate treatment scheme consisting in lime precipitation followed by a pre-filtration prior to RO at pilot scale. In their work, the process combination would make it possible to reach global conversion rates close to 90%. It was difficult to compare the obtained global recovery with other hybrid systems since most of the studies reviewed do not report global recovery values. Wang et al. (2014) presented an integrated system anoxic/aerobic granular active carbon assisted membrane bioreactors (A/O-GAC-MBR) coupled to NF and RO presenting treated water meeting industrial reutilization standards but there is no mention of the global recovery of the system in their work. Another hybrid system was tested at lab scale by Zhang et al. (2013) in which the combined process Fenton–SMBR–RO provided high quality effluent meeting water reuse standards and presented low membrane fouling. The authors did not report global recovery for that system. On the other hand, Abood et al. (2014) also presented a combined leachate treatment system of agitation-coagulation-SBR and filtration which effectively eliminated pollutant loading from landfill leachate although global recovery of their system was not mentioned. Thus, combined processes show promising results for landfill leachate treatment. Concretely, the treatment scheme presented in this work exhibits encouraging results according to the high recovery obtained.

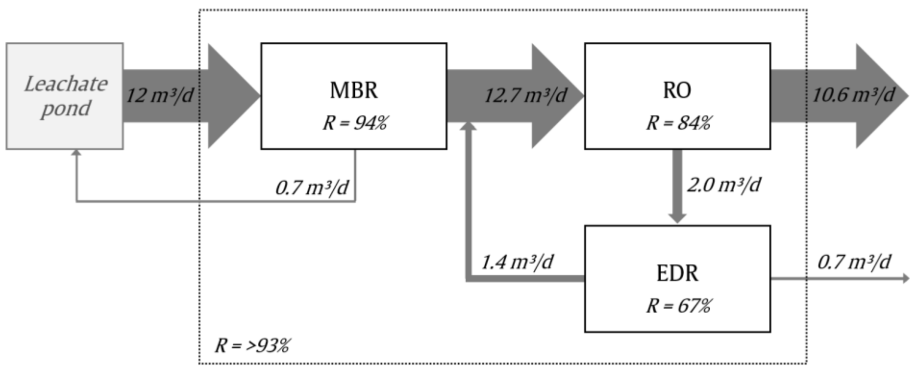


Figure 6.10. Average partial and global recoveries of the pilot plant.

6.3.5. Life cycle assessment

6.3.5.1. Proposed new treatment scheme

The analysis of the proposed treatment prototype was performed including five subcategories: auxiliary equipment (i.e. piping, electrical box, maritime tanks), MBR (module 1), RO + EDR (module 2), chemicals used and sludge management. Modules 1 and 2 included capital goods and energy consumption for each category. Brine treatment environmental impact was based on a stabilization process using lime while permeate treatment was based on a treatment of the permeate in a conventional WWTP.

Within the prototype, the highest impact was derived from modules 1 and 2 in all impact categories (Table 6.8). Concretely, module 1 contributed to 50-67% of the impact, while module 2 contribution ranged from 25% to 32%, depending on the impact category. Thus, module 1 and 2 together contributed to >75% in FE, FET, HT-C and HT-NC categories while in the case of GW, OD, PM, FS and CED categories, contributed to >94%. These results are mainly due to energy consumption of both modules. Concretely, module 1 contribution to energy consumption accounted for 66% and module 2 for 31%. In a secondary level, auxiliary equipment was the third contributor to overall impact, with values ranging from 1% as is the case of CED to 22% in the case of HT-C.

Regarding the evaluation of the impact of the whole system, including leachate treatment (prototype), permeate and brine treatments; the highest contributor to environmental impact was the prototype. Leachate treatment in the prototype represented an average impact of 84%. Impact categories that had higher relevance in leachate treatment (>94%) were GW, PM, FET, FS and CED. Nevertheless, permeate treatment was also relatively relevant (16% in average) and especially significant in OD, FE and HT-NC impact categories, in which its contribution was 47%, 47% and 27%

respectively. Brine treatment presented the lowest contribution to the environmental impact categories (<1.3%). This low contribution was ascribed to the high recovery of the system and thus, to the low volume of brine generated. Therefore, permeate and brine post-treatments had a low impact in the whole analysis given that leachate treatment was the main contributor in all categories.

Table 6.8. Proposed treatment environmental impact.

Impact category	Units	Prototype						Brine treatment	Permeate treatment	TOTAL
		Auxiliary Equipment	MBR	RO and EDR	Chemicals	Sludge Management	Prototype Subtotal			
GW	kg CO2 eq	3.54E-01	1.10E+01	5.18E+00	6.11E-02	2.17E-03	1.65E+01	2.22E-01	4.01E-01	1.71E+01
OD	kg CFC11 eq	1.33E-07	5.08E-06	2.40E-06	4.74E-08	3.38E-09	7.66E-06	4.28E-08	6.89E-06	1.46E-05
PM	kg PM2.5 eq	1.24E-03	2.03E-02	9.50E-03	1.30E-04	1.77E-06	3.11E-02	1.04E-04	5.62E-04	3.18E-02
FE	kg P eq	2.35E-04	3.04E-03	1.43E-03	4.69E-05	8.24E-07	4.75E-03	8.74E-06	4.21E-03	8.97E-03
FET	kg 1,4-DCB eq	3.31E-02	2.01E-01	9.46E-02	2.89E-03	8.93E-05	3.32E-01	9.48E-04	1.88E-02	3.52E-01
HT-C	kg 1,4-DBC eq	1.13E-01	2.58E-01	1.30E-01	1.31E-02	1.19E-04	5.14E-01	1.69E-03	6.10E-02	5.76E-01
HT-NC	kg 1,4-DBC eq	4.12E+01	1.28E+02	6.09E+01	3.24E+00	4.65E+00	2.39E+02	1.27E+00	8.95E+01	3.30E+02
FS	kg oil eq	8.11E-02	2.92E+00	1.40E+00	2.10E-02	7.79E-04	4.41E+00	3.48E-02	4.82E-02	4.49E+00
CED	MJ	2.84E+00	2.07E+02	9.73E+01	8.03E-01	2.38E-02	3.08E+02	1.71E+00	3.15E+00	3.13E+02

6.3.5.2. Comparison between PT, AOP and RO

Proposed treatment environmental impact was compared to AOP and RO configurations (Figure 6.11). When PT was compared to AOP treatment, a clear impact decrease was observed, with an average reduction of 93%. Main impacts of AOP analysis are related to chemical and energy consumption during leachate treatment. Therefore, clear environmental impact reduction over AOP technology could be achieved by implementing the innovative proposed treatment. On the other hand, when PT was compared with RO, environmental impact reduction was also achieved although in a lower percentage. Average impact reduction of 26% was calculated for PT over RO configuration. PT showed lower environmental impact than RO in all the impact categories (with values from 25 to 49%) except for OD and FE. Regarding OD, PT presented an environmental impact 2% higher than RO. In addition, FE in PT was 26% higher than in RO case. Considering that in FE category permeate treatment had a great relevance, its higher impact in the PT could be explained by the high recovery of PT process and thus the high amount of permeate generated.

Energy requirement was the main contributor to global impact in the three studied configurations. According to that, lower environmental impact in PT configuration was linked to its lower energy consumption. Concretely, energy requirement of PT was 7% lower than RO and 91% lower than AOP. Moreover, it is worth mentioning that PT reduced 91% of CO_2 emissions when compared to AOP and 3.2% when compared to RO configuration. Thus, promising results aroused from the LCA supporting the implementation of the proposed treatment in terms of decreasing the environmental impact of landfill leachate treatment.

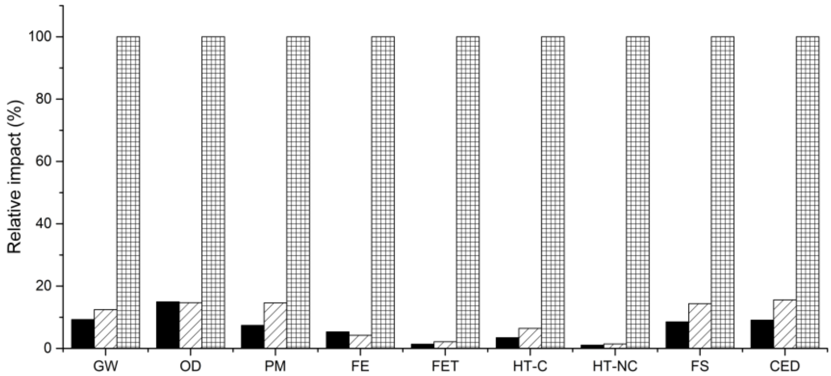


Figure 6.11. Impact analysis of proposed treatment (black), RO (lined pattern) and AOP (crossed pattern) configurations.

6.4. Conclusions

Total removal of inorganic carbon in the biological treatment was achieved by removing the stoichiometric ammonium concentration in the nitrification process in the MBR treatment. Nitrogen removal, by the complete nitrification-denitrification processes, achieved a percentage of 85% at the end of operation. Improvements of these processes could include, on one hand, the addition of an inorganic carbon source to allow the complete ammonium removal and, on the other hand, the addition of easily biodegradable organic matter such as an organic waste to enhance the denitrification process. Instead, partial nitrification could also be employed, thus decreasing the amount of inorganic carbon and organic matter required for the biological process. Alternatively, Sharon-Anammox process could be used for nitrogen removal, avoiding the need of organic matter.

Regenerated spiral-wound RO membranes were capable of treating landfill leachate with a recovery above 80% and a salt rejection above 90%. Thus, waste that would be landfilled was transformed into a reusable resource closing the circular economy loop. In order to release the permeate obtained to natural water bodies, an extra refining step, such as a second RO pass was required. EDR technology in semi-continuous mode was proven viable for RO brine further concentration, reaching 67% of recovery in this stage of the treatment scheme. Thus, when using the proposed new treatment scheme, a global recovery of above 90% was achieved, meaning only less than 10% of the original leachate volume would have to be managed as a residue later.

From LCA analysis, it was demonstrated that proposed new treatment had a lower environmental impact than conventional treatments currently used in landfill facilities (RO and AOP). Concretely, for the nine impact categories evaluated, the proposed treatment presented an average impact reduction of 93% compared to an advanced oxidation system and an average reduction of 26% when compared to a conventional RO treatment. Moreover, a reduction of 91% of CO_2 emissions when compared to AOP and 3.2% when compared to RO configuration was calculated.

Accordingly, it was demonstrated that proposed new treatment was technically viable and had lower environmental impact than conventional treatments currently used in landfill facilities.

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CHAPTER 7

Conclusions and future perspectives

This chapter gives an overview of the main achievements of this work and points out the topics for future research. Conclusions are summarised based on the objectives that have been defined previously.

Objective 1. To improve the performance of UASB systems in terms of start-up, solids hydrolysis and effluent quality by innovative configurations for municipal wastewater treatment under psychrophilic conditions.

Previous acclimation of the reactors biomass to the temperate treatment conditions, even with a 4-month inoperative period, proved to help the quick start-up of the three UASB configurations tested, that is, UASB with flocculent biomass (F-UASB) and UASB-AnMBR with flocculent and granular biomass (F-AnMBR and G-AnMBR). Results obtained for the first 45 days of operation of the three configurations showed that solids management was critical for anaerobic treatment of domestic wastewater using UASB reactors. Solids, colloids and particles needed to be retained in the reactor to increase the efficiency of solids hydrolysis and thus, membrane systems were necessary. In addition, flocculent biomass promoted slightly higher hydrolysis than granular biomass since flocculent sludge acted as a fine filter while granular acted as a coarse filter. From the results obtained, F-AnMBR showed a better performance in terms of solids hydrolysis and effluent quality for the treatment of domestic wastewater at 10°C. Therefore, a configuration based on flocculent UASB-AnMBR is appropriate for the treatment of urban wastewater with low COD content (sCOD 54.1 ± 10.3 mg/L; pCOD 84.1 ± 48.5 mg/L), at a temperature around 10°C.

Currently, further research is being carried out at Cranfield University on assessing the long-term stability of the three configurations. Thus, the definition of the best UASB configuration for urban wastewater treatment under psychrophilic conditions will be derived both from the results obtained in the start-up stage and from long-term performance of F-UASB, F-AnMBR and G-AnMBR reactors.

Objective 2. To demonstrate the technical feasibility of AnMBR for the recovery of resources (energy and water) in the treatment of cheese whey.

One-stage AnMBR proved being effective for the resource recovery from cheese whey. High COD removal (91% ± 7%) was observed with a biogas production of 0.2 – 0.9 m^3 biogas/kg COD removed in which the methane content ranged from 51 to 73%. Therefore, high energy recovery could potentially be obtained when using this process with a mean value of 2.4 kWh/kg COD removed. Employing the examined methodology, a maximum OLR of 8.4 kg COD/(m^3·day) was achieved with no decrease of the AnMBR performance.

The application of the described AnMBR technology at a larger scale, constituting a compact and decentralized treatment of cheese whey, was feasible given that it did not require the residue to be refrigerated. Therefore, AnMBR showed promising results for its implementation for resource recovery in cheese whey treatment. Although energy recovery was directly validated, several limitations were detected regarding water reuse. The product water did not meet water reuse standards in terms of electrical conductivity and turbidity derived from salt precipitation. Hence, process effluent would require a post-treatment prior to its reuse to meet water reuse standards.

Further research in this topic is required to determine the most suitable technology for permeate post-treatment to satisfy the demands of existing Spanish water reuse legislation (RD 1620/2007). Technology used for salt removal in this case could be either EDR or RO with regenerated membranes such as those used and described in Chapter 6. In addition, a study of the composition of the brine obtained in this processes should be undertaken in order to assess its potential use as a fertiliser given that all dissolved salts would remain in the mentioned brine. Regarding process performance, OLR could be further increased to assess the maximum treatment capacity of the technology and its co-digestion could be further tested by using other wastes or changing its proportions. In addition, upscaling of this technology was assessed (not reported in this thesis) obtaining poor results due to poor membrane performances (Deliverable D1.3., FP7 DEMOWARE). For this reason, upscaling of the technology focusing on membrane fouling and membrane performance should be developed prior to its real scale implementation.

Objective 3. To optimise the pre-treatment of petrochemical wastewater for in-situ water reclamation.

Coagulation-flocculation Actiflo® technology showed a decrease in wastewater turbidity but no significant DOC removal. Regarding biological treatment, the importance of using adapted inoculum was relevant for this case. When treating previously flocculated-coagulated petrochemical wastewater in the MBBR system, high sCOD removal efficiency (80-90%) was maintained with a maximum OLR of 3.25 kg/(m^3 d) and a minimum HRT of 4.5h. Thus, only 10-20% of organic matter present in the wastewater mixture was refractory to biological treatment. The MBBR proved to be also effective when treating raw wastewater as well as when feed wastewater effluent proportions were changed. Therefore, the MBBR system is a robust technology able to cope with changes in the wastewater composition. Turbidity and TSS in MBBR effluent were slightly dependent on the feeding flow, reaching values above 100 NTU and above 150 mg TSS/L in some periods. These results proved that a further step was needed before reverse osmosis could be applied to wastewater. Subsequently, the pre-treatment was successfully optimised by calculating the organic load that the MBBR could degrade (4.96 ± 0.01 kg/(m^3 d) at 23 ± 2°C) as well as by suggesting the bypass of the previous coagulation-flocculation process.

Currently, further research is being carried out in this field. A pilot plant has been designed, built and installed at the facilities of a petrochemical industry. Following the results obtained in Chapter 5, an adaptable system has been installed. Thus, flocculation-coagulation step has been placed before and after the MBBR. Results obtained in Chapter 5 suggested omitting the coagulation-flocculation step prior to MBBR. However, careful monitoring should be performed when treating raw wastewater in order to determine possible long-term inhibitions. Moreover, results also suggested MBBR effluent should be further treated to be able to undergo a reverse osmosis step. Thus, in the pilot prototype, coagulation-flocculation system placed after MBBR treatment is meant to condition wastewater prior to UF, which is aimed to decrease TSS and turbidity to values acceptable for RO feeding (i.e. < 1 mg/L and < 1 NTU).

Objective 4. To minimise the environmental impact in landfill leachate treatment by increasing the recovery of current membrane-based systems and using tailor-made regenerated membranes.

Total removal of inorganic carbon in the biological treatment was achieved by removing the stoichiometric ammonium concentration in the nitrification process in the MBR treatment. Nitrogen removal, by complete nitrification-denitrification processes, achieved a percentage of 85% at the end of operation. Moreover, regenerated spiral-wound RO membranes were capable of treating landfill leachate with a recovery above 80% and a salt rejection above 90%. Thus, waste that would be landfilled was transformed into a reusable resource closing the circular economy loop. Due to high nitrogen content in permeate, an extra refining step, such as a second RO pass was required. However, the biological pre-treatment should be optimised to enhance nitrogen removal in order to meet discharge standards. In addition, EDR technology was proven adequate for RO brine further concentration, reaching a recovery of 67% in this stage of the treatment. Thus, when using the proposed new treatment scheme, a global recovery of above 90% was achieved, meaning only less than 10% of the original leachate volume would have to be managed as a residue.

From LCA analysis, it was demonstrated that proposed new treatment had a lower environmental impact than conventional treatments currently used in landfill facilities (RO and AOP). Concretely, for the nine impact categories evaluated, the proposed treatment presented an average impact reduction of 93% compared to an advanced oxidation system and an average reduction of 26% when compared to a conventional RO treatment. Moreover, a reduction of 91% of CO_2 emissions when compared to AOP and 3.2% when compared to RO configuration was calculated.

Further research in this hybrid treatment system should include the improvement of the biological treatment. On one hand, it should include the addition of an inorganic carbon source to allow the complete ammonium removal and, on the other hand, the addition of easily biodegradable organic matter such as an organic waste to enhance the denitrification process. Instead, partial nitrification could also be employed, thus decreasing the amount of inorganic carbon and organic matter required for the biological process. Alternatively, Sharon-Anammox process could be used for nitrogen removal, avoiding the need of organic matter. An improvement in this biological pre-treatment could help improve the whole system performance and avoid a second pass RO, although its effect in the LCA analysis should also be taken into account. Alternatively, tailor-made regenerated membranes aimed at nitrate and nitrite removal could be also developed to meet discharge standards in the permeate obtained.

Objective 5. To define the characteristics of the microbial community in the bioreactors to evaluate the effect of microbial ecology in performance.

Determination of the characteristics of microbial structure in bioreactors has been previously reported to be of special importance. This importance is related to the close relation between performance and stability of biological wastewater treatment systems, microbial community structure and dynamics of bioreactors. As for this thesis, microbial community characteristics of the different reactors partially explained the bioreactor performances. While bacterial microbial population was easily defined when sequencing 16S gene, archaeal and fungal sequences detected were not easily classified. Although this work does not use the obtained data to enhance the bioreactors performance, its reporting and thus the contribution to scientific knowledge can help optimise biological treatment in the future.

It was observed that there is still a lack of annotated sequences of *mcrA* (for methanogen identification) and *ITS* (for fungal identification) genes that allow classification of the sequences obtained. In the future, when the number of annotated sequences is higher, microbial identification will be improved as it will be able to include all kind of microorganisms that take part in the biological reactions occurring in the reactors.

General conclusions

As stated previously in this work, current challenges in wastewater treatment such as higher environmental standards, complex effluents to be treated and reused have boosted the interest for novel treatment systems. Accordingly, these novel treatments should be able to beat the limitations of conventional treatment systems. As it has been demonstrated in this thesis, hybrid systems are a promising solution to these challenges. Hybrid systems allow an increase of efficiency and operability of wastewater treatment in a synergic manner, reinforcing the processes involved in the system. The combined effect of these systems comes either from higher pollutant removal, reduced or removed adverse effects of some treatments or boosted global functionality of the entire process.

It has been proved that hybrid systems involving biological processes can be broadly used and that they are as well very versatile for the treatment of different effluents. A broad range of urban and industrial wastewater can be treated by using multiple combinations of bioreactors with membrane processes. The fact of coupling both biological and membrane processes can enhance wastewater treatment strategies by providing excellent water quality, which can be reused for different purposes. In the same manner, from a resource recovery perspective, hybrid systems are key. That is, hybrid systems achieve higher water quality while, in some cases, allow energy and/or nutrient recovery.

On one hand, AnMBR can be used to treat wastewater from different origins, being suitable for those that have organic content. This AnMBR technology can treat wastewater with high organic content, as cheese whey, or low, as urban wastewater. Anaerobic treatment has the potential to reuse energy contained in wastewater and thus decrease the overall energy consumption in wastewater treatment. AnMBR can also produce reclaimed water, although inorganic content of the wastewater should be considered and it should be removed by auxiliary technologies.

On the other hand, MBBR has been proven to be effective in removing organic content from potentially toxic and recalcitrant wastewater and to be robust and able to cope with changes in the wastewater composition. It is a suitable option when organic wastewater content needs to be reduced prior to a membrane step such as reverse osmosis. This system could be applied to multiple industrial wastewater reuse schemes such as textile industry and tannery, among others.

Furthermore, the successful use of regenerated membranes in hybrid systems is of great importance as a membrane that would have been landfilled is recovered and reused in wastewater treatment. The feasibility of creating tailor-made regenerated membranes able to cope with different effluents is highly significant in the framework of circular economy. These membranes can be employed in a vast number of applications either in urban or industrial wastewater treatment systems.

Finally, it is worth mentioning that by using advanced technologies working symbiotically, that is hybrid systems, challenges related to wastewater treatment are faced and reduced.