Universitat Autònoma de Barcelona

ESTABLINT LES BASES DELS SISTEMES BIOELECTROQUÍMICS COM A TRACTAMENT INNOVADOR D'AIGÜES RESIDUALS

ÈDGAR RIBOT i LLOBET

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Tesi Doctoral

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Tesis presentada en compliment dels requisits pel
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GENOCOV
Departament d'Enginyeria Química, Biològica i Ambiental
Escola d'Enginyeria
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Èdgar Ribot i Llobet

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CERTIFIQUEM:

Que l'enginyer químic Èdgar Ribot i Llobet ha realitzat sota la nostra direcció, el treball que amb títol "Establint les bases dels sistemes bioelectroquímics com a tractament innovador d'aigües residuals", es presenta en aquesta memòria, i que constitueix la seva Tesi per optar al Grau de Doctor per la Universitat Autònoma de Barcelona dintre del Programa de Doctorat en Ciència i Tecnologia Ambiental.

I perquè en prengueu coneixement i consti als efectes oportuns, presentem a l'Escola de Postgrau de la Universitat Autònoma de Barcelona l'esmentada Tesi, signant el present certificat a

Bellaterra, 1 de juliol de 2022

Dr. Albert Guisasola i Canudas Dr. Juan Antonio Baeza Labat

Als meus pares Cristina i Joan,
als meus germans Pol i Mariona,
i a l'Ester, companya de vida.

Poder viure prop d'aquest camí,
poder veure l'aigua d'aquest riu,
sentir la pluja com cau i ens mulla,
i tenir-te al meu costat agafant-te de la mà,
per si no ho podem fer demà...

- Això es pot salvar, Sau, 1992 -

Agraïments

Voldria començar donant les gràcies a l'Albert i en Juan per confiar en mi quan els vaig dir que volia fer el doctorat. Recordo com si fos avui la conversa que vàrem tenir mentre dinàvem plegats al restaurant del rectorat. Podríem dir que allà vam firmar el contracte en el paper de la safata. Gràcies per la vostra paciència i el vostre suport en totes les decisions que vaig prendre al llarg d'aquest camí. També a en Javier, per portar la seva energia sempre que ens feia falta.

També voldria agrair a les meves companyes inseparables d'investigació, la Núria, la Yolanda i la Laura. Junts vam passar tant temps al laboratori amb els nostres "bitxos" que se'm fa impossible comptar les hores. Pel cap em ronden mil i una històries: preparant els primers escovillons de grafit a mà, calibrant el cromatògraf per n-èssima vegada perquè no sortia cap pic, posant dos quilos de cinta de tefló a les juntes de la cel·la perquè no s'escapés l'hidrogen, la frustració conjunta quan no obteníem el que esperàvem i l'eufòria dels dies en que tot sortia i ens creiem els millors! I quines coses... vaig ser el primer a entrar i seré l'últim a sortir! Heu estat les millors companyes que he pogut tenir. Mil gràcies!

Vull agrair també als companys de grup: Javi, Carlota, Edu, Mabel i en Luís les estones i xerrades compartides al laboratori, als passadissos i al menjador. A les meves companyes de despatx la Marina, la Lucía, la Belén i la Raquel; recordeu: knock the door please!

Thanks professor Logan to let me spend half year in your research team, it was a great honor and one of the best experiences in my life. Thanks to Joo Young Nam and Justin Tokash for your help every time I needed it. Schzemeck and Elvira, you were the best neighbors in whole State College, still remember the Chirstmas dinner at 4PM! Merve, Hannah, Bouha, Nima and all the people from European Club of the Penn State, I will never forget the barbecues, and the crazy Mad Mex dinners!

Per descomptat que no em vull oblidar dels millors companys de pis: en Vicent, l'Helena i en Raúl, quina colla de freaks estàvem fets tots plegats! Impossible oblidar: martes locos, la roba estesa una setmana, temporada de piel de sapo, competicions de sing star, converses filosòfiques fins que sortia el sol, les festes de Bocairent... bazinga per a tots vosaltres!

També vull donar les gràcies a una colla d'amics molt especials: els de la uni. Ens vam conèixer l'any 2004 i des de llavors ens hem fet inseparables. Vam compartir tardes de pràctiques i d'informes, dies d'estudi a la biblioteca de ciències, nits en vela resolent problemes impossibles, plats combinats d'en Txema, 3x1 a zona hermètica, sopars al Wilson, castanyades a la cívica, festes majors de l'autònoma,... vam celebrar tot el que es podia celebrar i més: aniversaris, caps d'any, Sant Joans, fi d'exàmens, festes majors, calçotades...; vam viatjar junts per tot Europa i més enllà. Van ser tantes coses les que vam compartir que al final va passar el que era inevitable, que ens convertíssim en una colla d'amics inseperables! Etser@s, sou els millors!

Per descomptat que no puc acabar els agraïments sense donar les gràcies a les persones que m'han recolzat durant tot el camí fins arribar aquí. Els meus pares i els meus germans.

Pol: per no xivar-te quan ens posàvem a mirar la tele en comptes de fer els deures, per cada matí en bici fins al cole, pels berenars dels divendres, per les voltes al mercat per anar a veure els nimalets, pels dinars al conan den pollo, pel teu mimi inseparable, per organitzar les festes d'aniversari més llargues, pel muro a llet, pel teu bon humor incombustible, per jugar amb mi a arqueòlegs enterrant objectes a l'hort de l'avi, per haver lluitat pel que volies i per haver-ho aconseguit.

Mariona: pels minimois, per les dues cuetes, pel nervi, pel tovalló màgic, perquè ets una ballarina fantàstica, perquè seràs una psicòloga brillant, per la ninua, pel dofí i els forats, perquè estàs molt mimada, pels dies que estava a la nuversitat, perquè t'agraden les mates encara que ho neguis, perquè sempre seràs la petita i perquè, tot i això, ja ets tot una dona.

Mama i papa: per cada bronca quan no feia els deures, per animar-me a anar a l'autònoma quan jo no ho veia clar, per pagar-me els estudis, per portar-me

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A tots vosaltres: per l'estima immesurable.

I finalment a l'Ester. Crec que podria omplir totes les pàgines d'aquesta tesi dient mil raons per les quals t'estic agraït però molt em temo que no serien suficients. Gràcies a la uni ens vam conèixer, vam fer els nostres millors amics i vam passar una època fantàstica. Aquesta tesis ens va portar a viure una aventura plegats als Estats Units, una decisió que en el seu moment no va ser fàcil, però que tornant la vista enrere va ser la millor que vam poder prendre. Allà va ser on per primera vegada vam (con)viure plegats i des de llavors ja no ens hem separat ni un sol dia. Recordaré sempre la conversa que vam tenir amb en Vijay (el llogater de l'apartament a State College) l'últim dia quan li vam entregar la clau: "Només una clau? Al final no us vaig donar la segona? Em sap molt de greu! De debò que heu pogut viure tot aquest temps amb una sola clau? Això vol dir que passareu tota la vostra vida junts". Jo no en tinc cap dubte, t'estimo.

Resum de la tesi

L'energia necessària per a tractar les aigües residuals és actualment el principal cost associat a aquest procés. Els sistemes bioelectroquímics permeten tractar les aigües residuals alhora que són capaços d'extreure'n part de l'energia emmagatzemada. Per una banda les cel·les microbianes de combustibles (MFC, *Microbial Fuel Cells*), és una tecnologia que permet obtenir electricitat directament del procés de tractament i, per una altra, les cel·les l'electròlisi microbiana (MEC, *Microbial Electrolysis Cells*), que basant-se en la tecnologia anterior i realitzant una petita modificació, se'n pot obtenir hidrogen gas com a vector energètic o altres productes de valor afegit. Aquesta tesi ha indagat en alguns aspectes innovadors dels sistemes biolectroquímics que poden ser útils per millorar el seu desenvolupament.

El primer punt d'estudi es va centrar en la part comuna d'ambdues tecnologies: els bacteris exoelectròtrofs. Es va seleccionar biomassa exoelectròtrofa a partir de llots anaerobis per tal que formessin una biopel·lícula al voltant de l'ànode. Es va utilitzar un mètode ràpid, efectiu i de cost reduït utilitzant un càtode flotant de llana d'acer inoxidable i un ànode format per un escovilló de grafit submergit en llots anaerobis.

Posteriorment, durant l'estada a la Penn State University, es va posar el focus en l'estudi de les MEC. Concretament, es va avaluar l'efecte del pH inicial del càtode en absència de solució tamponadora i el comportament de quatre materials diferents sota aquestes condicions: platí (utilitzat com a referència), sulfat de molibdè, escuma de níquel, i llana d'acer inoxidable. Es va comprovar que la influència del pH inicial del càtode era pràcticament nul·la i que en canvi el major efecte el tenia el material escollit. Es va veure com el platí obtenia els millors resultats com ja era d'esperar, i que després d'aquest l'escuma de níquel oferia uns resultats força prometedors, que tot i tenir un cost inferior al platí continua essent elevat. És per això que es va concloure que l'elecció final del material havia de ser una solució de compromís entre eficiència, producció i cost.

El tercer punt d'estudi va ser avaluar la resistència dels microorganismes durant diferents períodes sense substrat. Els experiments es van dur a terme en dues

condicions. Per una banda en condicions de circuit obert, és a dir, el càtode i l'ànode desconnectats, i per l'altra en condicions de circuit tancat, el càtode i l'ànode connectats. Els resultats van mostrar que en condicions de circuit tancat, el temps d'absència de substrat no tenia una afectació significativa sobre l'activitat de la biomassa, tan sols en el cas d'haver estat 21 dies en condicions de fam, les cel·les van mostrar una baixada reversible de la seva activitat. No obstant això, en el cas en que les cel·les es van deixar en circuit obert durant els períodes d'absència de substrat, a partir del cinquè dia ja mostraven una baixada notable de la seva activitat si bé al cap de dos cicles amb substrat es tornaven a recuperar. En canvi al cap de 21 dies, es va causar un dany irreparable a l'ànode. Per tant, es va concloure que per llargs períodes en absència de substrat era millor mantenir les cel·les en circuit tancat.

Finalment es va estudiar el consum de metanol en MFC. Es va escollir aquest substrat degut a que és un possible candidat a tractar com a subproducte de certs processos industrials. A més a més es va voler estudiar fins a quin punt els microorganismes presents a les cel·les serien capaços de consumir un substrat força tòxic i difícilment assimilable per molts microorganismes donat que solament té un àtom de carboni. Per dur-ho a terme es van dissenyar i avaluar tres estratègies d'inoculació: la primera va ser substituir directament l'acetat per etanol, la segona fer una substitució progressiva de l'acetat cap a etanol i la tercera crear un consorci sintròfic de bacteris exoelectrògens i bacteris fermentadors de metanol, amb la idea que els exoelectrògens consumissin els subproductes tals com l'acetat produïts pels fermentadors. El millor sistema dels tres va resultar ser el del consorci sintròfic, demostrant alhora que bacteris exoelectrògens poden treballar en sintròfia amb altres comunitats bacterianes presents al medi.

Resumen de la tesis

La energía necesaria para tratar las aguas residuales es actualmente el coste principal asociado a este proceso. Los sistemas bioelectroquímicos permiten tratar las aguas residuales y a la vez son capaces de extraer parte de la energía almacenada. Por un lado, las celdas microbianas de combustible (MFC, *Microbial Fuel Cells*), son una tecnología que permite obtener electricidad directamente del proceso de tratamiento y, por otro lado, las celdas de electrólisis microbianas (MEC, *Microbial Electrolysis Cells*), que basándose en la tecnología anterior y realizando una pequeña modificación, permiten obtener hidrógeno gas como vector energético. Esta tesis ha indagado algunos aspectos innovadores de los sistemas bioelectroquímicos que pueden ser útiles para mejorar su desarrollo.

El primer punto de estudio se centró en la parte común de ambas tecnologías: las bacterias exoelectrógenas. Se seleccionó biomasa exoelectrogénica procedente de lodos anaerobios con el objetivo de formar una biopelícula alrededor del ánodo. Se utilizó un método rápido, efectivo y de coste reducido que consistió en un cátodo flotante de lana de acero inoxidable y un ánodo formado por un escobillón de fibras de grafito sumergido en lodos anaeróbicos.

Posteriormente, durante una estancia en la Penn State University, se puso el foco en el estudio de las MEC. Concretamente, se evaluó el efecto del pH inicial en el cátodo en ausencia de una solución tamponadora, así como el comportamiento de cuatro materiales diferentes bajo estas condiciones: platino (utilizado como referencia), sulfato de molibdeno, espuma de níquel y lana de acero inoxidable. Se comprobó que la influencia del pH inicial del cátodo era prácticamente nula y, en cambio, el material escogido tenía un efecto mayor. Se comprobó que el platino obtenía los mejores resultados, tal como era de esperar, y que después de este la espuma de níquel ofrecía unos resultados prometedores, que aun teniendo un coste inferior al platino continuaba siendo elevado. Es por este motivo que se llegó a la conclusión que la elección del material tenía que ser una solución de compromiso entre eficiencia, producción y coste.

El tercer punto de estudio fue la evaluación de la resistencia de los microorganismos durante diferentes periodos sin sustrato. Los experimentos se llevaron a cabo en dos condiciones. Por un lado, en circuito abierto (es decir cátodo y ánodo desconectados) y, por otro lado, en circuito cerrado (cátodo y ánodo conectados). Los resultados mostraron que, en condiciones de circuito cerrado, el tiempo transcurrido sin sustrato no tenía un efecto significativo sobre la actividad de la biomasa, Tan solo en el caso de haber estado 21 días sin sustrato, las celdas mostraron una bajada reversible de su actividad. No obstante, en el caso de las celdas que se dejaron en circuito abierto durante periodos de ausencia de sustrato, a partir del quinto día ya mostraban una bajada notable de su actividad, aunque al cabo de dos ciclos con sustrato se recuperaban de nuevo. En cambio, al cabo de 21 días, se causó un daño irreparable al ánodo. Por tanto, se concluyó que por largos periodos de ausencia de sustrato era mejor mantener las celdas en circuito cerrado.

Finalmente se estudió el consumo de metanol en las celdas MFC. El motivo fue que el metanol es un posible candidato a tratar debido a que es un subproducto de ciertos procesos industriales, y a la vez para comprobar hasta qué punto los microorganismos presentes en las celdas serían capaces de consumir un sustrato fuertemente tóxico y difícilmente asimilable para muchos microorganismos, dado que solo contiene un átomo de carbono. Se evaluaron tres estrategias de inoculación: la primera fue sustituir directamente el acetato por etanol, la segunda fue una sustitución progresiva de acetato hacia etanol y la tercera fue crear un consorcio sintrófico de bacterias exoelectrógenas y bacterias fermentadoras de metanol, con la intención que las exoelectrógenas consumiesen los subproductos tales como el acetato producido por los fermentadores. El mejor sistema de los tres resultó ser el del consorcio sintrófico, demostrando a la vez que las bacterias exoelectrógenas pueden trabajar en sintrofía con otras comunidades bacterianas presentes en el medio.

Thesis summary

The energy required to treat wastewater is currently the main cost associated with this process. Bioelectrochemical systems allow treating wastewater and, at the same time, are capable of extracting part of the energy present in the wastewater. Microbial fuel cells (MFC) are a technology that allows the production of electricity obtained directly from the treatment process. On the other hand, microbial electrolysis cells (MEC), based on the previous technology and making a small modification, allow obtaining hydrogen gas. This thesis has investigated some innovative aspects of bioelectrochemical systems that may be useful to improve their development.

The first point of study was focused on the common part of both technologies: exoelectrogenic bacteria. Exoelectrogenic biomass from anaerobic sludge was selected with the objective of forming a biofilm around the anode. A fast, effective, and low-cost method was used. This method consisted of a floating stainless steel wool cathode and an anode formed by a graphite fibre brush immersed in anaerobic sludge.

Later, during a stay at Penn State University, the focus of study was placed on MEC. Specifically, the effect of the initial pH in the cathode in the absence of a buffer solution was evaluated, as well as the behaviour of four different materials under these conditions: platinum (used as a reference), molybdenum sulfate, nickel foam and stainless steel wool. It was found that the influence of the initial pH of the cathode was practically null and instead the chosen material had a greater effect. It was found that platinum obtained the best results as expected, and after it the nickel foam offered promising results, which even having a lower cost than platinum it continued to be high. It is for this reason that it was concluded that the choice of material had to be a compromise solution between efficiency, production, and cost.

The third point of study was the evaluation of the resistance of the microorganisms during different periods without substrate (starvation). The experiments were carried out under two conditions. On the one hand in an open circuit, that is, the cathode and anode disconnected, and on the other hand in a

closed circuit, the cathode and anode connected. The results showed that under closed circuit conditions, the time elapsed without substrate did not have a significant effect on the biomass activity; only in the case of maintaining 21 days under starvation, the cells showed a reversible decrease in their activity. However, in the case of the cells that were left in open circuit during periods of starvation, from the fifth day they already showed a notable decrease in their activity, although after two cycles with substrate they recovered again. Nevertheless, after 21 days, irreparable damage was done to the anode. Therefore, it was concluded that for long starvation periods it was better to keep the cells in a closed circuit.

Finally, the consumption of methanol in the MFC cells was studied. The reason was that methanol is a possible candidate to be treated because it is a by-product of certain industrial processes, and at the same time to check if the microorganisms present in the cells would be capable of consuming a highly toxic substrate that is difficult to assimilate for many species since it only has one carbon atom. To design the experiment, three inoculation strategies were evaluated: the first was to directly substitute acetate for ethanol, the second was a progressive substitution of acetate for ethanol, and the third was to create a syntrophic consortium of exoelectrogenic bacteria and methanol-fermenting bacteria. With the last one it was intended that exoelectrogenic bacteria would consume the by-products such as acetate produced by the fermenters. The best system of the three turned out to be the syntrophic consortium, demonstrating at the same time that exoelectrogenic bacteria can work in syntrophy with other bacterial communities present in the medium.

Índex

Capítol 1

Introducció General

Capítol 1. Introducció general

1.1 Energia i residus. Context global

Durant les últimes dècades hem experimentat uns avenços tecnològics sense precedents. No obstant això, i malauradament, no sempre han anat acompanyats d'un desenvolupament sostenible. És per aquest motiu que actualment la nostra societat ha de fer front a dos grans reptes: minimització/tractament de residus i producció energètica sostenible (Rao et al., 2017) .

Tot i que ens els darrers anys hi ha hagut alguns avenços en aquesta direcció, avui dia continuem essent una societat depenent d'energies no-renovables (petroli, carbó, gas, urani) i la quantitat de residus per càpita que es generen en el planeta continuen augmentant any rere any (World Bank, 2018).

Aquests dos efectes no solament provoquen un problema ambiental greu sinó que també són preocupants des d'un punt de vista sòcio-econòmic. Per una banda les energies no-renovables són altament contaminants ja sigui per les seves emissions a l'atmosfera com pels subproductes generats, a més de suposar una sobreexplotació d'uns recursos naturals limitats. Els residus produïts arrel de la nostra societat altament consumista fa que la capacitat d'autoregeneració dels ecosistemes no sigui suficient com per poder eliminar-los de manera natural.

Pel que fa els efectes socioeconòmics, aquests són igualment preocupants. Degut a que les fonts d'energia no-renovables són limitades, és sabut que tard o d'hora aquests recursos s'esgotaran. De fet, durant els últims anys ja s'han pogut veure efectes socials de l'escassetat com guerres en el Pròxim Orient que pretenen controlar territoris rics en petroli; els conflictes en el mar de la Xina per la sobirania d'illots inhabitats però que podrien comptar amb importants reserves de cru i de gas; i més recentment el conflicte entre Rússia i Ucraïna ha portat a l'encariment sobtat del preu de l'energia, i de retruc dels béns de consum.

D'altra banda no acabem de ser conscients de la quantitat de residus que s'han de produir per continuar amb el ritme de vida actual. Això provoca per una banda que el nivell de vida s'encareixi degut al cost de tractar tots aquest residus, i per altra banda generi unes diferències socials entre països de primer i tercer món, on els primers embruten i els segons reben les deixalles.

Una altra causa que està generant un gran impacte en el planeta és l'emissió de gasos d'efecte hivernacle procedents de la crema de derivats del petroli, gas i carbó que estan contribuint a l'escalfament global del planeta i al canvi climàtic (Manabe, 2019).

Institucions d'arreu del món són conscients de les nefastes conseqüències que pot tenir en un futur no molt llunyà la dependència econòmica del petroli i actualment s'estan invertint molts esforços per tal de canviar els hàbits actuals i poder oferir alternatives basades en fonts d'energia renovables.

És sabut que per tal de pal·liar el canvi climàtic és necessari disminuir les emissions de gasos d'efecte hivernacle. Per tal d'aconseguir aquesta fita, l'any 1998 una sèrie de països de tot el món van firmar el protocol de Kyoto (United Nations, 1998), on es comprometien a revisar a la baixa les emissions de gasos. Posteriorment la Unió Europea va aprovar el programa 20-20-20 reduir un 20% les emissions de gasos d'efecte hivernacle respecte l'any 1990, augmentar un 20% l'ús d'energies renovables i millorar un 20% l'eficiència energètica per a l'any 2020 (Comisión Europea, 2010). L'any 2021 la Unió Europea informa que s'han aconseguit els objectius, tot i que reconeix que en part és gràcies a la desacceleració econòmica provocada per la Covid-19. Un cop finalitzat aquest programa el nou objectiu de la UE és la reducció d'un 55% conegut amb el nom de *Fit for 55* de les emissions de gasos d'efecte hivernacle amb l'objectiu d'arribar a la neutralitat climàtica de la UE l'any 2050 (European Environment Agency, 2021).

Segons dades de l'Eurostat, el percentatge d'energia renovable està augmentant any rere any (Figura 1.1) tant a la Unió Europea com a l'estat espanyol, arribant al 22,1% i 21,2% respectivament l'any 2020 (Eurostat, 2021).

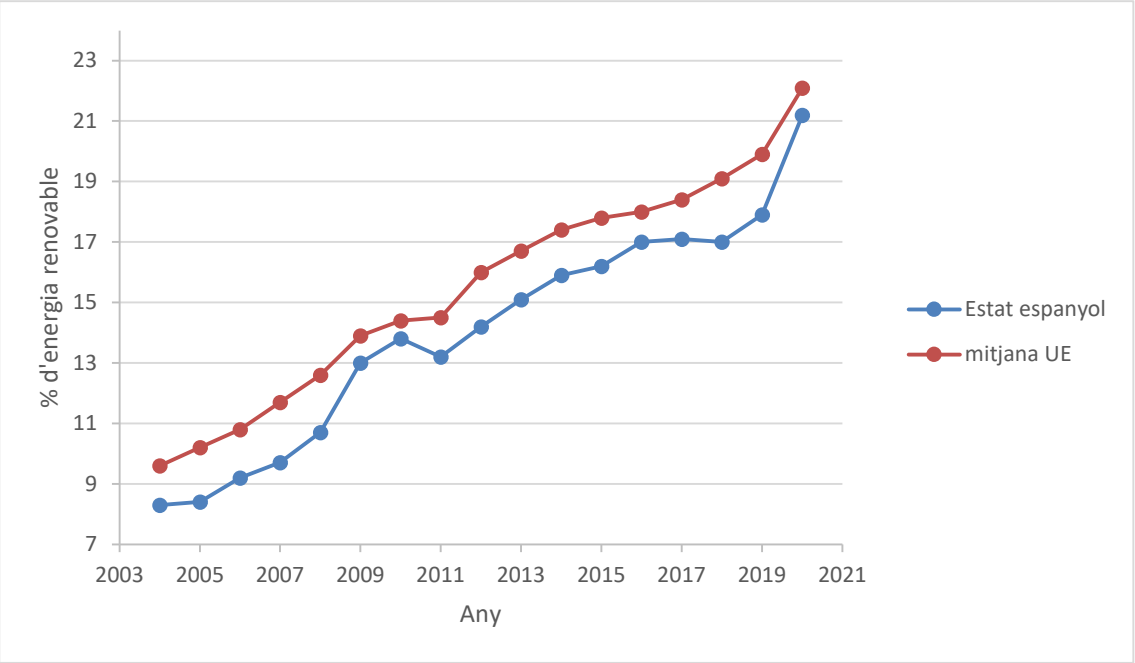

Figura 1.1 Percentatge d'energia renovable a la unió europea i a l'estat espanyol des de l'any 2004 fins l'any 2020.

Les fonts d'energia alternatives desenvolupades fins a dia d'avui es troben en diferents nivells. L'energia solar i eòlica són les més populars i les més extenses dins d'aquesta categoria, no obstant això la seva fluctuació i els problemes d'emmagatzematge així com d'espai encara són punts a resoldre.

Una altra categoria és l'energia provinent de combustibles renovables com el biodièsel i la biomassa, però un dels majors problemes que representen ambdues tecnologies és la gran quantitat de terreny que es necessita per produir-les. A més a més, competeixen econòmicament i social amb productes alimentaris de primera necessitat pel que podria provocar un desabastament als països més pobres (Renzaho et al., 2017).

1.2 Energia a partir d'aigües residuals

El Pla de Sanejament de Catalunya inclou 538 depuradores (abril de 2022) que sanegen les aigües residuals del 97% de la població catalana i tracten anualment gairebé 700 hm³ d'aigua (Agència Catalana de l'Aigua, 2022). El tractament d'aigües residuals urbanes mitjançant el procés de llots actius té un alt cost

operatiu bàsicament derivat de: aireació, bombeig i tractament de sòlids (fangs). Solament a Catalunya l'any 2018 es van tractar 1.775.463 m^3 d'aigua diàriament, que amb un cost estimat de tractament de 0.67Kwh/m^3, significa que aproximadament un 0.8% de l'energia total consumida a Catalunya es fa servir per depurar aigües residuals (Institut d'Estadística de Catalunya, 2021)

En un futur pròxim la situació no sembla millorar ja que a nivell global la població del planeta està en augment, i per tant també augmenten les necessitats de tractament d'aigua residual. A més a més, tenint en compte l'increment del preu de l'energia fa que el preu de tractament d'aigua s'incrementi any rere any, i per tant que incrementi conseqüentment el preu de l'aigua (Figura 1.2). Si bé els últims anys hi hagut una estabilització del valor unitari gràcies a l'optimització dels processos en el tractament de l'aigua, aquest efecte es pot veure superat degut a l'actual tendència alcista del preu de l'electricitat.

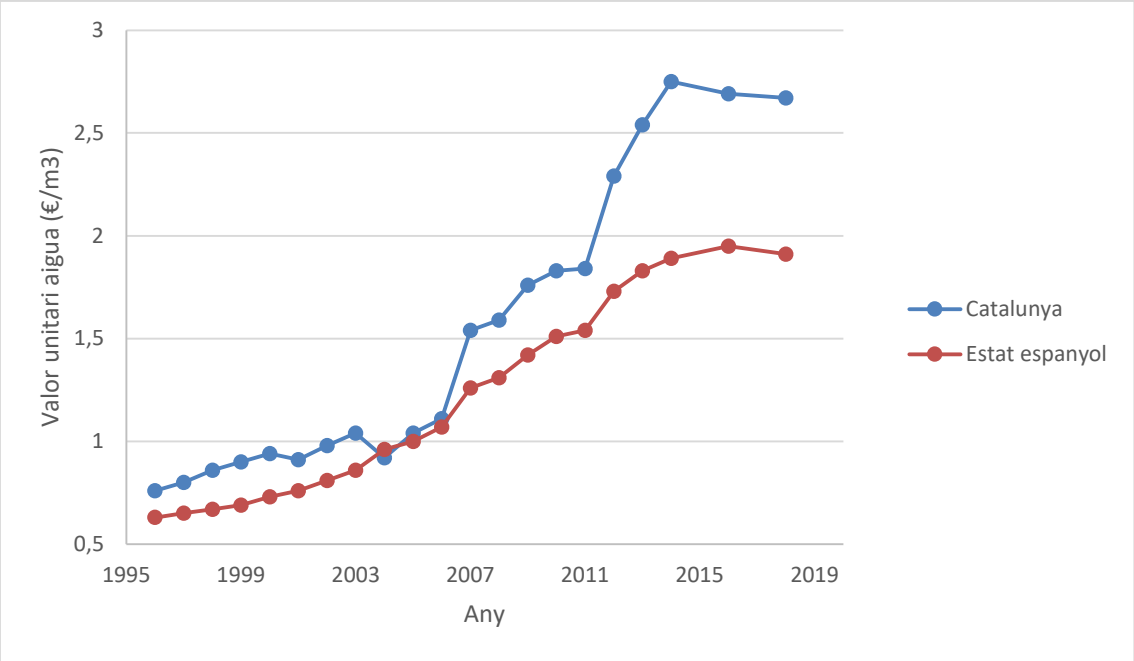

Figura 1.2 Valor unitari de l'aigua a Catalunya des de l'any 1996 fins 2018

No obstant això, el potencial energètic de l'aigua residual és 10 vegades superior a l'energia necessària per tractar-la, per tant el procés de depuració d'aigües residuals podria ser possible a cost 0 o fins i tot tenir un balanç d'energia positiu i produir energia (Heidrich et al., 2011; Shizas and Bagley, 2004).

La digestió anaeròbia podria ser una alternativa al tractament amb llots actius per a l'aigua residual urbana, ja que produeix metà (que posteriorment es pot fer servir com a vector energètic) a partir de la matèria orgànica present a l'aigua. Aquest metà posteriorment es pot fer servir per produir electricitat amb una eficiència en torn al 35% (McCarty et al., 2011). No obstant això, aquesta tecnologia encara té algunes barreres a superar: l'eliminació de matèria orgànica pot no ser suficient pel que és necessari un post-tractament, i a més que baixes temperatures i concentracions de matèria orgànica s'ha vist que afecten negativament al procés (McCarty et al., 2011).

La previsió de l'escassetat de combustibles fòssils i l'impacte negatiu del seu ús en el medi ambient impulsen la necessitat de buscar fonts alternatives de combustible sostenible (Barreto, 2018). En aquest marc, els sistemes bioelectroquímics (BES, de l'anglès *BioElectrochemical Systems*) estan emergent com una tecnologia prometedora en el camp de la valorització d'aigües residuals. Es consideren sistemes amb potencial d'ocupar un lloc destacat en la futura generació d'energia renovable, bioremediació i tractament d'aigües residuals (Ivase et al., 2020). Les oportunitats que ofereixen els BES resideixen en la seva capacitat de convertir l'energia química dels substrats no fermentables i fermentables en electricitat o altres productes d'alt valor afegit. No obstant això, els BES encara estan a les beceroles i queda molt de camí per tal que sigui una tecnologia aplicable a escala industrial.

1.3 L'hidrogen com a vector energètic

Entre totes les fonts d'energia renovables, l'hidrogen gas és una de les alternatives més atractives per a la comunitat científica gràcies al seu gran potencial com a combustible en un futur. És un vector energètic net, sense cap mena d'impacte en l'emissió de gasos d'efecte hivernacle. A més, té una calor de combustió molt elevada (-143 kJ/g) en comparació amb d'altres biocombustibles com el metà (-50kJ/g) o l'etanol (-26,8 kJ/g) (Perry and Green, 1997).

D'altra banda l'hidrogen pot ser transformat de manera eficient en electricitat mitjançant piles de combustibles (Pham et al., 2006). A més de ser un vector energètic important, és també una matèria prima molt utilitzada en la indústria química.

Avui en dia la majoria d'hidrogen es produeix a partir del *steam reforming* del metà en el qual el vapor d'aigua reacciona amb el metà per acabar donant hidrogen i monòxid de carboni. Aquest procés es duu a terme a altes temperatura (700-850ºC) i sota pressions que van des dels 3 fins als 25 bar en presència de catalitzadors metàl·lics. L'hidrogen també es pot produir a partir de la gasificació del carbó. Degut a que aquests dos últims processos no són sostenibles a causa que depenen de matèries primes no renovables, la recerca està centrada en buscar processos de producció d'hidrogen alternatius i renovables.

Entre les alternatives hi ha l'electròlisi de l'aigua que consisteix en descompondre la molècula d'aigua en oxigen i hidrogen aplicant un voltatge mínim teòric de 1,23V. Aquest procés sols es pot considerar renovable quan l'energia subministrada prové d'energies renovables tals com l'energia eòlica o solar.

L'hidrogen també es pot produir a partir de processos biològics, els quals tenen grans avantatges sobre el *steam reforming* del metà, ja que l'hidrogen seria un combustible renovable i sense carboni. La producció d'hidrogen d'origen biològic es pot aconseguir mitjançant: la fotosíntesi, fermentació fosca i la bioelectroquímica. Malgrat els seus gran avantatges, la seva producció a nivell industrial encara no està optimitzada degut a les limitacions inherents de les tecnologies mencionades anteriorment. (Rajesh Banu et al., 2021).

En la fotosíntesi, l'aigua es dissocia en oxigen i hidrogen mitjançant l'energia solar i organismes fotosintètics com les algues verdes i els cianobacteris. La hidrogenasa i la nitrogenasa són els enzims implicats en la producció d'hidrogen. Tot i això, ambdós enzims són molt sensibles a la presència d'oxigen, aquest és el major repte que ha d'afrontar aquesta tecnologia

L'hidrogen també es pot produir quan els bacteris utilitzen els protons com a acceptor d'electrons durant la fermentació fosca de substrats orgànics. Els principals avantatges d'aquest procés són les elevades taxes de producció

d’hidrogen i la possibilitat de produir-lo a partir de fonts orgàniques complexes. La contrapartida en aquest cas és que s’obtenen conversions baixes. (Rafal et al., 2018).

Finalment, la producció d’hidrogen a partir de cel·les bioelectroquímiques representen una tecnologia atractiva des del punt de vista econòmic ja que la quantitat d’energia produïda en forma d’hidrogen és superior a l’electricitat subministrada. (Cusick et al., 2010). En el següent apartat s’explica el seu funcionament.

1.4 Sistemes bioelectroquímics

Els BES són una tecnologia emergent capaç de transformar l’energia química present en aigües residuals en electricitat, en el cas de les cel·les microbianes de combustible (MFC, *Microbial Fuel Cells*) o en altres productes d’interès, en el cas de les cel·les microbianes d’electròlisis (MEC, *Microbial Electrolysis Cells*) utilitzant microorganismes com a catalitzadors del procés (Benetto, 1987, 1990; Hong Liu, Shaoan Cheng, & Bruce E. Logan, 2005; Rozendal, Hamelers, Euverink, Metz, & Buisman, 2006). Durant els últims anys, s'ha realitzat una àmplia investigació sobre els BES amb l'objectiu de generar electricitat o produir productes d’alt valor afegit, com l'hidrogen (Kadier et al., 2015)

La MFC està basada en una catàlisis microbiana de les reaccions electroquímiques que tenen lloc a la cel·la, representa una millora de l’actual procés de depuració d’aigües al permetre la generació simultània d'energia en el tractament de les aigües residuals urbanes. El funcionament bàsic de les MFC (veure figura 1.3, esquema superior) és el següent: la matèria orgànica actua de donadora d'electrons a un ànode insoluble (generalment grafit). Aquest procés és catalitzat per una biomassa disposada a l'ànode en forma de biopel·lícula. Els electrons circulen a través d'una resistència fins al càtode, on es forma aigua electroquímicament a partir de l'oxigen subministrat i dels protons generats a l'ànode. La circulació d'electrons de l'ànode al càtode és el que produeix l’electricitat. La investigació en MFC està millorant l'eficiència d'aquest processos

i fent servir combustibles sintètics (glucosa, acetat...) s'ha arribat a valors superiors a 5W/m^2 ànode (Ren et al., 2016).

Posteriorment a aquesta tecnologia, es pensà en un sistema de producció d'hidrogen gas fent servir un procés electrolític catalitzat per microorganismes semblant al descrit en la MFC. Aquest procés anomenat MEC produeix hidrogen electroquímicament a partir dels protons i els electrons generats en la oxidació electrolítica catalitzada microbiològicament.

Així doncs, en les MFC, la presència d'oxigen en el càtode provoca la producció de corrent i el flux d'electrons. En el cas de les MEC, l'absència d'oxigen evita la generació espontània d'electricitat. La realitat és que quan s'aplica un petit voltatge (>0.2V, en la pràctica) es pot arribar al potencial necessari per reduir els protons formats a l'ànode i formar hidrogen gas.

1.4.1 Funcionament de les MFC i MEC

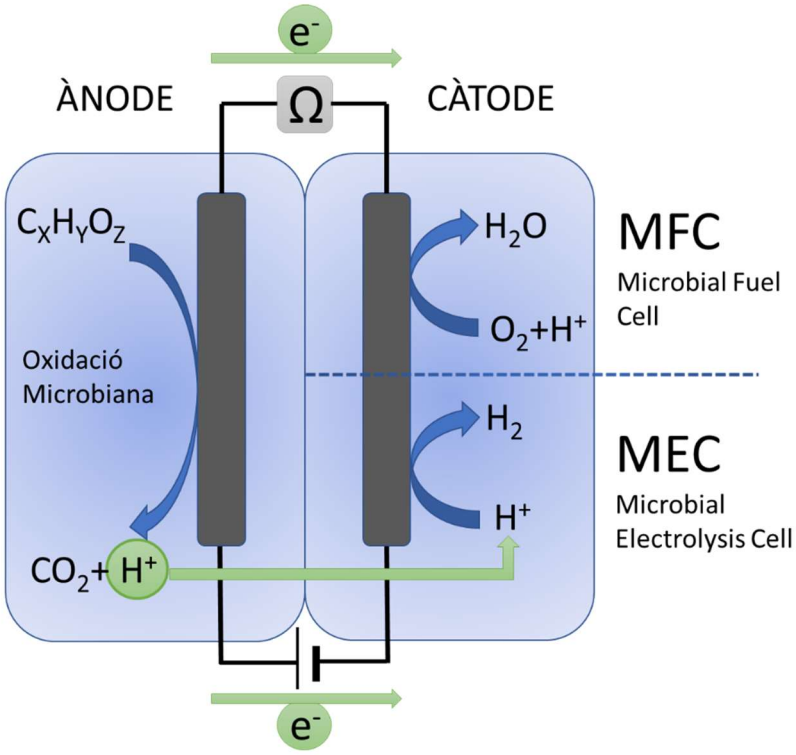

Figura 1.3 Diagrama esquemàtica d'una MFC i una MEC

La taula 1.1 mostra un exemple numèric del funcionament de les MFC i de les MEC amb acetat com a substrat. La força electromotriu (emf*, electron motion force*) de la reacció en un sistema com els descrits anteriorment es pot calcular segons l'equació de Nernst: emf = ΔG/nF, on ΔG és l'energia lliure de Gibbs per a la reacció, n el número d'electrons implicats en la reacció redox i F la constant de Faraday (96485.3C/mol). Tal i com es pot veure, a partir de l'energia lliure de Gibbs o a partir de la emf, el procés que té lloc en una MFC és espontani, mentre que en una MEC no ho és, per que requerirà una aportació externa d'energia.

	MFC	MEC
Càtode	$2\ O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$	$8H^+ + 8e^- \rightarrow 4H_2$
Ànode	$CH_3COO^- + 4\ H_2O \rightarrow 2HCO_3^- + 9\ H^+ + 8e^-$	$CH_3COO^- + 4\ H_2O \rightarrow 2HCO_3^- + 9\ H^+ + 8e^-$
Global	$CH_3COO^- + 2\ O_2 \rightarrow 2HCO_3^- + 1\ H^+$	$CH_3COO^- + 4\ H_2O \rightarrow 2HCO_3^- + 1\ H^+ + 4H_2$
Gibbs i emf	ΔG = -847,60 kJ/mol; emf = 1,10 V	ΔG = 93,14 kJ/mol, emf = -0,12 V

Taula 1.1 Exemple de funcionament de les MFC i les MEC amb acetat com a donador d'electrons.

Si bé, tal i com s'ha dit, les MEC poden produir altres productes d'interès a part de l'hidrogen, en el transcurs d'aquesta tesi ens centrarem concretament en aquest producte. El motiu és que l'hidrogen és un vector energètic excel·lent, malauradament la seva producció requereix processos amb un alt impacte ambiental, bàsicament a partir de combustibles fòssils, pel que és necessari trobar alternatives (Nikolaidis and Poullikkas, 2017).

L'electrohidrogènesi és un nou enfocament per a la producció d'hidrogen a partir de diferents fonts orgàniques biodegradables utilitzant cel·les d'electròlisi microbiana (MEC) (Liu et al., 2005b; Logan et al., 2008; Rabaey and Rozendal, 2010; Rozendal et al., 2006). Els bacteris exoelectrògens poden transferir electrons fora de la cèl·lula a un ànode sòlid alhora que converteixen la matèria orgànica en CO_2, alliberant protons en solució i electrons a l'elèctrode (Bond and Lovley, 2003; Logan, 2009; Reguera et al., 2005). Degut a que la reacció no és espontània, cal una entrada d'energia. Es requereix un potencial de més de 0.114 V, a més del produït pels bacteris que utilitzen acetat com a font de carboni, però

en la pràctica s'utilitzen tensions més elevades a causa del sobrepotencial elèctric (Logan, 2008). El platí té un sobrepotencial molt baix, però es necessiten alternatives degut l'alt cost d'aquest metall preciós (Cheng and Logan, 2008; Hu et al., 2010, 2009; Roche and Scott, 2008; Selembo et al., 2009a; Wang et al., 2012; Zhang et al., 2010)

1.4.2 Bacteris exoelectròtrofs

La clau dels BES és l'enriquiment de l'ànode amb bacteris exoelectròtrofs (també coneguts en anglès com *anode respiring bacteria*, ARB) que tenen la capacitat de transferir els seus electrons fora de la cel·la microbiana a un ànode sòlid (Sleutels et al., 2012).

Els ARB es poden trobar en indrets diferents del medi natural, tal com als sediments del fons marí o bé en llots anaerobis. S'ha vist que aquesta habilitat no pertany solament a un grup específic, sinó que podem trobar bacteris de diferents famílies capaços de donar electrons. Alguns dels bacteris més comuns són: Geobacter (Dumas 2008, Basseguy, & Bergel, 2008; Kim & Lee, 2010; Derek R Lovley, 2006; Yi et al., 2009), Shewanella (Biffinger et al., 2008; Kim et al., 2002) i Rhodoferax (Liu and Li, 2007). També en trobem d'altres com: Pseudomonas aeruginosa, Desulfuromonas acetoxidans, o Geothrix fermentan (Hurst and Crawford, 2007).

La primera vegada que es va publicar un estudi on es parlava d'aquest bacteris va ser Potter l'any 1911 (Potter, 1911), però no va ser fins a final dels 90 que el metabolisme d'aquest bacteris i les implicacions tecnològiques van ser descrites i investigades amb major profunditat (Benetto, 1990; Han'guk Sanŏp Misaengmul Hakhoe. et al., 1999; Lovley et al., 2004; Stirling et al., 1983).

S'ha vist que la gran majoria de ARB prefereixen substrats simples com l'acetat però alguns d'ells són capaços d'utilitzar substrats més complexos com: propionat, butirat, lactat i glucosa (Debabov, 2008; Holmes et al., 2004; Lovley et al., 1993).

Alguns estudis indiquen que si bé per poder cedir els electrons han d'estar en condicions estrictament anaeròbies, algunes comunitats han mostrat

comportaments facultatius, és a dir, que en presència d'oxigen canvien el metabolisme i s'adapten a les condicions, i quan els torna a mancar oxigen reprenen l'activitat exoelectrògena.

Inicialment, per tal que els bacteris alliberessin electrons fora de la cel·la i els donessin a un ànode s'afegia una substància química que actuava com a transportador d'electrons entre el bacteri i l'ànode el qual van anomenar mediador (Derek R. Lovley, 2006). Posteriorment, després de prescindir de l'ús de mediadors químics afegits, varen veure que l'activitat bioelectroquímica continuava, llavors van proposar que els mateixos bacteris eren capaços de produir els mediadors de manera natural (Borole et al., 2011; Busalmen et al., 2008). Finalment, quan la tecnologia va avançar es va observar que la comunitat bacteriana tendia a formar un biopel·lícula al voltant de l'ànode (Figura 1.4). Després d'analitzar detingudament aquestes formacions amb l'ajuda de microscòpics electrònics van comprovar com certs bacteris estaven en contacte formant un biopel·lícula conductor i per tant cedien els electrons directament, i d'altres tenien uns micro-fils capaços de transportar els electrons, tal com un cable elèctric, fins l'ànode (Figura 1.5).

Cal dir que els mecanismes exposats no pertanyen exclusivament a una o altra espècie sinó que pot ser que n'utilitzin més d'un i dependrà de les condicions d'operació.

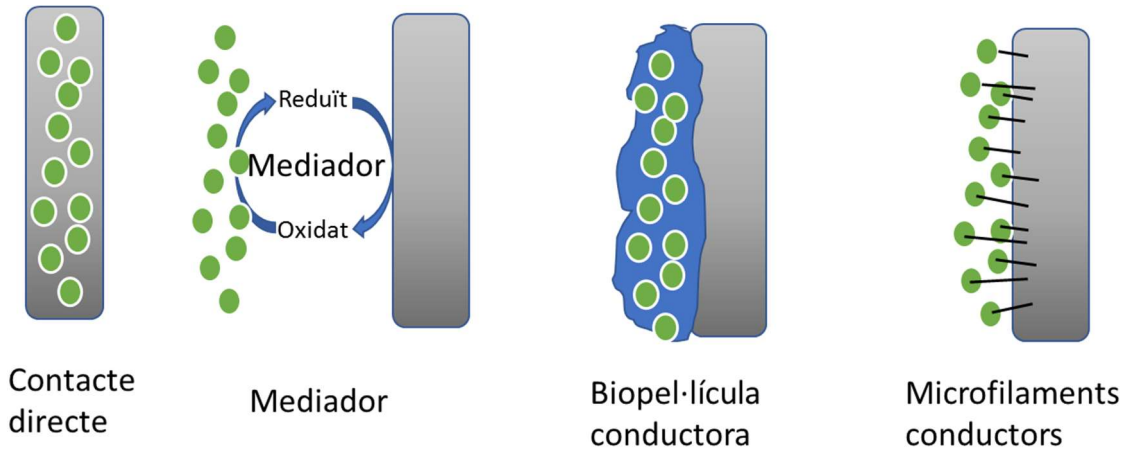

Figura 1.4 Diferents mecanismes de transport extern d'electrons.

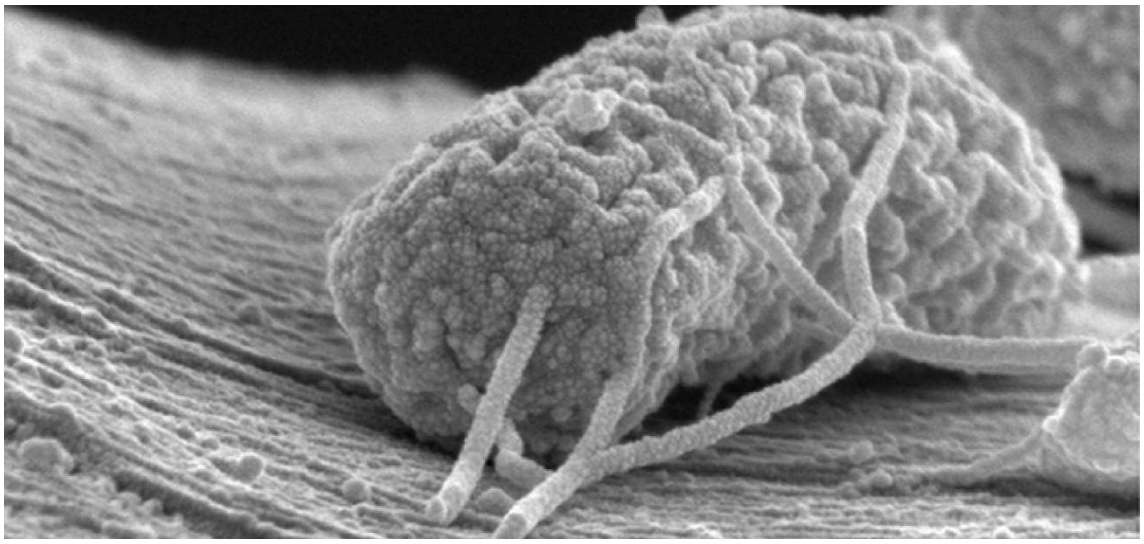

Figura 1.5 Fotografia d'un bacteri on es poden apreciar els microfilaments. Fotografia: Xing Xie, Stanford Engineering

Capítol 2

Abast de la tesi

Capítol 2. Abast de la tesi

El grup de recerca GENOCOV (Grup de recerca en tractament biològic d'efluents líquids i gasos. Eliminació de nutrients, olors i compostos orgànics volàtils) del departament d'Enginyeria Química, Biològica i Ambiental de la Universitat Autònoma de Barcelona va ser fundat l'any 1994 amb l'objectiu de millorar els processos biològics en el tractament d'efluents líquids i gasos urbans i industrials. L'any 2009, va néixer la línia de recerca de sistemes bioelectroquímics amb un nou objectiu dins del grup: no només optimitzar el tractament d'aigües residuals, sinó també recuperar la major part de l'energia que conté l'aigua en forma d'electricitat i d'hidrogen. Aquesta tesi està emmarcada en l'inici d'aquesta línia de recerca, amb l'objectiu de fer les primeres passes dins del grup en aquesta tecnologia emergent.

2.1 Motivacions de la recerca

Un dels principals objectius que ha perseguit el món del tractament de residus, especialment en aigües residuals, ha estat la optimització dels processos des d'un punt de vista energètic. És sabut que actualment el tractament de les aigües residuals ja siguin urbanes o industrials, té un alt cost econòmic pels ciutadans i les empreses, i gran part d'aquests costos provenen de l'energia necessària per dur a terme tot aquest procés.

És doncs en aquest context, que emergeixen noves tecnologies per tal de no només tractar els residus sinó a més extreure'n energia. En aquesta línia s'emmarquen els sistemes bioelectroquímics, per una banda tracten l'aigua residual i per l'altre s'obté energia en forma d'electricitat o hidrogen. Ara bé, com totes les tecnologies emergents, encara falta un gran recorregut per tal d'entendre exactament com funciona, quins són els paràmetres limitants i com es poden superar.

El motiu principal que va impulsar aquesta tesi va ser la d'iniciar una nova línia de recerca dins el grup GENOCOV. Fins aquell moment no s'havia fet cap treball relacionat amb aquesta tecnologia, així que era necessari obrir camí. El primer

repte que vam haver d'afrontar va ser la de muntar un laboratori de bioelectroquímica des de zero. Per descomptat que en aquell moment no érem conscients d'això, sinó que el nostre objectiu era construir una MFC. Per fer-ho vam passar pràcticament 4 mesos llegint, comentant i discutint. En primera instància, i donat el bagatge del grup, es va donar prioritat en obtenir una biomassa exoelectrògena més o menys estable, llavors ens vam centrar en qüestions com l'inòcul del qual partiríem, la configuració del sistema; és a dir, les condicions per aconseguir-ho.

Cadascuna de les passes que fèiem, ens portava a noves preguntes que calia resoldre, algunes d'elles llegint molts articles d'autors més experimentats, però en d'altres ocasions amb experiments, els quals molts ells no han estat publicats, però que per a nosaltres van ser molt importants en el seu moment per poder avançar.

2.2 Objectius

Els objectius d'aquesta tesi es van centrar en iniciar la nova línia de recerca en bioelectroquímica del grup de recerca GENOCOV. És per això que els punts detallats més a baix es centren en aspectes de recerca bàsics tals com selecció i comportament de la biomassa, configuracions i materials. Els objectius específics van ser els següents:

- Desenvolupar estratègies de selecció de biomassa exoelectrògena a partir de diferents procediments i condicions.

- Assajar diferents materials en el càtode amb doble objectiu: obtenir el màxim rendiment possible i reduir costos.

- Assajar la potencialitat d'aquesta tecnologia utilitzant diferents substrats alhora que s'observa l'aclimatació de la biomassa.

- Entendre com es comporta la biomassa en diferents períodes d'absència de substrat, sota diferents configuracions.

2.3 Estructura de la tesi

Aquest document està dividit en 9 capítols. El capítol 1 és una introducció general, en primer lloc sobre el context de la producció de residus i energètic global i en segon lloc un repàs breu teòric sobre els sistemes bioelectroquímics estudiats en aquesta tesi. En el capítol 2, on aquesta secció està inclosa, es presenten els objectius generals d'aquest treball. En el capítol 3 es detallen els materials i mètodes generals fets servir en els experiments conduits en el marc d'aquesta tesis. El capítol 4 presenta la primera tècnica que es va utilitzar per seleccionar biomassa exoelectrògena, es tracta d'una tècnica senzilla, de baix cost i versàtil. El capítol 5 discuteix sobre la viabilitat econòmica d'aquesta tecnologia centrada en l'ús de diferents materials en el càtode. El capítol 6 es centra en l'estudi de la utilització de metanol com a font de substrat. El capítol 7 s'estudia la resistència de la biomassa en absència de substrat sota diferents

condicions de configuració. El capítol 8 presenta les conclusions generals d'aquesta tesi. Finalment en el capítol 9 es poden trobar totes les referències.

2.4 Llistat de publicacions

Part dels resultats obtinguts en aquesta tesi han estat publicats en diferents articles. A continuació es detallen:

Ribot-Llobet, E., Montpart, N., Ruiz-Franco, Y., Rago, L., Lafuente, J., Baeza, J. A., & Guisasola, A. (2014). Obtaining microbial communities with exoelectrogenic activity from anaerobic sludge using a simplified procedure. Journal of Chemical Technology and Biotechnology, 89(11), 1727–1732.

Ribot-Llobet, E., Nam, J. Y., Tokash, J. C., Guisasola, A., & Logan, B. E. (2013). Assessment of four different cathode materials at different initial pHs using unbuffered catholytes in microbial electrolysis cells. International Journal of Hydrogen Energy, 38(7), 2951–2956.

Montpart, N., Ribot-Llobet, E., Garlapati, V. K., Rago, L., Baeza, J. A., & Guisasola, A. (2014). Methanol opportunities for electricity and hydrogen production in bioelectrochemical systems. International Journal of Hydrogen Energy, 39(2), 770–777.

Ruiz, Y., Ribot-Llobet, E., Baeza, J. A., & Guisasola, A. (2015). Conditions for high resistance to starvation periods in bioelectrochemical systems. Bioelectrochemistry, 106, 328–334.

Capítol 3

Materials i Mètodes

Capítol 3. Materials i Mètodes

3.1 Medi

El medi de treball estàndard de les cel·les va ser el següent (excepte en aquells experiments en que les concentracions varien o s'afegeixen o s'eliminen components i que s'indica en cada cas):

- Tampó de fosfat salí (PBS, phosphate buffer saline): 80 NaCl, 2 KCl, 14.4 Na_2HPO_4, 2.4 KH_2PO_4 (0.1M, pH 7.4)
- Solució de macronutrients (g/L): 11.33 $NaCH_3COO{\cdot}3H_2O$, 0.19 $CaCl_2{\cdot}2H_2O$, 1.2 $MgSO_4{\cdot}7H_2O$, 1.02 NH_4Cl
- Solució de micronutrients (g/L): 1.5 $FeCl_3{\cdot}6H_2O$, 0.15 H_3BO_3, 0.03 $CuSO_4{\cdot}5H_2O$, 0.18 KI, 0.12 $MnCl_2{\cdot}H_2O$, 0.06 $Na_2MoO_4{\cdot}2H_2O$, 0.12 $ZnSO_4{\cdot}7H_2O$, 0.15 $CoCl_2{\cdot}6H_2O$, 0.12 $AlCl_3$, 0.12 $NiCl_3$ and 10 EDTA (Lovley and Phillips, 1988).

La concentració estàndard final d'acetat va ser 1,4g/L. En alguns experiments es va afegir 10 mM o 50mM de 2-bromoetà sulfonat (BES) per tal de suprimir l'activitat metanògena tal i com s'ha descrit prèviament en altres treballs (Chidthaisong and Conrad, 2000; Nielsen et al., 2002) (Parameswaran et al., 2011).

La conductivitat es va ajustar al voltant de 10-15 mS/cm i el pH entre 7 i 7.0. Les cel·les van treballar a temperatura ambient, al voltant d'uns 21ºC durant tot el període d'operació.

3.2 Monitorització

La tensió a través de la resistència externa a la Sed-MFC i l'AC-MFC (veure seccions 4.4.1, 6.4.1 i 7.4.1) es va monitorar mitjançant una targeta d'adquisició de dades de 16 bits (Advantech PCI-1716, Taiwan) connectat a una computadora personal amb el programari AddControl desenvolupat a LabWindows CVI 2010 per a l'adquisició i el seguiment de dades.

3.3 Càlculs

A partir de les dades obtingudes mitjançant el seguiment de la tensió i dels anàlisis químics es van calcular diferents paràmetres tal i com s'especifica a continuació.

$$I = V/Rext \qquad \text{Eq.1}$$

$$P = V \cdot I \qquad \text{Eq.2}$$

On V és la diferència de potencial en la resistència en volts, Rext és la resistència externa en ohms, I és la intensitat de corrent i P és la potència en watts.

L'eficiència coulombica es defineix com la fracció d'electrons recuperats en forma de corrent respecte els que inicialment hi ha en forma de substrat. Es calcula com:

$$CE = \frac{\int Idt}{F \cdot b \cdot \Delta S \cdot V_R} \qquad \text{Eq. 3}$$

On t és el temps en segons, F és la constant de Faraday (96485 C/mol e^-), b és el nombre estequiomètric d'electrons produïts per un molt de substrat (8mol e^- /mol acetat), ΔS és el consum de substrat (mol/L) i V_R és el volum de líquid.

La recuperació de gas catòdic (rCAT) es calcula com la proporció de mols d'hidrogen mesurades i mols d'hidrogen que es poden produir en funció de la intensitat de corrent mesurada, tal com es presenta:

$$r_{CAT} = \frac{n_{H2}}{\frac{\int_{t0}^{t} I(t)dt}{2F}} \qquad \text{Eq. 4}$$

on nH_2 és la quantitat de moles d'hidrogen mesurada i es calcula d'acord amb la

llei de gasos ideals (PV = nRT) sabent el volum d'hidrogen mesurat. 2 és la quantitat de moles d'electrons per mol d'hidrogen.

L'eficiència global (rH_2) es calcula tal com s'indica a continuació:

$$r_{H_2} = r_{CAT} \cdot CE \qquad \text{Eq. 5}$$

La recuperació d'energia, és a dir, la quantitat d'energia afegida al circuit per la font d'alimentació i el substrat que es recupera com a hidrogen es calcula a partir de l'entrada d'electricitat (ηw) i de les entrades d'electricitat i substrats (ηws) d'acord amb les equacions següents:

$$\eta_W = \frac{n_{H2}}{n_{in}} \qquad \text{Eq. 6}$$

on n_{in} és la quantitat de moles basades en l'entrada d'energia elèctrica, calculada com:

$$n_{in} = \frac{\int_{to}^{t}(I \cdot E_{ps} - I^2 R_{ext})dt}{\Delta H_{H2}} \qquad \text{Eq. 7}$$

on Eps és la tensió aplicada (V), Rext és la resistència externa (Ω) i $\triangle H_{H2}$ és la calor de la combustió per a l'hidrogen (286 kJ / mol).

$$\eta_{WS} = \frac{\Delta H_{H2} \cdot n_{H2}}{\int_{to}^{t}(I \cdot E_{ps} - I^2 R_{ext})dt + \Delta H_S \cdot n_S} \qquad \text{Eq. 8}$$

on ΔH_S és la calor de la combustió del substrat i n_S és el nombre de mols de substrat consumit durant el període de temps considerat.

3.4 Fabricació del càtode

Els càtode de platí sobre pols de carboni es va fer utilitzant un mètode descrit anteriorment (Nam and Logan, 2011) aplicant una pasta de catalitzador sobre

una malla d'acer inoxidable de 12cm^2 (tipus 304, mida de malla nº60, diàmetre de filferro 0.019cm, mida de prous 0.0023cm; McMaster-Carr) al costat de la malla de dins de la cel·la. L'altre costat estava pintat amb grafit pols i Nafion. La pasta de Pt catalitzadora (5mg/cm^2 10% Pt Vulcan XC-72) contenia una barreja de 19.8mg de pols de platí, 400mL de Nafion I 200mL d'isopropanol.
També es va preparar, seguint el mateix procediment, un càtode de MoS_2 en comptes de platí tal i com esta descrit per Tokash and Logan, (2011)

3.5 Configuracions

3.5.1 Tipus H

La literatura clàssica, partia de la base que una cel·la de combustible posseeix un ànode un càtode separats per una membrana d'intercanvi d'ions. D'aquí va néixer la primera MFC batejada com a "H" degut a la seva forma.

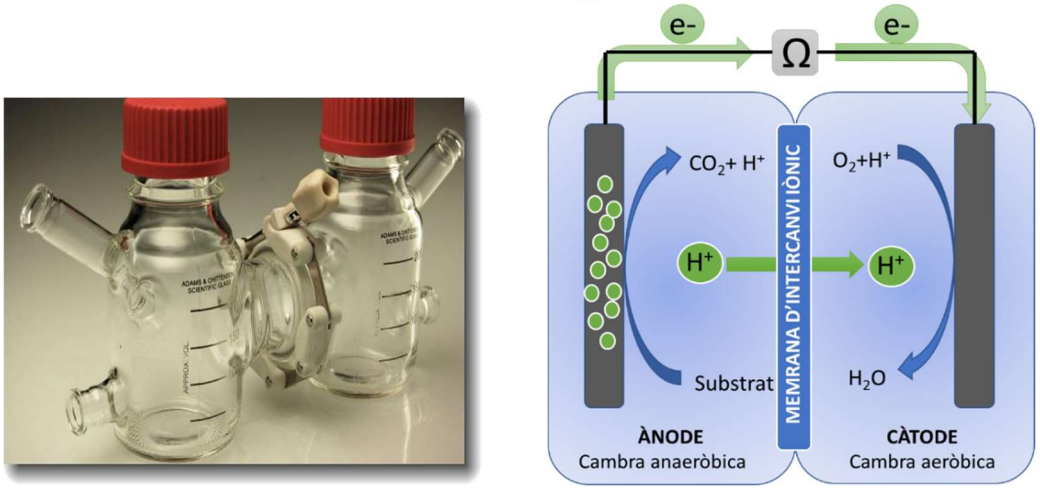

Fig. 3.1 Fotografia i esquema d'una MFC tipus H.

En l'esquema es pot veure com a l'esquerra hi ha l'ànode, en un primer moment de paper de grafit i filferro de coure i posteriorment de fibres de grafit i filferro de titani. A la dreta el càtode connectat amb l'ànode mitjançant un resistència externa de valor entre 500 i 1000 ohms. La configuració H té la particularitat que necessita oxigenar el catòlit per tal que hi hagi una concentració d'oxigen suficient per dur a terme la reacció química. Això s'aconseguí amb una línia d'aire comprimit juntament amb un difusor. Aquesta va ser la primera configuració d'èxit.

3.5.2 Tipus G

Si bé la configuració H funcionava, hi havia la necessitat de tenir preparats ànodes per poder fer diferents experiments. Degut a que la configuració H tenia un cost elevat, es necessitava una configuració més econòmica per poder inocular ànodes de manera massiva. Va ser llavors que es va pensar en adaptar les bentic-MFC en un sistema reproduïble en un laboratori a petita escala. Així van sorgir les Sed-MFC o MFC de sediments.

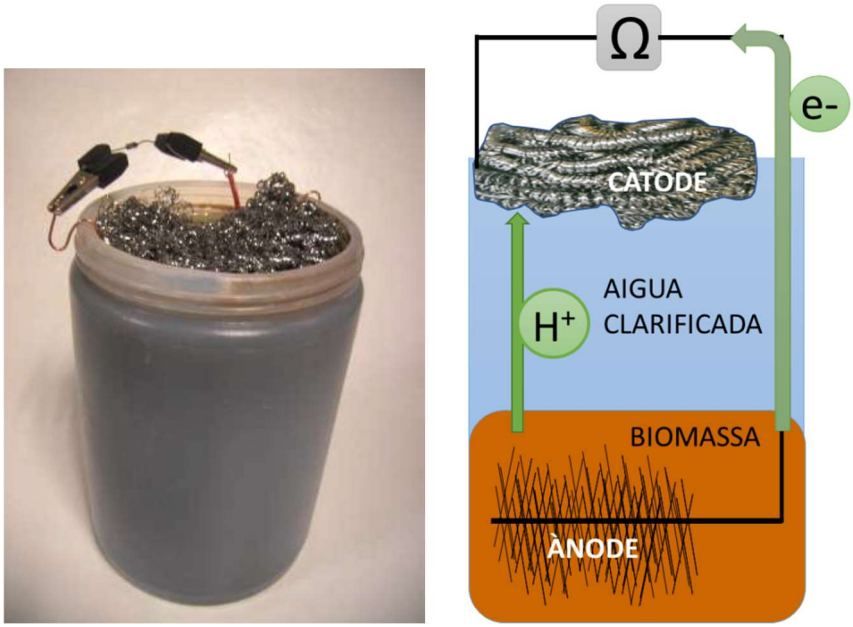

Figura 3.2 Fotografia i esquema d'una MFC tipus G.

En aquest cas l'ànode es troba situat a la part inferior de la cel·la i el càtode en la part superior en contacte amb el medi i l'aire. Degut a la falta d'agitació els llots anaerobis descansen sobre l'ànode i el càtode rep l'oxigen directament de l'aire. Aquest sistema de baix cost va permetre tenir desenes d'ànodes inoculant que posteriorment s'utilitzarien en d'altres configuracions per dur a terme diferents experiments.

3.5.3 MFC d'una cambra i càtode a l'aire

A mesura que la recerca avançava i anàvem ampliant coneixements sobre el tema, es va observar que una configuració anomenada air-MFC estava donant un resultats prometedors. A diferència de la H on s'havia de dissoldre oxigen en

el càtode mitjançant aire comprimit (tal com un reactor aerobi de llots actius clàssic), en aquest cas s'eliminava la cel·la aeròbia i sols quedava un reactor d'una sola cambra anaeròbia (*single chamber*), i en una de les seves parets es disposava d'una membrana semipermeable on la part exterior deixava passar l'oxigen i la part interior hi tenia lloc la reacció d'oxidació.

L'AC-MFC (figura 3.3) va consistir en un recipient de vidre de 400 ml amb una obertura lateral de 7 cm de diàmetre on es va muntar el càtode. El càtode estava fet amb tela de carbó recoberta de pols de carboni i suspensió de platí en el costat intern, mentre que el costat exterior estava recobert amb una solució de politetrafluoroetilè (PTFE, Teflon) (Cheng et al., 2006b, 2006a). A l'ànode es va utilitzar l'escombreta de fibra de carboni procedent de la Sed-MFC. Ambdós elèctrodes es van connectar a través d'una resistència de 560 Ω i es va controlar l'evolució de la tensió.

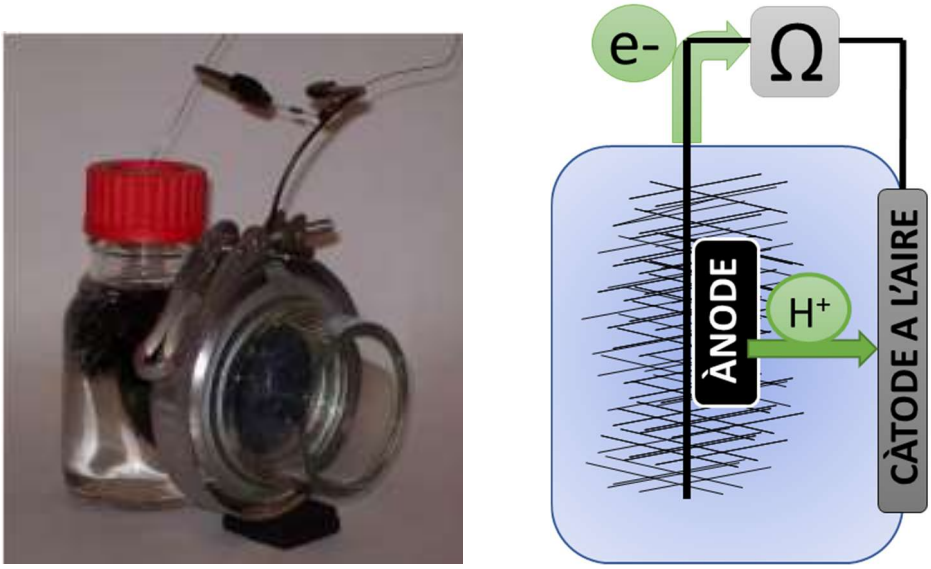

Figura 3.3 Fotografia i esquema d'una MFC tipus càtode a l'aire.

3.6 Evolució de l'ànode

El primer ànode que es fa fabricar va operar en una MFC tipus H i consistia en un rectangle de paper de grafit (*carbon paper*) enganxat amb una cola conductora a un fil de coure. Aquesta configuració va ser abandonada aviat perquè la cola conductora al cap dels dies acabava completament dissolta en

aigua i el paper de grafit i el coure s'acabaven separant. Si bé es va intentar acoblar el fil i el paper de grafit directament, no va ser possible ja que el paper de grafit es trencava al més mínim plec.

La solució va passar pel teixit de carboni. A diferència de l'anterior era molt millor estructuralment, no feia falta cap cola per unir-los ja que es podia lligar el filferro directament al teixit sense perill de ruptura. El que es va observar és que a la llarga el coure s'acabava oxidant, el que suposava dos problemes, per una banda des del punt de vista d'integritat dels materials que havien d'acabar essent substituïts, i el perill d'interferència en les lectures, i per tant en els resultats dels experiments, ja que s'estava donant lloc una reacció electroquímica no desitjada en el mateix circuit on es feien les lectures. Per aquest motiu es va decidir passar de coure a titani, un metall també conductor elèctric però molt més resistent a l'oxidació.

Si ja s'havien fet progressos importants en la configuració de l'elèctrode de l'ànode, érem conscients que quanta més àrea d'ànode hi hagués disponible, més gran seria la colònia de bacteris exoelectròtgens formant biopel·lícula al voltant del mateix. El teixit de carboni, tot i suposar una gran millora respecte el paper de carboni, l'àrea estava molt delimitada per les dimensions de la cel·la, per tant en aquest sentit es podia fer poca cosa més.

Posteriorment vam adoptar la utilització del *carbon brush anode* publicat per Logan (Logan et al., 2007). Aquest ànode consisteix en fibres de grafit entrelligades per un filferro de titani adoptant la forma d'escovilló. Aquest disseny va anar substituint la resta d'ànodes dissenyats prèviament. El motiu com es va poder comprovar va ser que gràcies a la seva gran superfície específica permetia la formació d'una biopel·lícula major, el que es traduïa amb un major rendiment de la cel·la.

3.7 Evolució del càtode

En un primer moment, durant les primeres experiències amb les MFC tipus H, es va utilitzar una placa de coure 2x4 cm, gruix 1mm puresa 99,99+%) soldada a un fil de coure (diàmetre 1mm, puresa 99,99+%), no obstant es va observar com

el coure es degradava i podia interferir en els resultats. Posteriorment va ser substituïda per paper de grafit tot i que va presentar les mateixes dificultats que en el cas de l'ànode. Finalment es va utilitzar tela de grafit, que si bé suposava una millora ja que no es degradava i estructuralment suportava les condicions, oferia uns rendiments inferiors als esperats.

Va ser llavors que es va decidir adquirir una placa de platí (2x4 cm, gruix 0,5 mm, puresa 99,999%, Goodfellow) la qual vam connectar amb un fil de titani (diàmetre 0,5 mm, puresa 99,99+%, Goodfellow) per tal que actués com a càtode. Amb aquest canvi, vam veure una millora notable en el rendiment de la MFC i per tant en el creixement de la biomassa, no obstant no era econòmicament viable comprar tants càtodes de platí com cel·les hi havia, per tant es va continuar buscant un material més idoni.

Es va optar per fabricar càtodes a l'aire (*air-cathode*) les quals consisteixen en tela de grafit amb una capa catalitzada amb carboni amb pols i platí i per l'altra una capa semipermeable. Aquest canvi no només va suposar la fabricació d'un nou càtode sinó canviar el disseny de la MFC del tipus H al tipus càtode a l'aire (air cathode), que ja s'han explicat anteriorment.

3.8 Mètodes analítics

3.8.1 Anàlisis d'àcids grassos volàtils

La concentració de àcids grassos volàtils (acetat, propionat, butirat i valerat) es van analitzar amb cromatografia de gasos (GC, Agilent Technologies, 7820-A) utilitzant una columna capil·lar PEG modificada amb àcid nitroteréptic (30 mx 250 µm x 0.25 µm; longitud x diàmetre intern x gruix de la pel·lícula) i un detector de ionització de flama. Es va injectar una mostra de 1 µl (liner 5183-4711, Agilent) a una temperatura de 275ºC en condicions de separació (29 psi). Es va utilitzar heli com a gas portador amb una proporció de 10:1. La temperatura del forn es va fixar a 85ºC durant 1min, seguit d'un primer augment de 3ºC per minut fins a assolir el valor estable de 130ºC, i després una rampa de 35ºC per minut fins a 220ºC. La temperatura del detector es va fixar a 275ºC, amb 350 mL d'aire per minut, 40 mL d'hidrogen per minut i 30 mL d'heli per minut. El temps d'execució

va ser de 18 min. En la preparació es filtra la mostra en un filtre de 0.22 μm, es pipeteja 0.6ml a un vial de 1,5 ml, s'afegeix una solució de 0,15mL d'una solució de conservació i 0.75mL d'aigua desionitzada, i la mostra es va guardar a -20ºC fins l'anàlisi. La solució de conservació afegida va ser destinada a protegir la mostra i a utilitzar-la com a estàndard intern. Contenia per litre de solució: 2 g d'HgCl2, 33.7 g d'àcid ortofosfòric i 2 g d'àcid hexanoic. Es recomana la sonicació de la solució en un bany ultrasònic per obtenir una dissolució completa. L'àcid hexanoic actua com a estàndard intern en l'anàlisi de quantificació de pics.

3.8.2 LSCV

La voltametria cíclica (LSCV, low-scan cyclic voltammetry) es va realitzar utilitzant un potenciostat μAutolab tipus II en el mode de tres elèctrodes a l'AC-MFC. L'ànode es va utilitzar com a elèctrode de treball i el càtode com a auxiliar. Es va utilitzar un elèctrode Ag / AgCl, KCl 3M (+210 mV vs. SHE) com a elèctrode de referència. El sistema estava sota condicions de circuit obert durant una hora just abans que comencés el LSCV. LSCV es va registrar a 0,1 mV / s des del potencial de circuit obert de l'ànode -0,50 V, fins a 0,3 V vs. Ag / AgCl.

3.9 Inoculació

L'objectiu d'inoculació era doble, per un banda seleccionar les poblacions bacterianes amb activitat exoelectrògena i per altra que aquestes comunitats colonitzessin l'ànode formant una biopel·lícula. Per fer-ho es van seguir dues estratègies depenent de l'estudi.

Per una banda s'inoculaven ànodes a partir de cel·les tipus G. Un cop havien passat entre 20 i 30 dies ja estaven llestes per operar en qualsevol tipus de configuració. La segona, era agafar aigües sortints d'una cel·la ja operativa on hi havia ARB en suspensió i afegir-les al medi d'un nova cel·la per a la seva posada en marxa.

Capítol 4

Obtaining microbial communities with exoelectrogenic activity from anaerobic sludge using a simplified procedure

El contingut d'aquest capítol es troba publicat segons la referència que segueix:

Ribot-Llobet, E., Montpart, N., Ruiz-Franco, Y., Rago, L., Lafuente, J., Baeza, J. A., & Guisasola, A. (2014). Obtaining microbial communities with exoelectrogenic activity from anaerobic sludge using a simplified procedure. Journal of Chemical Technology and Biotechnology, 89(11), 1727–1732. https://doi.org/10.1002/jctb.4252

Capítol 4. Obtaining microbial communities with exoelectrogenic activity from anaerobic sludge using a simplified procedure

4.1 Abstract

The microbial fuel cell (MFC) technology transforms the chemical energy present in substrates into electricity. Starting-up these systems, i.e. enriching the anodic community in exoelectrogenic bacteria, is a lengthy process or requires expensive equipment.

An easy and low-cost procedure based on a sediment MFC was developed to select microbial communities with exoelectrogenic activity from the anaerobic sludge of a waste water treatment plant (WWTP). The configuration was based on a simple vessel working as a single chamber MFC with a cathode of stainless steel wool in the liquid surface and a submerged graphite fibre brush as anode. In 30 days of operation, a biofilm with remarkable exoelectrogenic activity was grown on the anode of the MFC. This graphite fibre brush anode was able to supply 0.9 W m^{-2} when working in an air-cathode MFC (AC-MFC) for 45 days.

The procedure presented was demonstrated to be a successful, low-cost and low-maintenance procedure to obtain exoelectrogenic activity and had performances comparable with other more costly and complex inoculation procedures. The Sed-MFC does not require a potentiostat, external aeration, stirring, membranes or an enriched inoculum in the exoelectrogenic biomass.

4.2 Introduction

The starting-up of a bioelectrochemical system, i.e. enriching the anodic community in ARB, usually takes weeks. The most common inoculum consists of using either the liquid effluent or some scrapped biofilm from the anode of an existing bioelectrochemical system. Another common start-up technique is controlling the anode at a fixed potential: i.e. the anode inoculated with anaerobic sludge is immersed into a substrate solution and poised at a certain potential. The choice of the optimal anode potential is a controversial issue (Torres et al., 2008; Wagner et al., 2010). An anode with a more positive potential would theoretically result in a higher microbial diversity because different microorganisms would obtain a high yield of energy transferring their electrons to the anode. However, lower anode potential would select those more specialized bacteria able to use a minimal amount of energy to grow releasing electrons to an anode. In any case, working at a fixed anode potential requires an expensive potentiostat, which can be particularly costly if high amounts of ARB are needed. A cheaper option would be using an MFC with a selected external resistor resulting in a desired potential range (Kim et al., 2005) studied several inoculation techniques using a classical two-chamber MFC configuration. They were able to increase the power from 22 to 30 mW/m^2 using ferric iron-coated carbon electrodes (Liu et al., 2008) demonstrated that the performance of mixed culture microbial biofilms could be improved by a consecutive, purely electrochemical selection and biofilm acclimatization procedure. Their method was shown to be very efficient but it also required a multipotentiostat.

Despite these advances recently made, MFCs and MECs still face significant challenges for large-scale real-world applications (Zhou et al., 2013). For example, when moving bioelectrochemical systems into pilot or industrial scale (Cusick et al., 2011; Logan, 2010), the development of a low-cost and reliable procedure to obtain ARB-enriched biofilms on large anodes will be essential. The selected procedure should not require either ARB-enriched cultures or expensive equipment as for example potentiostats or selective membranes. In this sense, the aim of this study was to develop an efficient (simplified, successful and scalable) technique to select ARB in a graphite fibre brush anode suitable for

different bioelectrochemical systems (MFC or MEC). The developed method is based on sediment/benthic MFC and uses anaerobic sludge as inoculum. In short, a benthic MFC harvests energy from natural environments by placing an electrode in the sediment (anode) and connecting it with an electrical circuit to another electrode (cathode) situated on the overlying water layer (Dumas et al., 2007; Hong et al., 2009, 2008; Ren et al., 2013; Zhao et al., 2012). This work proposes the adaptation of the benthic MFC concept to a simplified lab-configuration (hereafter named Sed-MFC). In short, the Sed-MFC configuration consists of a single chamber MFC where the anode, a brush graphite, is buried into settled anaerobic sludge meanwhile the cathode, a stainless steel wool mesh, floats on the upper layer of the cell, thus in contact with the medium and the atmosphere. Then, the Sed-MFC corresponds to an air cathode configuration.

To the best of our knowledge, this is the first report of a methodology based on benthic MFC to obtain anodes with increased exoelectrogenic activity from raw anaerobic sludge.

4.3 Materials and methods

4.3.1 Sed-MFC construction and operation

The proposed Sed-MFC consisted of a conventional plastic vessel (1L) with an anode, a cathode and an electrical wire connection (Fig. 4.1). The anode was a graphite fibre brush (70 mm diameter x 70 mm length) made with fibres of diameter 7.2 μm (type PANEX33 160K, ZOLTEK, Hungary) and titanium wire. The brush was thermally treated at 440ºC for 30 minutes to increase further microbial adhesion (Bruce Logan, Shaoan Cheng, Valerie Watson, & Estadt, 2007). The cathode was commercial SSW placed in the air/liquid interface and connected to a copper wire over the water surface to avoid undesired copper corrosion that could affect MFC performance (Zhu and Logan, 2014). This low cost cathode provided high specific area, which balanced the overpotential losses (Ribot-Llobet et al., 2013).

Fig. 4.1. Schematic representations (Left) and pictures (Right) of the Sed-MFC (Top) and the AC-MFC (Bottom)

Acetate was selected as electron donor and 2-bromoethanesulfonate (BES) was added to prevent methanogenesis. The cell inoculation comprised 500 mL of anaerobic sludge, 125 mL of acetate solution, 0.38 mL of micronutrient solution and 100 mL of phosphate buffer solution (PBS). Then, it was filled up with deionized water up to 1000 mL. The anaerobic sludge was obtained from an anaerobic digester of an urban WWTP (Manresa, Barcelona).

The SSW cathode was immersed 50% in the liquid, and the other 50% exposed to the atmosphere. Then the circuit was closed connecting the titanium wire from the brush and the copper wire from the steel wool through a 560 Ω resistance.

4.3.2 Air cathode MFC (AC-MFC) description

Power and polarisation curves could not be done in Sed-MFCs due to their lack of homogeneity (i.e. the liquid was not stirred). For this reason, when these curves were needed, the brush from the Sed-MFC was slightly rinsed to remove all the non-attached bacteria and was placed in a conventional AC-MFC using a fresh medium with the desired initial acetate concentration.

Thus, the Sed-MFC has the anode buried in anaerobic sludge and a SSW-based cathode while the AC-MFC has the enriched anode and a Pt-based cathode. The main goal of the Sed-MFC is to enrich the anode in exoelectrogenic bacteria for its posterior use in another MFC.

Other details have been detailed previously in the materials and methods chapter.

4.3.3 Chemical analyses and monitoring

Detailed previously in the materials and methods chapter.

4.3.5 Scanning Electron Microscopy

Samples of graphite fibre brush were collected and fixed with a solution of 2.5% glutaraldehyde and 2% paraformaldehyde. Samples were treated with osmium tetraoxide, dehydrated with ethanol and dried at critical point with carbon dioxide (BAL-TEC CPD030; Bal-Tec). Then, the samples were coated with few nanometers of Au-C (E5000 Sputter Coater, BIO-RAD, California, USA) to increase signal detection and visualized on a Scanning Electron Microscope (Hitachi S-70, Japan).

4.4 Results and discussion

4.4.1 Sed-MFC development and performance

Anaerobic WWTP sludge was inoculated in three identical Sed-MFCs with an external resistance of 560 ohm. Fig. 4.2a shows the voltage profiles obtained during more than 30 days. The initial voltage around 150 mV decreased during the first days of operation due to the acclimatization period. Moreover, residual oxygen presence in the water and the sediments also favoured this initial slow response. After approximately 4 days, the voltage increased linearly (around 0.6 mV/h) which led to an increase in intensity, indicating the development of exoelectrogenic activity. This linear increase period reached fairly high voltage values, up to 300 mV (0.94 A/m^2 and 0.3 W/m^2). A constant water loss was observed due to evaporation, which was detrimental for the Sed-MFC operation, since low water levels prevented the correct contact between the cathode and the medium (i.e. the cathode surface in contact with water decreased). To avoid complete substrate depletion and ensure good contact between the water and the cathode, fresh medium was periodically added causing some oxygen diffusion and partial ARB inhibition. The systems recovered their working voltage some days after the medium addition.

These Sed-MFCs also allow inoculating at different external resistance and thus providing different external conditions that can induce the growth of different microbial communities in the anode. For example, Fig. 4.2b shows the voltage profiles obtained in another experiment with three cells under the same operational conditions except for the different external resistances used. As can be observed, the potential increases when the external load increases, in agreement with the theoretical background. In this case, the cells with higher external resistances gave similar power results (around 0.17 mW).

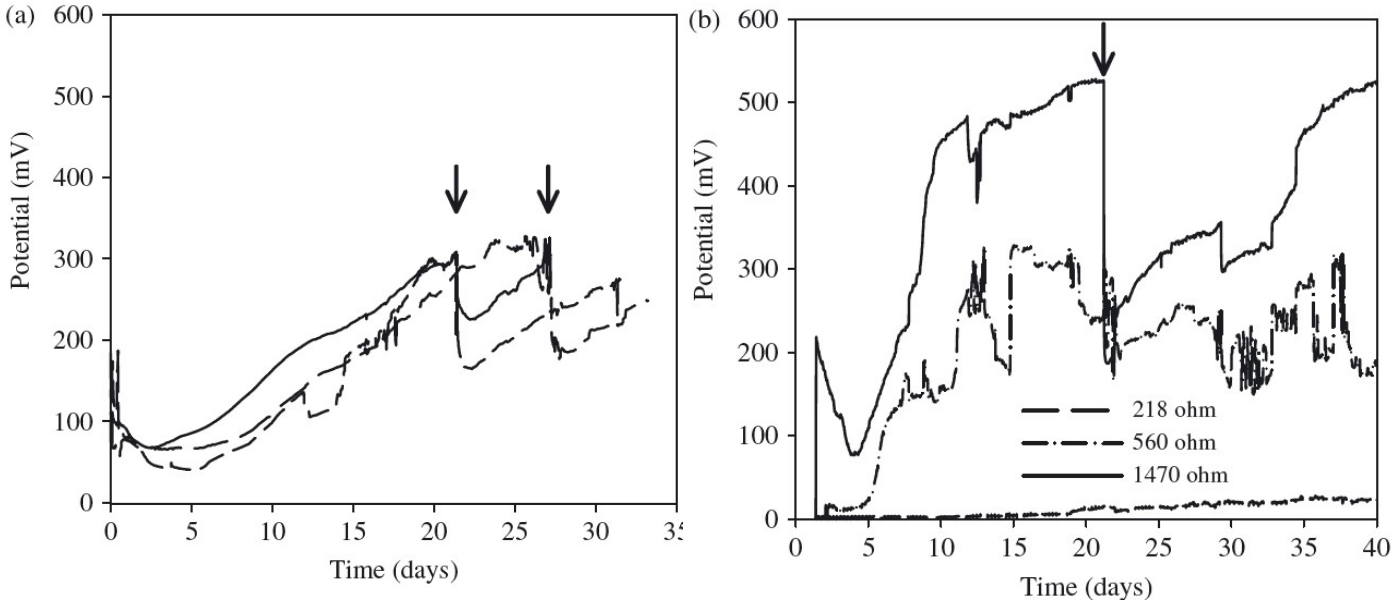

Figure 4.2. (a) Monitored voltage across 560_ resistance for three different Sed-MFCs with identical inoculation. (b) Experimental profiles for three different Sed-MFC with different external resistances. Arrows indicate substrate addition.

The time needed to develop a significant amount of exoelectrogenic biofilm is an essential parameter for the design of Sed-MFCs. To this aim, five graphite fibre brushes were developed in Sed-MFCs for different time periods. Each brush was transferred directly to an AC-MFC, where power density curves were determined (Figure 4.3). After these evaluations, it was concluded that a 30-day operational period ensured an acceptable biofilm development.

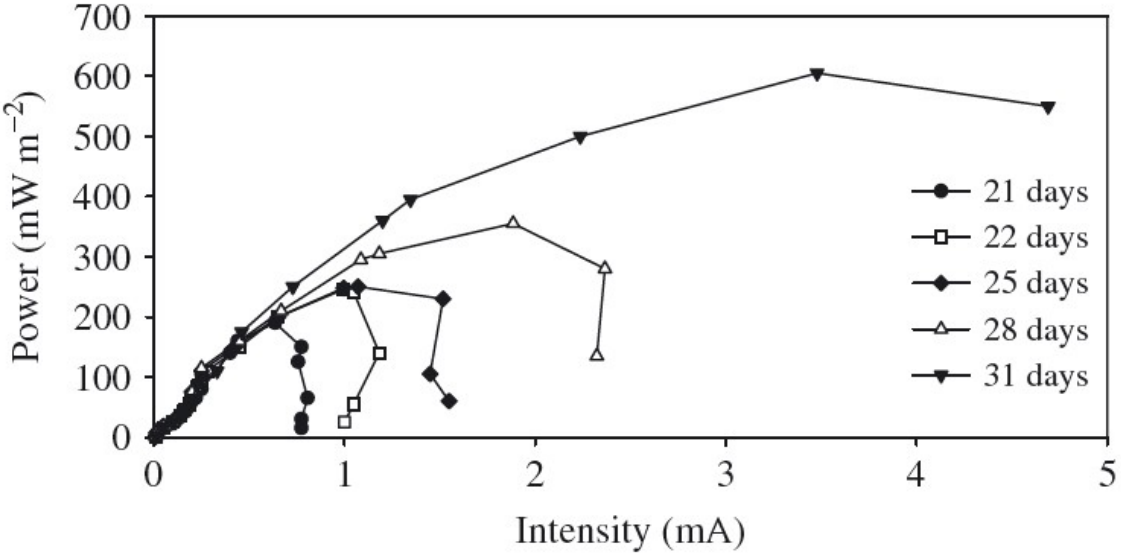

Figure 4.3 Power curves in AC-MFC with anodes developed in Sed-MFCs with different inoculation periods.

Another experiment was designed to determine if the Sed-MFC could be further simplified. Ensuring microbial adhesion is essential and thus, a thermal treatment of the graphite fibres was initially performed. Thermal treatments are recommended to enhance microbial adhesion since i) solvents and lubricants (from the anode manufacturing) are washout from the anode surface and ii) active area is increased due to microfractures generation, but this treatment increases the construction costs of MFC. Considering that our Sed-MFC architecture was different from other reported MFC (volume, distance between electrodes and electrodes surface are higher), an experiment was performed to study if the positive effect of the thermal treatment was significant in the Sed-MFC configuration. Then, a thermally treated graphite fibre brush and an untreated brush were inoculated in a Sed-MFC for 25 days. After this period, both anodes were placed in two different AC-MFCs. Fig. 4.4 shows the power and polarisation curves obtained with both brushes. The maximum powers reached by the untreated and treated graphite fibre brush were 312mW/m^2 and 903mW/m^2, respectively. The thermal treatment resulted in not only three times higher power but also in a significant internal resistance decrease: 362Ω for the untreated brush versus 151 Ω for the treated brush. Therefore, these results corroborate the better performance of the thermally treated brush and hence this treatment is recommended for the Sed-MFC. In this sense, the SEM microphotographies (Fig. 4.4b) for treated fibres corroborate the good colonization of the brush anode.

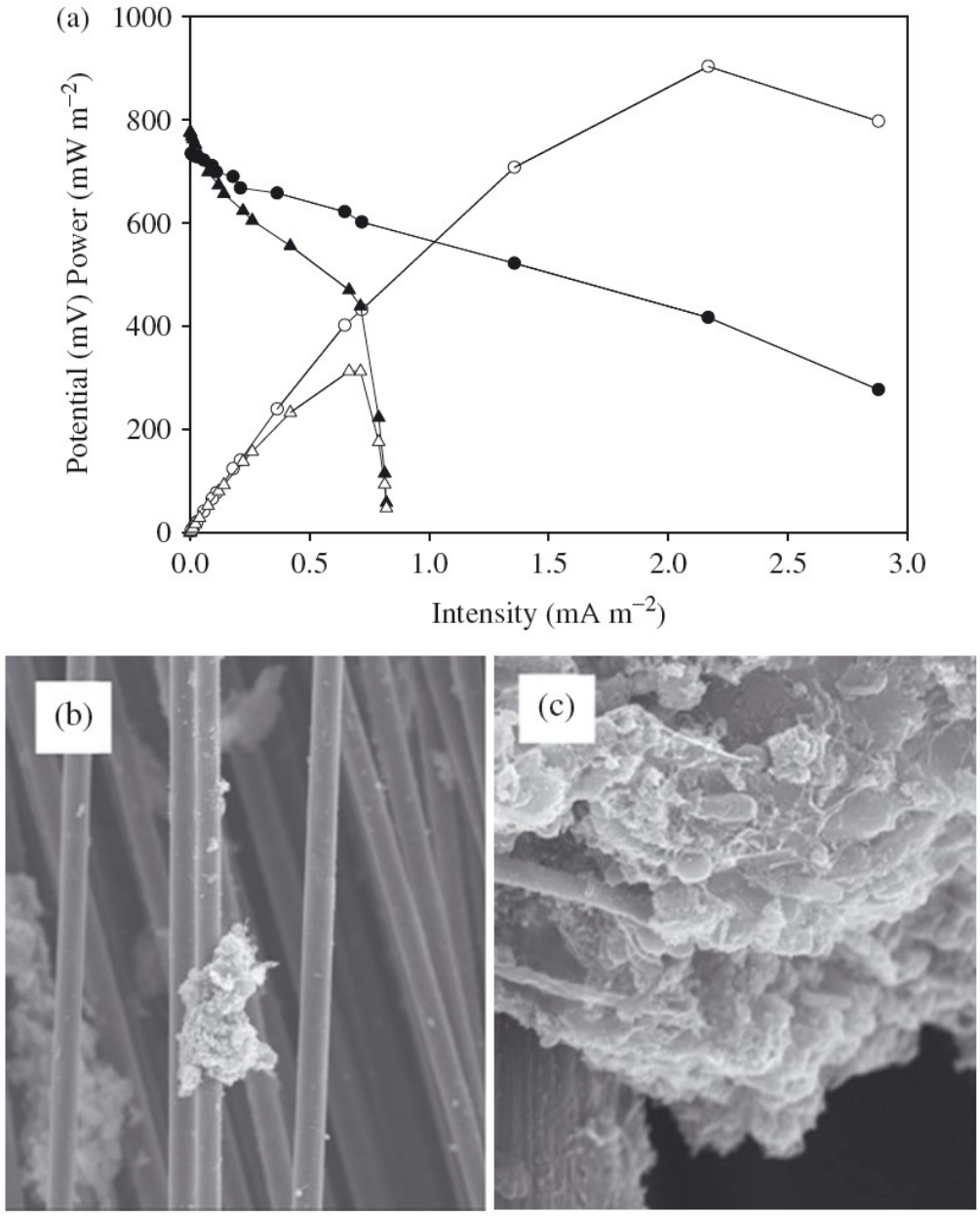

Figure 4.4. (a) Power (white symbols) and polarization (black symbols) curves for thermally treated (circles) and untreated (triangles) graphite fibre brush. (b) and (c) SEM photos of a colonized treated fibre.

4.4.2 From Sed-MFC to AC-MFC

The extent of exoelectrogenic activity obtained in the Sed-MFC was evaluated by moving an anodic brush which had been placed in a Sed-MFC for 30 days into a conventional AC-MFC under the same operational conditions (Fig. 4.5a). The first cycle (from day 0 to 14) corresponds to an acclimation cycle whereas the results from the second cycle (from 14 to 17.5 days) onwards were already promising. A high coulombic efficiency (51%) was achieved, the voltage reached 480 mV and the cycle length was 2.5 days. The experimental voltage ranged between 370 and 450 mV and an average coulombic efficiency of 55% was obtained. Then, only one cycle was needed to adapt the anode brush from the Sed-MFC to an AC-MFC operation. The AC-MFC system performance was very satisfactory, achieving maximum values up to 0.134 A/m^2 ($P = 0.07$ W/m^2) with a reasonably fair coulombic efficiency.

The exoelectrogenic activity was also evaluated through LSCV by comparing an anodic brush obtained from a Sed-MFC and stabilized in an AC-MFC for 48 hours to a non-inoculated brush (Fig. 4.5b). The inoculated brush exhibited one order of magnitude higher exoelectrogenic activity than the obtained with the non-inoculated brush, which showed negligible activity. The inoculated anode showed one typical oxidation peak at -0.25 V vs Ag/AgCl. The value of the anode potential giving half of the maximum current density, known as E_{kA}, was around -0.37 V, which is in agreement with the results found for acetate-fed *Geobacter* pure culture systems (Torres et al., 2009). The LSCV also showed a high capacitive current for the inoculated anode, indicating the presence of a conductive biofilm attached to the anode surface (Srikanth et al., 2008).

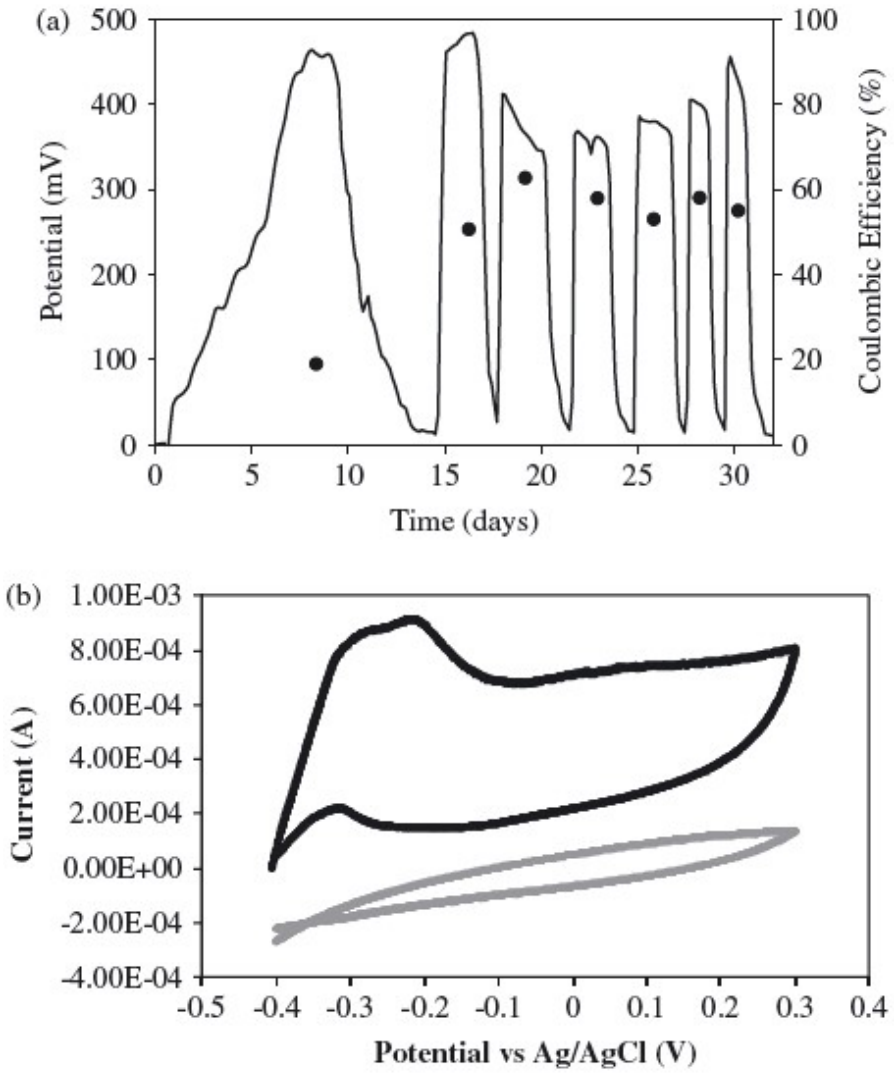

Figure 4.5. (a) Experimental voltage profiles (line) andCoulombic efficiencies (dots) for each batch cycle of an AC-MFC using an anode brush inoculated for 30 days in a Sed-MFC. (b) LSCV of an anode brush in an AC-MFC, 48h after being removed from a Sed-MFC (black), and anode brush without bacteria (grey).

4.4.3 Comparison with other works

The proposed inoculation procedure is based on placing a graphite fibre brush in a Sed-MFC with an anaerobic sludge blanket during 30 days. Anaerobic WWTP sludge is a good candidate for inoculation because it is easy to obtain and contains a high diversity of bacterial communities, including electrochemically active strains of bacteria (Kim et al., 2005). The Sed-MFC methodology has several advantages with respect to other MFC configurations. No external aeration is required, as the cathode is directly exposed to air resulting in significant aeration savings. The internal resistance is minimised because the electrodes can be located nearby. The system has low maintenance

requirements, as only the level of liquid must be supervised with low periodicity. Neither stirring nor proton exchange membrane (PEM) are required which decreases the operational costs. The main purpose of the PEM is to avoid oxygen entering to the anode. With the proposed configuration, the amount of oxygen in contact with the sludge blanket is negligible, particularly taking into account that the system is not stirred. Moreover, if some oxygen entered, it would be consumed in the upper layer of the blanket, maintaining the lower layer (where the brush is placed) under the required anaerobic conditions.

Reported configurations in the literature (Wagner et al., 2010) propose an initial polarisation period where a certain potential is applied to the cell in order to enhance ARB growth on the anode. This external voltage is reported to increase the ARB growth at the expense of increasing the cost. However, the proposed Sed-MFC does not consider the polarisation period since the objective is to develop an efficient (i.e. simplified, successful and scalable) procedure to obtain anodic microbial communities with exoelectrogenic activity using anaerobic sludge. The total cost of the cell materials is practically due to the titanium wire (around 166€/m, 0.5 mm diameter titanium) used to build the anode brush. Table 4.1 compares the performance of the presented procedure with other reported works. This comparison is not a straightforward issue since a wide range of reactor types, volumes, inoculum sources and substrates are found in the literature. In our case, we compare the experimental results obtained in the first batch with the AC-MFC when the anodic brush was transferred. In this study, a maximum power of 0.9 W/m^2 (Fig. 4.4) was reached, which is a fairly good result for a reactor volume of 400mL. (Wang et al., 2009b) presented a selection strategy able to reach half the power output of this study in about the same time, 35 days, and using a similar reactor volume, 480 mL. However they used a potentiostat, what increases considerably the cost of the inoculation process. Other studies where inoculation time was high, such as (Logan et al., 2007), obtained very high power output, nevertheless the volume was much lower, which obviously reduces power losses. (Kim et al., 2005) who worked with a similar reactor volume of 620mL, stated that 50 hours were needed for inoculation when anaerobic sludge was used as inoculum; however, power output was thirty times lower than the one observed in this study. Finally, (Wang et al., 2010) also

presented a work where inoculation time was very fast (60 hours) and power output was of the same order of magnitude as ours. However, the inoculum was coming from a previous working MEC with an already enriched exoelectrogenic environment that could have expedited the inoculation process.

Thus, our system, in comparison with others, seems to provide a fair amount of exoelectrogenic activity in a relatively high reactor volume when starting up from a poor ARB environment like anaerobic sludge from an anaerobic digester in a reasonable time frame.

Reference	[36]	[28]	[16]	[14]	This study
Volume (mL)	420	26	620	480	400
Internal Resistance (Ω)	N.D.	8	N.D.	91,84	133
Maximum Power (mW/m^2)	0,23	2,4	0,008-0,03	0,45	0,9
Reactor type	H-type	Cube air cathode	H-type	Cube-type	Air cathode
Inoculum origin	Previous MEC	Previous MFC	Anaerobic sludge	Anaerobic sludge	Anaerobic sludge
Cathode catalyst	Platinum	CoTMMPP	Platinum	Ferricyanide	Platinum
Substrate	Acetate	Acetate	Acetate	Glucose	Acetate
Polarization periode	No	No	No	Yes	No
Inoculation time	60 h	>6 months	50 h	35 days	30 days

Table 4.1 Comparison of different procedures aimed at increased exoelectrogenic activity.

4.5 Conclusions

A simplified and efficient procedure to increase the exoelectrogenic activity of anodic microbial communities from anaerobic WWTP sludge was developed. The Sed-MFC configuration was demonstrated to be a successful, low-cost and low-maintenance procedure to obtain exoelectrogenic activity. The anode graphite fibre brush developed in a Sed-MFC for 30 days provided satisfactory results and showed performance comparable with other more costly and complex inoculation procedures. The Sed-MFC does not require potentiostat, external aeration, stirring or membranes. The electrodes can be located nearby decreasing the internal resistance and the anaerobic sludge blanket allows the maintenance of strict anaerobic conditions in the anode.

Capítol 5

Assessment of four different cathode materials at different initial pHs using unbuffered catholytes in microbial electrolysis cells.

El contingut d'aquest capítol es troba publicat segons la referència que segueix:

Ribot-Llobet, E., Nam, J. Y., Tokash, J. C., Guisasola, A., & Logan, B. E. (2013). Assessment of four different cathode materials at different initial pHs using unbuffered catholytes in microbial electrolysis cells. International Journal of Hydrogen Energy, 38(7), 2951–2956. https://doi.org/10.1016/j.ijhydene.2012.12.037

Capítol 5. Assessment of four different cathode materials at different initial pHs using unbuffered catholytes in microbial electrolysis cells

5.1 Abstract

Nickel foam (NF), stainless steel wool (SSW), platinum coated stainless steel mesh (Pt), and molybdenum disulfide coated stainless steel mesh (MoS_2) electrodes have been proposed as catalysts for hydrogen gas production, but previous tests have primarily examined their performance in well buffered solutions. These materials were compared using two chamber microbial electrolysis cells (MECs), and linear sweep voltammetry (LSV) in unbuffered saline solutions at two different initial pHs (7 and 12). There was generally no appreciable effect of initial pH on production rates or total gas production. NF produced hydrogen gas at a rate of 1.1 m^3H_2/m^3d, which was only slightly less than that using Pt (1.4m^3H_2/m^3d), but larger than that obtained with SSW (0.52m^3H_2/m^3d) or MoS_2 (0.67m^3H_2/m^3d). Overall hydrogen gas recoveries with SSW (29.7 ± 0.5 mL), MoS_2 (28.6 ± 1.3 mL) and NF (32.4 ± 2 mL) were only slightly less than that of Pt (37.9 ± 0.5 mL). Total energy recoveries, based on the gas produced versus electrical energy input, ranged from 0.75 ± 0.02 for Pt, to 0.55 ± 0.02 for SSW. An LSV analysis showed no effect of pH for NF and Pt, but overpotentials were reduced for MoS2 and SSW by using an initial lower pH. At cathode potentials more negative than 0.85 V (vs Ag/AgCl), NF had lower overpotentials than the MoS_2. These results provide the first assessment of these materials under practical conditions of high pH in unbuffered saline catholytes, and position NF as the most promising inexpensive alternative to Pt.

5.2 Introduction

Two-chamber MECs have been used to improve hydrogen gas recovery compared to single-chamber systems. However, the use of a membrane results in the anode becoming more acidic due to the generation of protons, and the cathode becomes alkaline because protons are consumed (Rozendal et al., 2008). Optimum performance of exoelectrogenic bacteria requires a near-neutral or slightly alkaline pH, and therefore the anolyte often contains a buffer. The hydrogen evolution reaction does not require neutral pH conditions, although it is often buffered as well. Based on calculations using the Nernst equation, lower pHs should improve cathode performance due to high proton concentrations (Kyazze et al., 2010). However, recent studies have shown that there can be acceptable performance even at higher pHs when the catholyte is made highly conductive using salts such as NaCl, as this greatly reduces solution resistance (Merrill and Logan, 2009; Munoz et al., 2010). While many catalysts have been examined as alternatives to Pt, most of these have only been examined at neutral pH in well buffered systems (Nam and Logan, 2011). Therefore, there is no information on the performance of these alternative catalysts under more alkaline conditions in solutions lacking buffers. Some promising alternatives to Pt catalysts are nickel foam (NF), stainless steel (SS), and molybdenum disulfide (MoS_2). NF was examined due to its high surfaces area, and high current densities were recently achieved in MEC tests by others (Jeremiasse et al., 2010; Manuel et al., 2010). MoS_2 has recently been shown to be useful as a catalyst for hydrogen evolution, and it has a much lower overpotential than SS or nickel (Berit Hinnemann et al., 2005; Mehdi Afsahi et al., 2008; Tokash and Logan, 2011). High surface areas of SS can be used to produce high current densities (Call et al., 2009). Here, we examined the use of an SS wool as it is inexpensive (less than 0.10$ per gram), easy to produce, and it can be used with very high surface areas. These three catalysts were compared to Pt in MECs and in electrochemical tests under initially-neutral (pH= 7) or alkaline (pH=12) conditions in saline solutions lacking a buffer.

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5.3 Materials and methods

5.3.1 Reactor set-up

The anode (28 mL) and the cathode chamber (34 mL) of the MEC were separated by an anion exchange membrane (AMI-7001, Membranes international Inc.) (Nam and Logan, 2011). Reference electrodes (Ag/AgCl, MF-2052, BASi) were placed in each chamber. The anode was a heat treated (450 ºC, 30 min) carbon brush (25mm diameter, 25 mm length; 0.22 m^2 surface area; fiber type; PANEX 33 160 K, ZOLTEK). A glass tube was attached in the middle of the cathode chamber to collect gas, and it was sealed with a thick butyl rubber stopper and an aluminum crimp cap.

NF (Goodfellow, USA) cathodes had a size of 12 cm^2 and a specific surface area of 128 cm^2/cm^2 (area per projected area of the cathode) (Fig. 5.1). A total mass of 1.34 ± 0.02 g of stainless steel wool (SSW) was used for each cathode, producing an estimated surface area of 982 cm^2 based on an average filament size of 603 mm (Walmart, USA). Cathodes were made using MoS_2 and Pt based on previously used methods (Nam and Logan, 2011)

In order to ensure a good gas collection on the cathode, two little section (top and bottom) were cut and folded back for nickel foam, MoS_2 and Pt cathodes.

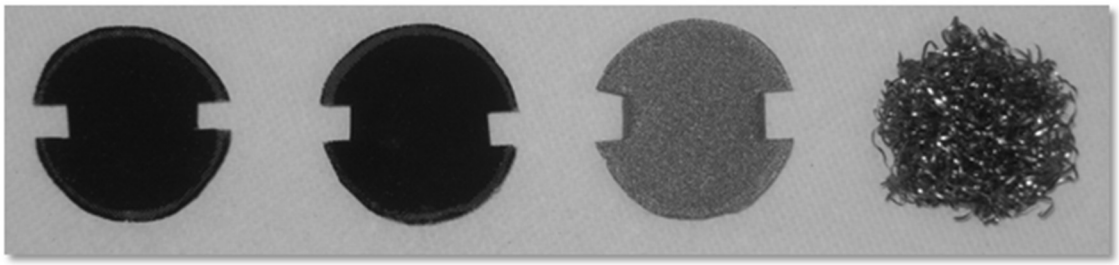

Fig. 5.1 Materials used in this study. Left to right: platinum coated stainless steel mesh (Pt), molybdenum disulfide coated stainless steel mesh (MoS2), nickel foam (NF), and stainless-steel wool (SSW). The notch helps to avoid trapped gas between the cathode and the reactor wall.

5.3.2 MEC operation

Anodes were pre-acclimated for 15 days in single chamber microbial fuel cells (1000 ohm external resistance) originally inoculated with domestic wastewater (Call and Logan, 2008; Cheng and Logan, 2007). The total voltage added to the circuit was fixed at 0.9 V using an external power supply (model 3645A; Circuit Specialists, Inc.), with the negative lead of the power supply connected to the cathode, and the positive lead to the anode. A 10 ohm resistor was included in the circuit in order to measure the voltage drop to calculate the current. The cathode solution was adjusted to a pH of 7 or 12 using NaOH, and then the initial conductivity adjusted to 8 mS/cm using KCl. When the current decreased to 0.1 mA, the cathode and anode chambers were replaced with fresh solutions and sparged with nitrogen gas to remove dissolved oxygen. All experiments were conducted using duplicate reactors at 30 ºC.

5.3.3 Linear sweep voltammetry set-up

Linear sweep voltammetry (LSV) was used to compare the cathode performance under well controlled solution conditions by using a potentiostat (BioLogic, Claix, France). Cathodes were placed in a two-chamber electrochemical cell with the anode and the cathode chambers separated by an AEM. Both two chambers were filled with unbuffered saline solution (8 mS/cm, pH 7 or 12), with reference electrodes in the cathode chamber (Ag/AgCl) and a platinumplate counter electrode. The scan rate was 0.1 mV/s over a potential range of 0 V to 1.4 V (vs. Ag/AgCl), which was chosen to represent reasonable conditions for the non-precious metal cathodic potentials in MEC experiments (1.2 V). Before starting

an experiment, N_2 gas was sparged in both chambers to remove oxygen. Current densities were normalized by the projected surface area of the cathode.

5.3.4 Measurements and calculations

Voltages across the external resistor and the electrode potentials versus the reference electrodes were recorded using a multimeter (Model 2700; Keithley Instruments, Inc.) connected to a personal computer. Gas production was measured using a respirometer (AER-200; Challenge Environmental), with the gas collected in a gas bag (0.1 L capacity; Cali-5-Bond, Calibrated Instruments Inc.) for analysis. Gases in the headspace and the gas bag were analyzed using a gas chromatograph (Model 8610B; SRI Instruments) equipped with a thermal conductivity detector, and Argon gas as the carrier. Samples were obtained (duplicates) using a gas syringe (250 mL, Hamilton Samplelock Syringe). The pH and conductivity were measured before and after each experiment (SevenMulti, Mettler-Toledo International Inc.). Total chemical oxygen demand (COD) of both chambers was measured at the beginning and end of each batch (TNT plus COD Reagent; HACH Company). Reactor performance was evaluated as previously described (Selembo et al., 2009a). Cathodic gas recovery (r_{CAT}) is the fraction of electrons recovered as hydrogen gas compared to the current generation. Coulombic efficiency (CE) is the ratio of coulombs recovered compared to those added in the substrate. The electrical energy recovery (η_E) is the ratio of energy recovered in hydrogen gas compared to the electrical energy added, and the overall energy recovery (η_{S+E}) is the energy in the hydrogen gas compared to the energy added as electrical energy and the energy in the substrate.

5.4 Results and discussion

5.4.1 Electrochemical analysis of cathodes using LSV

The solution pH slightly affected current densities produced by the SSW and MoS_2 cathodes, but it did not appreciably alter current production using the NF or Pt cathodes (Fig. 5.2). The NF and SS cathodes had the highest overpotentials

of -0.6 V over the measured range of potentials. MoS_2 produced a baseline current of -0.09 A/m2 at 0 V, which was much less than produced by Pt (0.4 A/m2), demonstrating that all alternatives to Pt had relatively high overpotentials. At potentials more positive than -0.9 V, the MoS_2 had the highest current density among the three alternatives to Pt, but at more negative potentials the highest current densities were produced by the NF, followed by MoS_2 and SS. This suggested that at cathode potentials of -0.6 to -0.85 mV, MoS_2 would work best among the three alternatives to Pt, but at more negative potentials NF would enable the highest current densities. SSW did not appear to be as effective a catalyst over this potential range, despite the much higher surface area of the material.

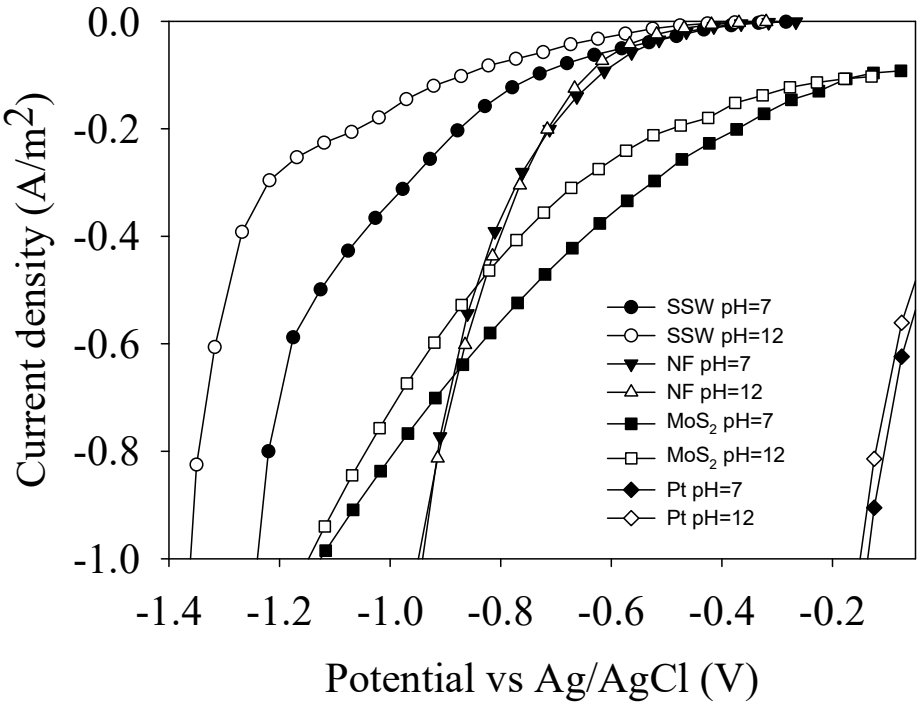

Fig. 5.2 LSVs for all materials at different initial cathodic pHs (filled symbols, pH=7; open symbols, pH=12).

5.4.2 Currents produced in MECs and total cycle times

In MEC tests, the current generated with a fixed applied potential was much different with the Pt catalyst than the three other materials (Fig. 5.3). With Pt, the initial current reached 3.4 A/m^2, while for the other catalysts the initial currents were much lower, with 2.6 A/m^2 for MoS_2, 2.4 A/m^2 for NF, and 1.7 A/m^2 for the

SSW at an initial pH of 7. The pH did not appreciably affect the current generated over time for using Pt or SSW cathodes, but higher initial currents were generated with MoS_2 and NF. This outcome was slightly different from that based on LSV tests, as it was expected that SSW and NF would show some initial differences with the lower initial pH conditions in the cathode chamber. As the cycle progressed, the pHs increased when the initial catholyte pH was 7, and slightly decreased when the initial catholyte pH was 12. At the end of the tests the catholyte pHs were all in the range of 11.3-11.7, independent of the initial pH (Table 5.1). The cycle time was the shortest in MECs with the Pt catalyst, with a 1 d cycle time at either initial pH (Table 5.1). The cycle time using the MoS_2 was similarly unaffected by the initial pH (1.6 d). However, the cycle times of the other two catalysts did not show any obvious trend with initial pH. The cycle time using the NF varied between 1.3 d (pH = 12) and 2.0 d (pH = 7). The MECs with the SSW had the longest cycle times of 1.8 (pH = 12) to 2.3 d (pH = 7). These shapes of the current profiles and the cycle time demonstrated that the MEC results with the anode generating a current produce a complex mixture of changes due to the initial pH and changes over the cycle, and the effect of the applied voltage on overpotential.

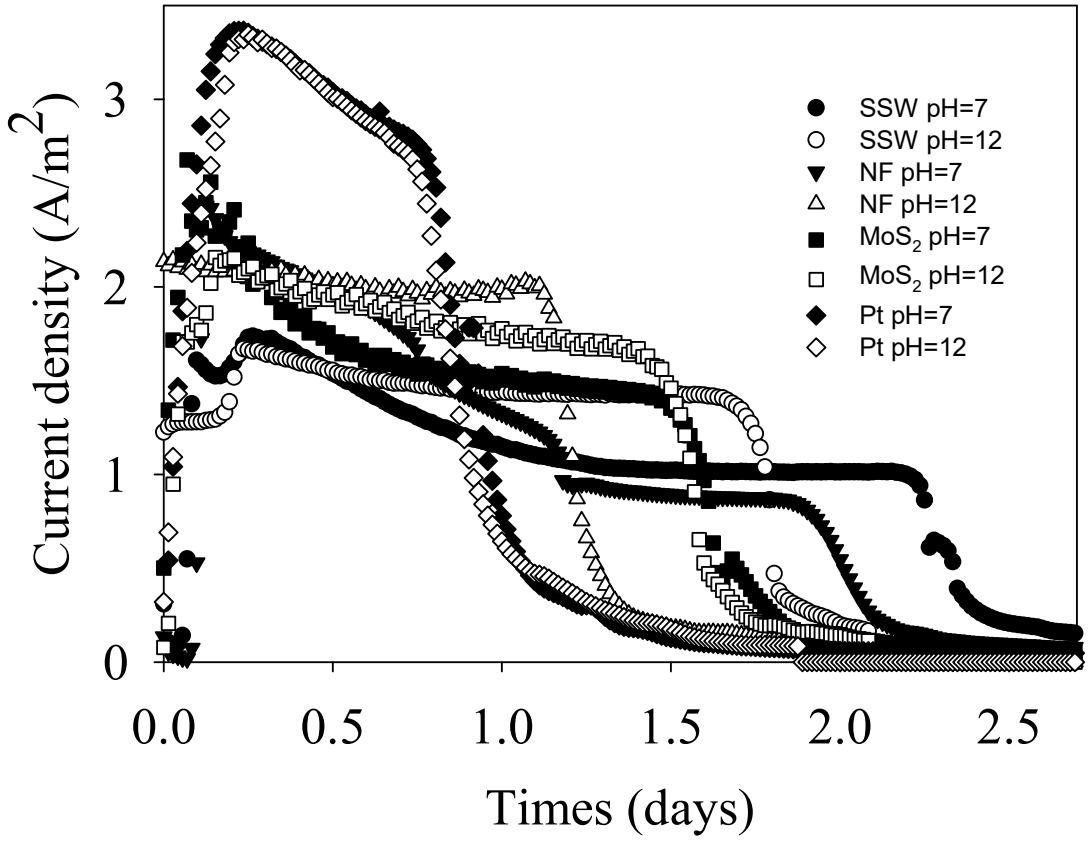

Fig. 5.3 Current densities for all materials at different initial pH in the cathode.

5.4.3 Hydrogen production volumes and rates

Pt produced the highest volume of recoverable hydrogen gas (37.9 ± 0.5 mL), followed by NF (34.5 ± 0.8 mL), with about the same amount of gas produced using either the MoS2 (29.9 ± 0.1 mL) or SSW (29.7 ± 0.5 mL) catalysts at pH = 12 (Fig. 5.4). The hydrogen yield was highest for Pt (2.6 mol H2/mol acetate) followed by NF (2.4 mol/mol), MoS_2 (2.0 mol/mol), and SSW (1.9 mol/mol). Despite the changes in the current profiles over time at the different pHs, there was very little effect of pH on the total amount of hydrogen gas produced at the two different initial pHs. Thus, from the perspective of the volume of hydrogen gas production, there is no need to set the initial pH. The maximum hydrogen gas production rates varied for the different catalysts from 1.37 m^3H_2/m^3d for Pt to 0.6 m^3H_2/m^3d for SSW at an initial pH = 7. The largest change in the maximum production rate was observed for the NF catalyst, which varied between 0.97 m^3H_2/m^3d at an initial pH = 12, compared to 0.6 m3 m^3H_2/m^3d at an initial pH =

7. Hydrogen production rates per volume could be increased by reducing the distance between the electrodes (3 cm here) or by using a buffer in the catholyte solution to control pH and decrease the internal resistance of the cell. However, the rates produced here are comparable to those in previous reports. (Wang et al., 2012) obtained 1.42 m^3H_2/m^3d using acetate as substrate and Pt with nanotubes as cathode catalyst, whereas (Rozendal et al., 2007) reported 0.3 m^3H_2/m^3d applying 1.0 V in a single chamber MEC. Other studies (Jia et al., 2010) working with actual wastewaters instead of acetate solutions, produced gas at lower rates (0.1 m^3H_2/m^3d).

Catalyst	Anode pH		Cathode pH		Cycle time (d)	
	Initial	Final	Initial	Final	pH = 7	pH = 12
Pt	7	6	7	11.5	1	1
	7	6.6	12	11.7		
MoS_2	7	6.3	7	11.4	1.6	1.6
	7	6.7	12	11.6		
NF	7	6.1	7	11.5	2	1.3
	7	6.7	12	11.6		
SSW	7	6.3	7	11.3	2.3	1.8
	7	6.8	12	11.3		

Table 5.1 Changes in pH and cycle times for the two different initial pH conditions in MEC tests.

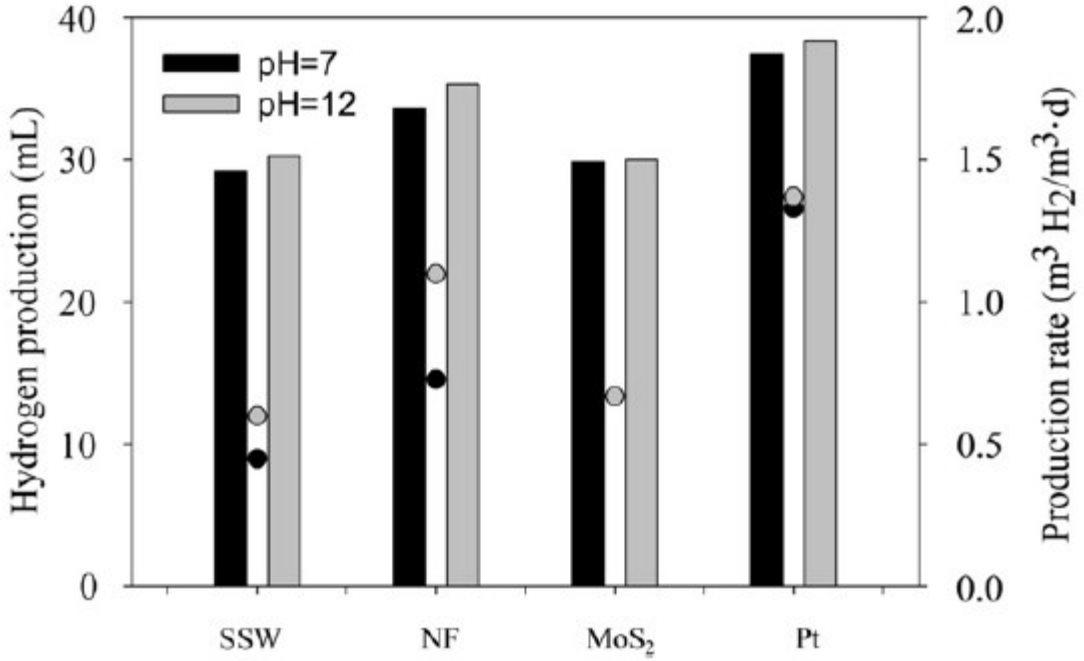

Fig. 5.4 Hydrogen production (bars) and production rate (circles) for each material at different initial cathode pHs

5.4.4 Operational efficiencies

Overall, there was little effect of the initial pH on cathodic or energy efficiencies. Cathodic hydrogen gas recovery were very high as a result of the use of a two-chamber system design, ranging from r_{CAT} =0.98±0.02 for Pt to 0.7±0.01 for SSW (Fig. 5.5). In single chamber MECs, cathodic recoveries are typically lower (87-65%) due to hydrogen losses to methanogens (Lu et al., 2011; Selembo et al., 2009b). Among the different types of catalysts, Pt had the highest electrical energy efficiencies, as expected based on the shortest cycle times and the highest peak currents, with η_E =1.74±0.09. NF reached the second best value (η_E =1.57±0.07) which was just 10% less than Pt. Lower electrical energy efficiencies were obtained with MoS2 and SSW (η_E =1.29±0.01 and η_E =1.33 ±0.09 respectively). Pt had the highest overall energy efficiency (η_{S+E} =0.75±0.02) followed by NF producing η_{S+E} =0.65±0.01 (13% less compared to Pt) and MoS_2 (η_{S+E} =0.57 ± 0.01). SSW, in spite of not being a high catalytic material, it had reasonably good efficiencies (hE=1.33 ±0.09, η_{S+E} = 0.55±0.02).

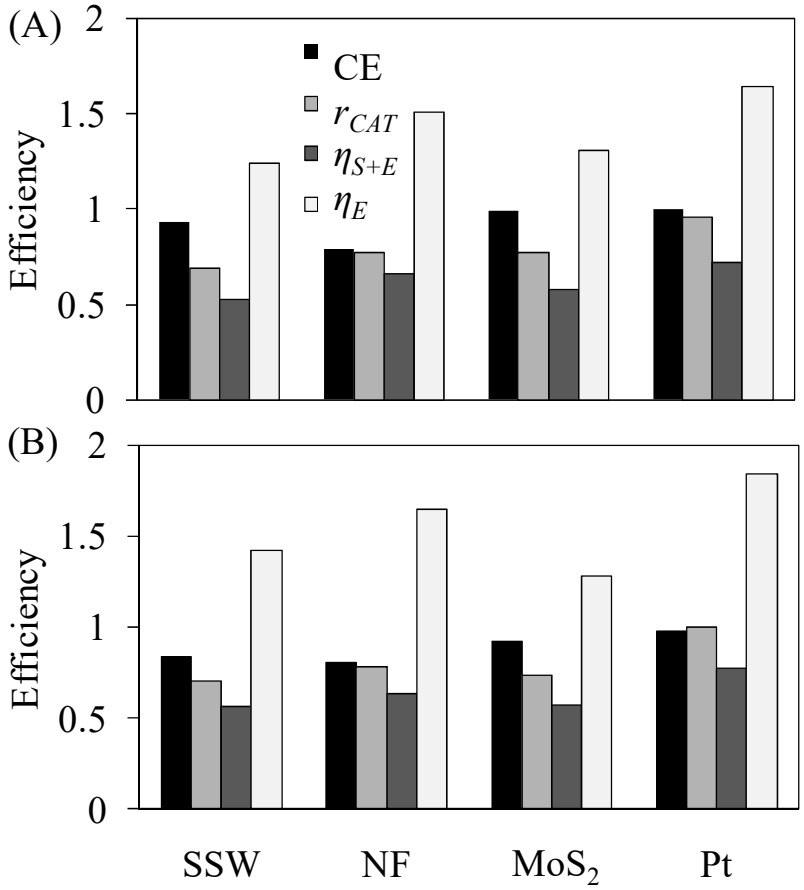

Fig. 5.5 Coulombic efficiency (CE), cathodic recovery (r_{CAT}), overall energy efficiency (η_{S+E}) and energy recovery (η_E) for all materials at initial cathodic (A) pH=7 and (B) pH=12.

5.5 Conclusions

The initial cathode pH had very little influence overall on hydrogen gas production with the different catalysts. Although electrochemical tests using LSV indicated changes with pH for two of the cathode materials (SSW and MoS_2), the overall results suggest that these initial pH conditions are not important for the overall MEC performance. In all cases, the final pH conditions were in the range of 11.3-11.7 independently of the startup at either a neutral pH of 7, or a more alkaline pH of 12. The type of catalyst was the most important factor in performance. Pt was always the best catalyst, as expected. Among all the materials tested, NF had the next best hydrogen production rate in MEC tests compared to Pt, followed by MoS_2, and SSW. In addition to hydrogen gas production rate and volume, the other main factor in performance is overall energy recovery. MoS_2 had the best energy recovery under the starting pH conditions. Although SSW had the lowest

performance values in terms of production, efficiencies and overpotentials, it is the cheapest catalyst examined here. Thus, the best alternative to Pt based on performance is NF, but based on cost it is SSW. The final choice of the catalyst is therefore a compromise between efficiencies, rate and material costs.

Capítol 6

Methanol opportunities for electricity production in bioelectrochemical systems

Part del contingut d'aquest capítol es troba publicat segons la referència que segueix:

> Montpart, N., Ribot-Llobet, E., Garlapati, V. K., Rago, L., Baeza, J. A., & Guisasola, A. (2014). Methanol opportunities for electricity and hydrogen production in bioelectrochemical systems. International Journal of Hydrogen Energy, 39(2), 770–777. https://doi.org/10.1016/j.ijhydene.2013.10.151

Capítol 6. Methanol opportunities for electricity production in bioelectrochemical systems

6.1 Abstract

An anodic syntrophic consortium (exoelectrogenic plus fermentative bacteria) able to use methanol as sole carbon source was developed for the first time in a bioelectrochemical system. In this frame, promising results were obtained in single chamber MFC, comparable to those obtained with readily biodegradable substrates. In a single chamber MFC, satisfying results (in terms of coulombic efficiency) were obtained even though energy recovery still restrained the feasibility of the process. The approach used in this work with methanol opens a new range of possibilities for other complex substrates as electron donors for bioelectrosynthesis.

6.2 Introduction

In determining the type of carbon source for BES, cost and availability impacts the total economy of the technology. Conversion of substrates other than volatile fatty acids (VFA) is essential in view of their practical implementation. ARB can use a limited range of substrates and fermentative bacteria do not have external electron transfer abilities. Nevertheless, the utilization of fermentable substrates (glucose, xylose, sucrose), non-fermentable substrates (acetate, propionate and butyrate) and wastewaters of domestic, swine, brewery, paper recycling, starch and food processing wastewaters for the generation of power or hydrogen through BES has been reported (Catal et al., 2008; Feng et al., 2008; Huang and Logan, 2008; Liu et al., 2005a; Logan et al., 2007; Oh and Logan, 2005).

Among all the different carbon sources used, methanol has never been reported to be a successful carbon source for BES. Understanding previous failures and achieving methanol-driven BES is interesting not only for potential methanol utilization but also as a pathway to follow for the utilization of other complex carbon sources. When compared to other alcohols such as ethanol, methanol is a more economical approach due to its availability from different sources. Biomethanol can currently be obtained from any organic waste source that can be first converted to synthesis gas (Kempegowda et al., 2012). Also, unlike ethanol, it does not interfere with human food chain and its purification process is simpler.

Methanol interaction in BES systems is also interesting in the frame of utilizing crude glycerol as carbon source, a target waste product to valorize. Crude glycerol as a raw material for processes such as BES for hydrogen production was reported to be an interesting carbon source (Escapa et al., 2009; Selembo et al., 2009b) but (Chignell and Liu, 2011) observed a decrease in hydrogen production yield when methanol was present in this waste stream. Direct utilization of methanol for operation of BES was attempted by (J. R. Kim et al., 2007), studying the feasibility of alcohols (ethanol and methanol) for power generation using double chamber MFC, succeeding with ethanol and reporting non-appreciable electricity generation with methanol. Finally, the utilization of

methanol in BES is a challenging task due to its possible inhibitory and toxic effect at high concentration.

Hence, in the present investigation, we have evaluated the performance of methanol in MFC for bioelectricity production with syntrophic consortia developed using ARB and anaerobic sludge. To the best of our knowledge, this is the first successful attempt of methanol utilization as a sole carbon source in BES.

6.3 Materials and methods

6.3.1 Microbial fuel and Electrolysis Cells

MFC were 28mL methacrylate vessels provided with a lateral aperture (3.8cm diameter), where a PTFE diffusion layer stuck to the cathode permitted oxygen diffusion into the cell while preventing water leakage (Cheng et al., 2006b; Shaoan Cheng, Hong Liu, & Bruce E. Logan, 2006). The anode was a titanium wire connected to a graphite fibre brush (20mm diameter x 30mm length; 0.21m^2 specific surface area made with fibres of diameter 7.2 μm (type PANEX33 160K, ZOLTEK). It was thermally treated at 450ºC for 30 minutes to enhance biomass adhesion and inoculated from an already working MFC (Wang et al., 2009a). The cathode consisted of graphite fibre cloth (3.8cm diameter, 7cm^2 total exposed area) coated with platinum (5mg Pt/cm^2, ElectroChem Inc.). The two electrodes, spaced 2.5 cm apart, were connected through a 1000Ω external resistance.

The cells operated with methanol as sole carbon source in fed-batch mode unless otherwise stated. The medium contained per litre: 1.6 g methanol, 172 mL PBS stock solution, 2.925 g $KHCO_3$ and 12.5 mL mineral media. The medium was completely replaced with fresh one when voltage response decreased below 20 mV. MEC were sparged with nitrogen for 10 minutes after feeding to guarantee anaerobic conditions. Cobalt (II) chloride was added to the system to enhance the growth of acetogens versus methanogens (Florencio et al., 1993). A 50mM 2-bromoethanesulfonate concentration was used according to the work of (Parameswaran et al., 2011), where it was stated that such concentration would selectively inhibit methanogenic bacteria. 2-bromoethanesulfonate had been previously stated to inhibit methanogenic activity (Nollet et al., 1997; Sparling et

al., 1997) and to be more effective than other chemical inhibitors or changes in system conditions such as pH and temperature (Chae et al., 2010). Cells were kept at room temperature during all the operational periods.

Voltage evolution was monitored by means of a 16-bit data acquisition card (Advantech PCI-1716) connected to a personal computer with a software developed in LabWindows CVI 2013 for data acquisition.

6.3.2 MFC start-up

During the start-up of the MFC, the cell was inoculated with the media removed from a previously working MFC (originally inoculated with anaerobic digester sludge) that had been running in fed batch mode for over one year. The MFC was fed with acetate as carbon source to enhance the growth of ARB and their enrichment in the anode. Once a stationary response in terms of current intensity was achieved (in about two weeks), the MFC was fed with methanol following three different strategies to obtain a methanol-driven MFC: i) direct replacement of acetate for methanol, ii) progressive replacement of acetate for methanol and iii) two-step consortium development with methanol fermenting bacteria and ARB. The methanol fermenting population was grown anaerobically at 37ºC in Schott bottles using anaerobic digester sludge (Granollers urban WWTP, Barcelona) as inoculum and operated under fed batch mode (5 days cycles). Every time the system was fed, the mixed liquor was centrifuged (4 minutes at 5000rpm) to enhance high biomass retention, the medium was discarded, and the sludge was resuspended in fresh medium identical to the one used for MFC and MEC. Methanol was used as sole carbon source and also a concentration of 50mM 2-bromoethanesulfonate was used to limit the methanogenic activity. Methanol and acetate concentrations were measured to assess the development of the fermenting community and gas analyses from the headspace allowed to ensure that no methane was being produced.

6.3.3 Chemical and electrochemical analyses

Methanol and acetate concentration was analysed with gas chromatography (Agilent Technologies, 7820-A) using a flame ionization detector and helium as

carrier gas. Hydrogen and methane were also measured with gas chromatography using a thermal conductivity detector and argon as carrier gas. Gas production was evaluated as in Ambler and Logan (Ambler and Logan, 2011). pH and conductivity were measured offline.

MFC internal resistance was assessed from polarization curves (Logan et al., 2006). The polarization curve was performed allowing the cell to reach the open circuit voltage for a period of one hour and then progressively changing the external resistance (from high to low resistance) and measuring the resulting cell voltage after 10 minutes. The set of external resistances used for the polarization curves were 470kΩ, 218kΩ, 44.2kΩ, 24.1kΩ, 12.1kΩ, 6.6kΩ, 3.3kΩ, 2.0kΩ, 1.65kΩ, 1.0kΩ, 825Ω, 470Ω, 250Ω, 218Ω, 100Ω, 50Ω and 25Ω.

6.3.4 Microbial analyses

High-throughput 16S rRNA gene pyrosequencing was performed in a 454 Titanium FLX system by the Research and Testing Laboratory (Lubbock, TX) based upon RTL protocols from cathode and anode DNA samples (20 ng/ μL, quality ratio of 1.8). Sequence checking, chimeras detection, sorting and trimming and quantitative assessment are detailed elsewhere (Rago et al., 2015).

6.4 Results and discussion

6.4.1 Development of syntrophic consortium for methanol utilization in BES

The development of a syntrophic consortium able to degrade methanol and generate current intensity in MFC was tested for three different strategies: the direct replacement of acetate for methanol (ST1), a progressive replacement of acetate for methanol (ST2) and a two-step consortium development bioaugmenting ARB with methanol fermenting bacteria (ST3). The idea beneath the syntrophic consortium of anaerobic methanol-degraders and ARB is that the anaerobic fraction (essentially, acetogens) would degrade methanol, while ARB would live off the degradation by-products (e.g. acetate) enabling

exoelectrogenesis. For this aim, a first step, where methanol-degrading acetogens were selected against other methanol degraders (essentially methanogens) from an anaerobic sludge, was necessary. Methanogens were absolutely undesired in this consortium since they could use both methanol and acetate for methanogenesis becoming, then, competitors to both ARB and acetogens. Once the anaerobic sludge was enriched in methanol-degrading acetogens, it was used as bioaugmentation agent in an MFC where an acetate-degrading population had been previously developed, i.e. an MFC already enriched in ARB.

Figure 6.1 presents the performance of three different MFC during the inoculation period using the three different strategies tested. It can be observed that for strategies ST1 (direct replacement) and ST3 (syntrophic consortium) an acclimation time was required before current intensity generation was boosted, which was shorter for ST3 (Figure 6.1A). On the other hand ST2 (progressive replacement) kept generating a much higher current intensity as a result of being fed also with acetate. After 80 days of operation under inoculation conditions, the cells were changed to the operational mode with methanol as sole carbon source (Figure 5.1B, change of axis scale to ease reading). The cell inoculated with ST2 (progressive replacement of acetate for methanol) suffered an abrupt decrease in cell performance. The current intensity with ST3 kept rising after the switch to methanol. The results indicated that the two-step consortium development was the most efficient in terms of higher CE, higher power density and lower internal resistance (Table 6.1).

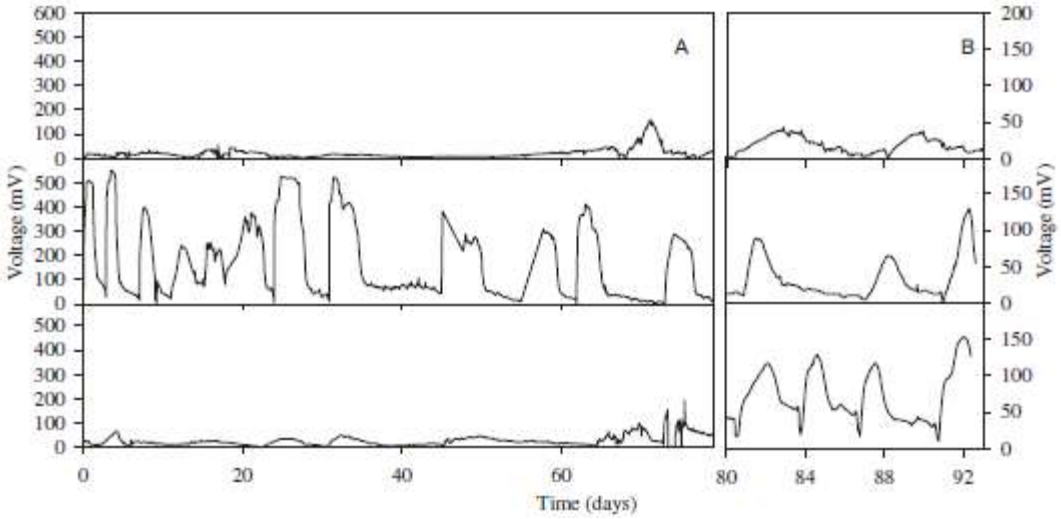

Fig. 6.1 MFC performance with the three different inoculation strategies tested. Top: ST1, direct replacement of acetate for methanol. Middle: ST2, progressive replacement of acetate for methanol. Bottom: ST3, syntrophic consortium. (A) Inoculation period. (B) Operation with methanol as sole carbon source

Strategy	CE	P_{MAX} (mW)	R_{INT} (Ω)	Maximum power density (mW/m²)
ST1 (Methanol)	13.4 ± 3.1	0.008 ± 0.003	3080	0.84
ST2 (acetate + methanol)	14.5 ± 1.2	0.017 ± 0.003	1575	1.19
ST3 (syntrophic consortium)	26.7 ± 1.0	0.021 ± 0.002	966	1.87

Table 6.1 MFC performance characterization with methanol as sole carbon source for the three inoculation strategies presented

The cell inoculated with ST3 was maintained for a longer term (Figure 6.2). Its performance was enhanced and reached an increase up to ten fold on power output (220μW). These results are comparable to previous values (250-300μW) obtained using acetate as sole carbon source in other studies with the same cells. In addition, these values are also comparable to those reported with conventional carbon sources. In analogous configurations, (Logan et al., 2007) obtained a power output of 325μW feeding acetate as carbon source and (Liu and Logan, 2004) obtained 270.4μW feeding glucose. The highly comparable values obtained here represent a high spot of this work since this is, to best of our knowledge, the first report of a methanol-driven MFC in the literature.

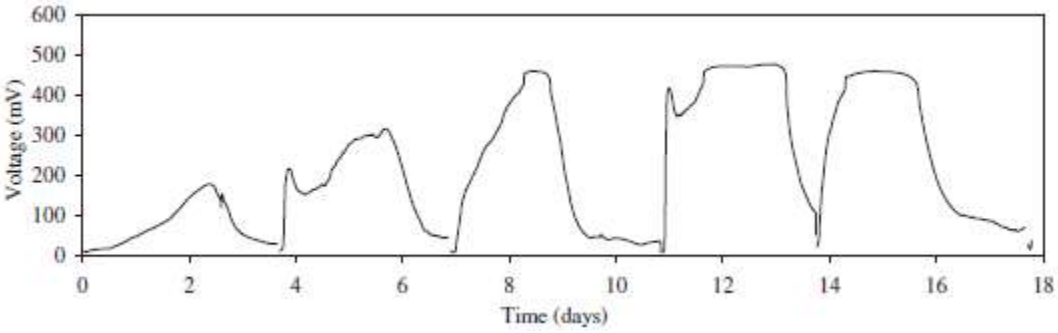

Fig. 6.2 Performance evolution of the methanol-driven MFC with a syntrophic consortium (ST3)

6.4.2 Practical implications

The results presented are not only significant in terms of methanol utilization in MFC systems but the approach used in this work could open a new range of possibilities and, similarly, other complex substrates can be used as electron donors for bioelectrosynthesis. The syntrophic consortium was developed in the biofilm (i.e. the biological activity in the suspended liquid was negligible). Growing the consortium as biofilm is interesting in view of practical implementation, because: (i) a pre-treatment tank to carry out the fermentation could be omitted, (ii) slow growing biomass in the biofilm is protected against washout when operating at low hydraulic retention times and (iii) operation at low hydraulic retention time would decrease the chances for other non-desired communities such as methanogenic bacteria to grow.

Methanol was not among the reported substrates in bioelectrochemical systems and therefore its potential for power generation in MFC was unknown. The work presented becomes relevant when the aim is using biodiesel waste water streams in BES, where methanol is commonly found. Glycerol from biodiesel, and methanol as impurity, could be effectively used a substrate for current production in single chamber MFC.

6.5 Conclusions

A syntrophic consortium of fermentative and exoelectrogenic bacteria was developed aiming at improving the starting-up step of a methanol-driven MFC. The cell inoculated with this consortium, reached about twofold CE and power output as well as lower internal resistance than other inoculation strategies concerning direct replacement of acetate for methanol and a progressive replacement of acetate for methanol.

The development of such anodic consortium allowed current generation in MFC, where homoacetogenic bacteria metabolized methanol to acetate, playing a key role in this system. Power output reached 220μW, values comparable to those obtained with readily biodegradable carbon sources.

Capítol 7

Conditions for high resistance to starvation periods in Microbial Fuel Cells

Part del contingut d'aquest capítol es troba publicat segons la referència que segueix:

Ruiz, Y., Ribot-Llobet, E., Baeza, J. A., & Guisasola, A. (2015). Conditions for high resistance to starvation periods in bioelectrochemical systems. Bioelectrochemistry, 106, 328–334. https://doi.org/10.1016/j.bioelechem.2015.06.010

Capítol 7. Conditions for high resistance to starvation periods in Microbial Fuel Cells

7.1 Abstract

The present work aims at understanding the performance of MFC when subjected to different starvation periods, which is relevant in view of their industrial application or use as biosensor. The results show that microbial fuel cells (MFC) could resist starvation periods up to 10-11 days without any significant decrease in their performance when endogenous consumption was enabled by closing the circuit in MFC. By contrast, starvation periods longer than 5 days when the flow of electrons from the anode to the cathode was not permitted, thereby avoiding endogenous consumption, led to a reversible decrease in the cells performance. A longer starvation period of 21-days under open-circuit caused an irreversible performance loss of the MFC.

7.2 Introduction

BES have been mainly applied for producing electricity from wastewaters. However, the enormous research conducted in the last years on this field has resulted in a plethora of alternative applications: from microbial electrosynthesis to bioremediation or biosensors development. With respect to the latter, different biosensors, in which anodic exoelectrogenic bacteria act as the biological sensing element, have been proposed so far for the measurement of biological oxygen demand (Chang et al., 2004; Di Lorenzo et al., 2009; Zhang and Angelidaki, 2011), microbial activity (Liu et al., 2011), toxicity (M. Kim et al., 2007; Patil et al., 2010; Nienke E Stein et al., 2012; Nienke Elisabeth Stein et al., 2012), dissolved oxygen (Zhang and Angelidaki, 2012) and single molecules such as glucose (Kumlanghan et al., 2007), lactate (J. M. Tront et al., 2008), volatile fatty acids (Kaur et al., 2013; J.M. Tront et al., 2008) and arabinose (Golitsch et al., 2013).

However, when dealing with biological processes, one has to be aware of the need to maintain bacterial activity. In this frame, starvation periods (i.e. periods in absence of substrate) can often occur in real systems due to technical plant stops. In this scenario, it is especially important to know i) how detrimental this period will be to biomass activity and ii) what are the best conditions for biomass maintenance if starvation is unavoidable. Regarding the particular case of biosensors, starvation periods will occur as part of their usual operation, during which the maintenance of an active ARB-enriched biomass must be ensured.

For this reason, it is essential to understand the behaviour of BES under different periods of starvation as well as to estimate how detrimental will these periods be to the biomass and, finally, which are the best conditions for the biomass in case starvation is unavoidable. To the best of our knowledge there is little information about starvation in both MFC and MEC. In (Oh and Logan, 2007) the relationship between starvation and voltage reversal in two MFC stacked together was studied and it was concluded that starvation periods up to 25 hours were detrimental for the MFC performance. However, this detrimental effect could have been caused not only by starvation but also by the voltage reversal experienced by the cell. In contrast, (Kaur et al., 2014) evaluated starvation as strategy to

avoid electron losses derived from methanogenesis and the results suggested that 12 days of starvation were not overly harmful for ARB in MFC. Nevertheless, only closed loop starvation conditions were tested and the causes of such high resistance to starvation were not addressed. (Gao et al., 2014) studied the syntrophy between biopolymer-accumulating bacteria and ARB both in MFC and MEC, which allowed current generation without the addition of an external substrate for several days. Moreover, the system recovered the initial current generation when external substrate was added. Experiments were conducted by operating both MFC and MEC in two chamber configuration and with a set anode potential at -0.4 vs Ag/AgCl, but no experiments were performed in open loop for MFC or without applied voltage in MEC. Therefore, considering the few works in the literature where BES starvation was studied and the lack of a systematic evaluation, the aim of this study is to shed light on the effect of starvation processes in MFC under a wider set of operating conditions. Open-circuit and closed-circuit conditions for MFC were studied in order to know which condition is better to maintain ARB activity and where is the limit of starvation time for each case.

7.3 Materials and methods

7.3.1 Reactors set-up

MFC consisted of a 30 mL cylindrical cell with a lateral 3 cm diameter aperture where the cathode was assembled (7.07 cm^2). The cathode was carbon cloth coated with carbon powder and platinum suspension on the inner side (0.5 mg/cm^2, ETEK C1-10 10% Pt on Vulcan XC-72, ElectroChem Inc), whereas the outer side was coated with a polytetrafluoroethylene (PTFE) solution, which permitted oxygen diffusion into the cell while preventing water leakage (Cheng et al., 2006c, 2006b). The anode was a carbon fibre brush (PANEX®33 160 K, ZOLTEK) (Logan et al., 2007) of 20 mm diameter x 25 mm length wound into a titanium core. It was thermally treated at 450 ºC for 30 min prior to its utilization to enhance biomass adhesion (Wang et al., 2009a). The two electrodes, spaced 2 cm apart, were connected through an external resistance (1000 Ω or 100 Ω). Current intensity was determined from the monitoring of the voltage drop across

this resistance. A reference electrode (Ag/AgCl NaCl 3M, model RE-1B, BAS Inc) was placed inside the cell.

Voltage evolution was monitored by means of a 16-bit data acquisition card (Advantech PCI-1716) connected to a personal computer with a software developed in LabWindows CVI 2013 for data acquisition.

7.3.2 MFC operation

Starvation tests were conducted in two MFCs. The effects of different starvation periods in MFCs were studied by maintaining the cell in closed-circuit (MFC_{CC}) and in open-circuit (MFC_{OC}) during these periods. The external resistance was 1000 Ω and 100 Ω for MFC_{CC} and MFC_{OC}, respectively. In the case of MFC_{OC}, the external resistance was only connected under feast conditions (i.e. substrate presence).

The cell was filled with medium without acetate before each starvation period to ensure fully starvation conditions. The medium was replaced with fresh medium with substrate for one batch cycle after each starvation period. Cycles after starvation periods were monitored to evaluate the effects of each starvation period on the cell performance. The cell was filled again with medium without acetate once current density started to decrease, provided that the performance was similar previous to any starvation period and a recovery time with substrate was not required. All experiments were conducted at room temperature (T = 25 ^{o}C).

7.3.4 Electrochemical analyses

Power and polarization curves were obtained with a multi-resistance board which allowed changing the external resistance between 470000 and 25 Ω. The cell was left in open circuit (OC) for 1 hour previous to any measurement. A 10 min period was used for voltage stabilization at each resistance. The voltage drop across each resistance was measured by means of a multimeter. Medium was renewed previous to polarization curves recording.

7.4 Results and discussion

7.4.1 Starvation in MFC systems

The effect of starvation periods was tested in MFCs under different conditions: (i) closed-circuit (MFC_{CC}) and (ii) open-circuit (MFC_{OC}) both in the short and in the long term.

Figure 6.1A summarizes the experimental current density profiles of MFC_{CC} subjected to different short term starvation periods. The starvation periods ranged from 3 to 11 days. Current density profiles did not show any significant variation with respect to the initial batch cycle, where a current density of 2.8 mA/m^2 was reached. This indicated that short term starvation periods (up to 11 days) under closed-circuit mode did not have any detrimental effect on the MFC operation. Moreover, during the starvation periods, the current density remained positive indicating that electrons were flowing from the anode to the cathode regardless of the lack of substrate in the medium.

Table 7.1 compares the experimental coulombic efficiency and maximum power of the different batch cycles. Both coulombic efficiency and power remained practically constant in all cycles in agreement with the observed current density profiles. Coulombic efficiency ranged between 23 and 27 %, whereas maximum power density was in a narrow range between 1.3 and 1.4 mW/m^2 in all batch cycles.

Under feast conditions, the anode potential (Figure 1B) reached a constant value in all the cycles (-210 mV vs Standard Hydrogen Electrode, SHE). Under starvation conditions (i.e. substrate absence), the anode potential showed a sharp increase to +260 mV vs SHE and, then, a slower increase. The anode potential profiles remained similar after each starvation period.

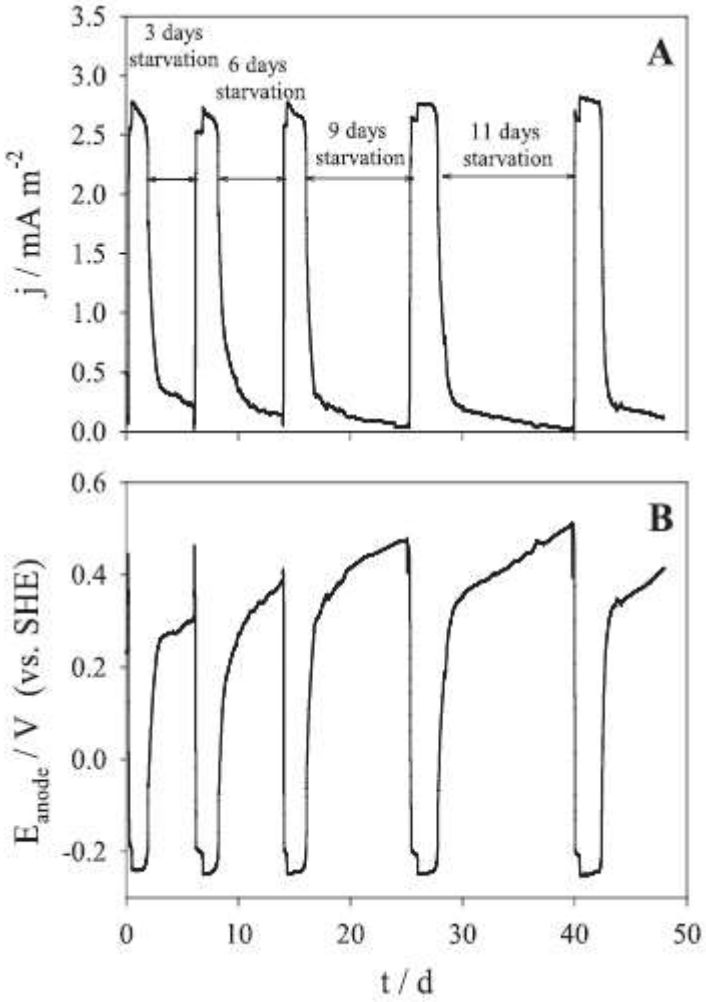

Fig. 7.1 Starvation experiments in MFC under closed-circuit conditions (MFC_{cc}). (A) Current intensity and (B) anode potential

Condition	Cycle	Starvation lenght (days)	CE (%)	Power density (mW/m^2)
MFC_{CC}	0	-	23.6	1.4
	1	3	24	1.3
	2	6	23	1.4
	3	9	26,7	1.4
	4	11	24,8	1.4
MFC_{OC}	0	-	38,9	1.2
	1	2	32,5	1.1
	2	5	35	1.1
	3	7	21,6	0.7

Table 7.1 Experimental coulombic efficiency (CE) and maximum power density of MFC after different starvation periods. Results for closed-circuit (MFCcc) and open-circuit (MFCoc) are presented.

Figure 7.2 displays the experimental current density profiles of MFC_{OC} obtained after short term starvation periods. MFC_{OC} was subjected to 2, 5 and 7 days of starvation. The selected starvation periods for MFC_{OC} were different than for MFC_{CC} since the resistance to starvation under open circuit was expected to be lower. Therefore, starting with a period of 2 days seemed more convenient. Moreover, starvation periods of both 9 and 11 days were not tested because, as discussed below, after 7 days without substrate, a performance decrease was already observed. The current density profile of those cycles after 2 and 5 days of starvation was very similar to that of the initial batch cycle, in which a current density of 8.3 mA/m^2 was achieved. However, the maximum current density after 7 days of starvation in open-circuit mode decreased to 6.1 mA/m^2, which means a decrease of more than 25 %. Hence, the activity was affected within one week of starvation. Nevertheless, subsequent cycles with substrate allowed a fast recovery of the initial cell activity (results not shown). This decrease in performance was not caused by oxygen diffusion to the anode. A natural biofilm on the cathode (which was visible with the naked eye) ensured fully anaerobic conditions in the anode even when substrate was not available (Ahmed et al., 2011; Montpart, 2014).

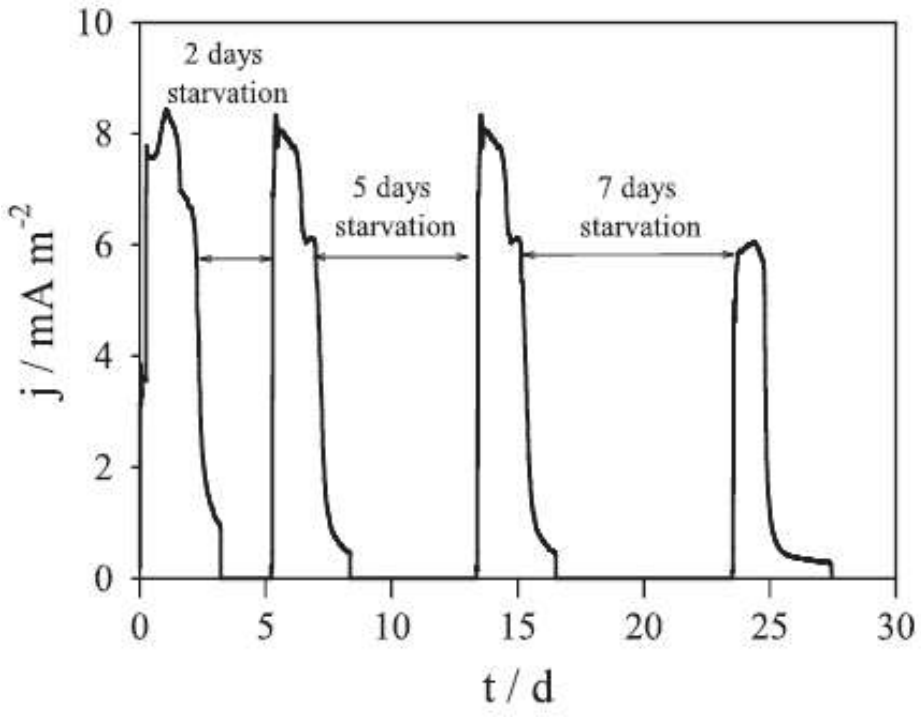

Fig. 7.2 Current density in MFC starvation experiments under open-circuit conditions (MFCoc)

Table 7.1 presents the coulombic efficiency and power of MFC_{OC} after the short-term starvation periods. As observed, the initial coulombic efficiency was 38.9 % and remained fairly constant after 2 (32.5 %) and 5 (35.0 %) days. The same occurred to the maximum power density, which remained practically constant (1.2-1.1 mW/m^2). Coulombic efficiency decreased to 21.6 % and power density to 0.67 mW/m^2 after 7 days of starvation, as expected according to the current density profiles. The initial coulombic efficiency and power values were again recovered after some cycles with substrate.

As previously stated, short term starvation did not show any irreversible negative effect on the MFC operation. MFC_{OC} performance only decreased after 7 days of starvation and could be easily recovered. Hence, it was decided to increase the starvation period to 21 days for both cases (closed and open-circuit). The power and polarization curves obtained before and after the long-term starvation experiment are shown in Figures 3A and 3B, respectively. The initial power curves reached a maximum power density of around 3.5 and 3.2 mW/m^2 for MFC_{CC} and MFC_{OC}, respectively. The initial polarization curves, meanwhile, showed similar open-circuit potentials (OCP) and maximum current density values. The maximum power density of MFC_{CC} after a 21-days starvation period severely decreased to a value of 1.7 mW/m^2 and the current density at this maximum power density was 3 times lower (3.3 mA/m^2) than the initial value (9.4 mA/m^2). The OCP value remained the same, although the maximum current density decreased to 6.1 mA/m^2.

Figures 3C and 3D show the experimental current density and anode potential profiles of MFC_{CC} during the long-term starvation experiment. After a 21-days starvation period, MFC_{CC} reached a lower current density (1.8 mA/m^2) with respect to the initial batch cycle (2.7 mA/m^2). Hence, 21 days of starvation under closed-circuit conditions were clearly negative for the cell operation. Nevertheless, as shown in Figure 3C, MFC_{CC} could recover its initial activity with an additional batch cycle with substrate.

Regarding open-circuit conditions (MFC_{OC}), the maximum power density after 21 days in starvation was far lower ($8 \cdot 10^{-3}$ mW/m^2) than its initial value (3.2 mW/m^2). The current density at this point fell from 8.9 mA/m^2 to 0.1 mA/m^2. The

polarization curve showed that both the OCP and the maximum current density drastically decreased to 170 mV and 0.1 mA/m^2, respectively. Several batch cycles with substrate were later performed but the cell could not recover its activity, suggesting an irreversible damage after a 21-days starvation period under open-circuit conditions.

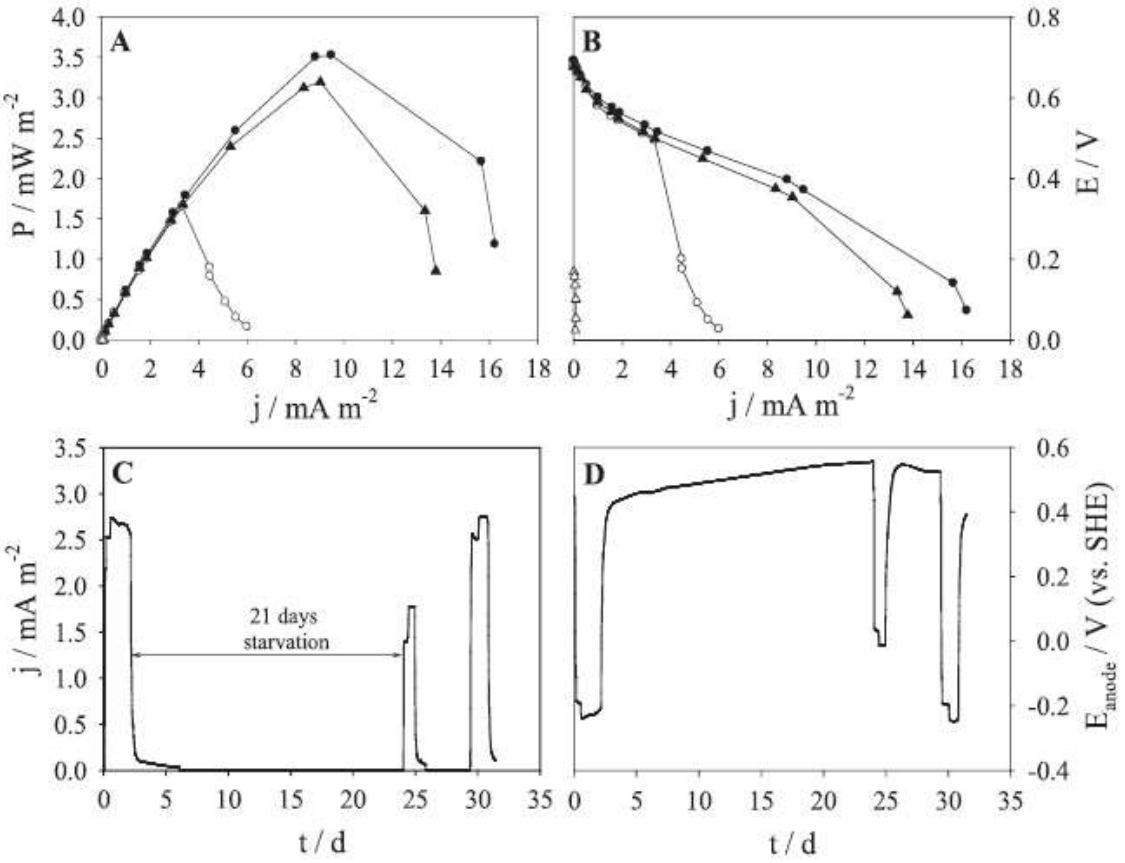

Fig. 7.3 MFC performance after a 21-days starvation period. (A) Power density and (B) polarization curves comparing the initial MFC performance (filled symbols) with the performance after the 21-days starvation period (open symbols) under closed (MFCcc, cyrcles) and open-circuit conditions (MFCoc, triangles). (C) Experimental current density and (D) anode potential of MFC under closed-circuit conditions (MFCcc)

The results show that short starvation periods (up to 11 days) are not detrimental to MFC performance under closed circuit conditions. Longer starvation periods (21 days) under closed circuit conditions negatively affected MFC performance, although it could be easily recovered after a short period with substrate. MFC are usually operated under batch mode and, as such, subjected under alternating feast and starvation conditions. Biomass can easily adapt to this scenario by

using storage polymers as carbon and energy source under famine conditions. This observation has been already detailed in conventional activated sludge systems (Sin et al., 2005). Hence, under alternating feast and starvation conditions, the population selected is able to survive under substrate limitation by accumulating substrate as a polymer inside of the bacterial cell under high substrate concentrations and consumed under starvation conditions. According to the literature this could be accomplished either by only ARB (Freguia et al., 2007) or by a syntrophic consortium (Gao et al., 2014). This endogenous behaviour can explain the observed current density under the starvation periods under closed-circuit conditions. The results in closed-circuit suggest that the performance decrease observed in (Oh and Logan, 2007) after 25 h of starvation were probably due to voltage reversal rather than the starvation itself. On the other hand, our results are in agreement with those in (Gao et al., 2014) and (Kaur et al., 2014), where acetate-fed MFC were subjected to 7 and 12 days of starvation, respectively. In the former case, the current density recovered its initial value, whereas in the second case, the performance of the cell was only slightly affected by 12 days without substrate. Moreover, (Chang et al., 2004) showed that an MFC usually fed with glucose and glutamic acid recovered its initial current intensity after 12 days of starvation. However, a recovery time was required in this case.

On the other hand, when the cell was maintained under open-circuit conditions, the utilization of substrate accumulation was not feasible because there was no electron sink. This is probably the reason why after only 7 days of starvation in open-circuit conditions MFC lost activity and after a 21-days starvation period the anode was irreversible damaged. Hence, this is the first report showing that the optimal way to maintain an MFC under starvation conditions is under closed-circuit conditions.

7.5 Conclusions

MFC under closed-circuit conditions during starvation periods displayed a high resistance to starvation, probably due to the consumption of the accumulated substrate as polymer inside the bacterial cell. In this sense, bacterial activity in

MFC was not affected by a period up to 11 days without substrate. Longer starvation periods (21 days) only showed a reversible decrease in its performance.

Nevertheless, when the accumulated substrate could not be consumed by exoelectrogenic bacteria during starvation periods, i.e. when the circuit was kept open, the performance after starvation periods drastically decreased. Starvation periods longer than 5 days produced a negative effect on the cells performance, although it was recovered after one or two cycles with substrate. However, a 21-days open-circuit starvation period in MFC caused an irreversible deleterious effect on the biological anode. Thus, for long starvation periods the utilization of the accumulated substrate has to be enabled by closing the circuit in MFC.

Capítol 8

Conclusions Generals

Capítol 8. Conclusions generals

El principal objectiu d'aquesta tesi va ser entendre i conèixer aspectes bàsics però de gran importància en el funcionament dels sistemes bioelectroquímics, per tal de poder iniciar una nova línia de recerca al departament.

Aquesta secció resumeix les principals aportacions d'aquesta tesi en el camp de la bioelectroquímica així com les conclusions que se'n poden extreure.

- Es va desenvolupar un procediment simplificat i eficient per a seleccionar bacteris exoelectrògens a partir de llots anaerobis formant una biopel·lícula al voltant de l'ànode. Va quedar demostrat que aquesta configuració era satisfactòria per seleccionar comunitats exoelectrògenes a baix cost i baix manteniment. Els ànodes inoculats amb aquest procediment van mostrar resultats comparables amb altres mètodes més costosos i complexes. El procediment no requereix potenciostat, aireació externa, agitació ni membranes. Els elèctrodes (l'ànode i el càtode) es poden posar pròxims un a l'altre per tal de disminuir la resistència interna i, al mateix temps, gràcies a que l'ànode està submergit en el llit de llots anaeròbics, fa que estigui en condicions estrictament anaeròbies.

- El pH inicial del càtode en una MEC de dues cambres sembla no tenir un efecte rellevant en l'activitat de la cel·la, tot i que els estudis electroquímics usant LSV indicaven canvis en el comportament del càtode per dos dels materials estudiats (llana d'acer inoxidable i sulfat de molibdè). Després dels experiments es va comprovar que independentment del pH inicial (7 o 12) tots els càtodes acabaven en valors compresos entre 11.3 i 11.7.

- El tipus de catalitzador escollit en el càtode d'una MEC de doble cambra és un dels factors més important pel que fa a l'activitat de la cel·la. Els resultats van mostrar que el platí és el material que obté produccions d'hidrogen i eficiències més altes tal i com ja era d'esperar. En segona posició va ser l'escuma de níquel, que actuava en dos sentits, per una banda el poder catalitzador el níquel, tot i que inferior a la del platí, es va veure compensat per l'alta àrea específica del material. Per altra banda, tot

i que la llana d'acer inoxidable va tenir l'activitat més baixa, s'ha de tenir en compte que és material de molt baix cost pel que disminueix considerablement el cost de la cel·la. Per tant, l'elecció final del catalitzador haurà de ser un compromís entre eficiència, producció i cost del material.

- Es va desenvolupar un consorci sintròfic constituït per bacteris fermentatius i bacteris exoelectrògens amb l'objectiu de maximitzar l'activitat d'una MFC alimentada amb metanol. Les cel·les inoculades amb aquest consorci van mostrar millors resultats en termes d'eficiència coulòmbica i potència generada que les altres dues estratègies plantejades, per una banda la substitució directa d'acetat per metanol, i la substitució progressiva d'acetat per metanol. El desenvolupament d'aquest consorci va permetre la generació de corrent en la MFC, on els bacteris homoacetògens metabolitzaven el metanol cap a acetat, jugant així un paper clau en el funcionament de la cel·la. El valor de potència obtingut (220 μW), va ser equiparable als valors obtinguts amb fonts de carboni ràpidament biodegradables.

- Les MFC en circuit tancat van mostrar tenir una alta resistència en períodes d'absència de substrat, segurament degut a que durant aquest període els bacteris van consumir el substrat acumulat en forma de polímer dins la cel·la microbiana. En aquest sentit, l'activitat bacteriana no es va veure afectada per períodes de fam de fins a 11 dies. En períodes més llargs (21 dies) solament va mostrar una disminució de la seva activitat però es va recuperar després d'un cicle amb substrat. Per contra, quan les MFC es trobaven en circuit obert, els bacteris exoelectrògens no podien consumir el substrat acumulat durant el període de fam i l'activitat de la cel·la va disminuir dràsticament. En períodes curts de fins a 5 dies, van necessitar entre 1 i 2 cicles amb substrat per poder recuperar l'activitat prèvia, mentre que un període de 21 dies va causar danys irreversibles en l'activitat biològica de l'ànode.

Capítol 9

Referències

Capítol 9. Referències

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