BIOELECTROCHEMICAL TRANSFORMATION OF CARBON DIOXIDE TO TARGET COMPOUNDS THROUGH MICROBIAL ELECTROSYNTHESIS

Pau Batlle Vilanova

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Bioelectrochemical transformation of carbon dioxide to target compounds through microbial electrosynthesis

Pau Batlle Vilanova

2016

EXPERIMENTAL SCIENCES AND SUSTAINABILITY PhD PROGRAMME

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PhD thesis submitted to aim for PhD degree for the University of Girona
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The present thesis has been written as published peer reviewed articles compendium based on the specific regulations of the PhD program of the University of Girona.

Peer reviewed publications that are presented as chapters of this thesis and the candidate PhD contribution in each publication is listed below:


Chapters of this PhD thesis are under preparation to be submitted as journal article and the candidate PhD contribution is listed below:

Author's contribution: Experimental design. Reactor operation and supervision. Writing the manuscript.

Author's contribution: Experimental design and performance. Data monitoring and reactor operation. Writing the manuscript.
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<td>AD</td>
<td>Anaerobic digestion</td>
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<tr>
<td>AEM</td>
<td>Anion exchange membrane</td>
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<tr>
<td>BE</td>
<td>Biphasic esterification</td>
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<tr>
<td>BES</td>
<td>Bioelectrochemical Systems</td>
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<tr>
<td>CA</td>
<td>Chronoamperometry</td>
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<tr>
<td>CCS</td>
<td>Carbon capture and storage</td>
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<td>CE</td>
<td>Coulombic efficiency</td>
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<tr>
<td>CEM</td>
<td>Cation Exchange membrane</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen demand</td>
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<tr>
<td>CCV</td>
<td>Closed circuit voltage</td>
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<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>CX</td>
<td>X-carbon compound</td>
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<tr>
<td>DET</td>
<td>Direct electron transfer</td>
</tr>
<tr>
<td>DIET</td>
<td>Direct interspecies electron transfer</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
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<tr>
<td>E₀</td>
<td>Standard electrode potential</td>
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<tr>
<td>Ean</td>
<td>Anode potential</td>
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<td>Ecat</td>
<td>Cathode potential</td>
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<td>Ecell</td>
<td>Cell potential</td>
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<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gases</td>
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<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
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<td>ME</td>
<td>Membrane electrolysis</td>
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<td>MEC</td>
<td>Microbial electrolysis cell</td>
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<tr>
<td>MES</td>
<td>Microbial electrosynthesis</td>
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<td>MET</td>
<td>Mediated electron transfer</td>
</tr>
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<td>Abbreviation</td>
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<tr>
<td>MIET</td>
<td>Mediated interspecies electron transfer</td>
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<tr>
<td>MFC</td>
<td>Microbial fuel cell</td>
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<tr>
<td>mM/MC</td>
<td>milimolar of carbon</td>
</tr>
<tr>
<td>NAC</td>
<td>Net anode compartment</td>
</tr>
<tr>
<td>NCC</td>
<td>Net cathode compartment</td>
</tr>
<tr>
<td>( \eta_E )</td>
<td>Energy efficiency</td>
</tr>
<tr>
<td>OCV</td>
<td>Open circuit voltage</td>
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<tr>
<td>Ox</td>
<td>Molecule in its oxidised state</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PCR-DGGE</td>
<td>polymerase chain reaction-denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>( P_{CO_2} )</td>
<td>Carbon dioxide partial pressure</td>
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<td>( P_{H_2} )</td>
<td>Hydrogen partial pressure</td>
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<tr>
<td>q-PCR</td>
<td>Quantitative real-time Polymerase chain reaction</td>
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<tr>
<td>RE</td>
<td>Reference electrode</td>
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<tr>
<td>Red</td>
<td>Molecule in its reduced state</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SHE</td>
<td>Standard hydrogen electrode</td>
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<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>WE</td>
<td>Working electrode</td>
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Certificate of thesis direction

El Dr. Sebastià Puig Broch, i el Dr. Jesús Colprim Galceran del Laboratori d’Enginyeria Química i Ambiental (LEQUIA) de la Universitat de Girona; i el Dr. Rafael Gonzalez Olmos de l’Institut Químic de Sarrià (IQS) de la Universitat Ramon Llull,

DECLAREM:

Que aquest treball, titulat “Bioelectrochemical transformation of carbon dioxide to target compounds through microbial electrosynthesis”, que presenta Pau Batlle Vilanova per a l’obtenció del títol de doctor/a, ha estat realitzat sota la nostra direcció i que compleix els requeriments per poder optar a Menció Internacional.

I perquè així consti i tingui els efectes oportuns, signem aquest document.

Dr. Sebastià Puig Broch

Dr. Rafael Gonzalez-Olmos

Dr. Jesús Colprim Galceran

Girona,
Als meus pares i avis, la Jas i la Laia,
Energy cannot be created or destroyed, it can only be changed from one form to another – Albert Einstein.
Agraiments/Acknowledgements

En aquest moment culminant arriba l’hora d’agrar les petites o no tant petites contribucions que d’alguna manera m’han ajudat a arribar fins al final d’aquest llarg camí.

Primer de tot els meus agraiments són pels meus directors de tesi: En Jesús, en Sebas i en Rafa. Recordo les classes a la carrera amb en Jesús, probablement són les que mes em van marcar, d’aquí que tot hi haver marxat de Girona durant un any decidís posar-me altra vegada en contacte amb ell. No sé si em podria definir com un trànsfuga de la institució, però en tot cas això no va ser cap impediment per a què el 2012 m’obris les portes del LEQUIA per començar el meu doctorat. A toro passat, també voldria agrair les dures apretades rebudes, que se’ns dubte m’han ajudat a millorar. A en Rafa, per la seva proximitat, sobretot durant els primers moments, que sempre són més durs. Vull agrair-li també la confiança, el positivisme i sobretot els ànims rebuts des del moment zero. També per les seves ganes de provar coses diferents, que tot i que la majoria de vegades no ha estat possible, crec que m’ha fet obrir els ulls cap a altres àrees de recerca. A en Sebas agrair-li sobretot la confiança i les ganes d’anar mes enllà que sempre té, el fet de no conformar-se amb el que tenim d’alguna manera se m’han acabat incultant a mi també. Les idees sonades, i les no tant sonades que hem tingut oportunitat de compartir durant aquest temps. L’esperit crític i el nivell d’exigència que m’ha imposat des del principi. El positivisme i consells els moments baixos, tant pel que fa a nivell científic, com personal. Crec que podríem dir que ha estat guia i company de trajecte. Finalment a la Marilós, tutora i directora a l’ombra, agrair-li la seva paciència, i la transferència d’aquesta vessant de la ciència que jo anomenaria “storytelling”. Si durant aquest temps he millorat pel que fa a l’escriptura es se’ns dubte gracies a ella. També el positivisme que transmet, que moltes vegades et fa sentir que les coses no són tant difícils com poden semblar en un primer moment. Un cop mes gracies a tots tres per tot el que m’heu transmès durant aquest temps.

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During my thesis I had the opportunity to undertake my research stay in Brisbane (Australia), which allowed me to learn how research is conducted in other places. For this, I acknowledge Dr. Bernardino Virdis and Dr. Stefano Freguia, who agreed to have me working in their labs. Special thanks to Dino, with who I had the opportunity to work more closely, learn from his experience, and share opinions and discussions. Also to my lab mates, and not lab mates who are made my stay in Australia undoubtedly more enjoyable: Dasomm, Elisa, Frauke, Guillermo, Ignasi, Igor, Mette, Natacha, Nils, Rita, Sergi, and Tim.

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A aquells companys, i amics de carrera i màster que em van fer donar una mica més de mi mateix, especialment Jaume i Miquel.

Finalment a la colla d’amics, que m’han ajudat a desconectar quan ho he necessitat, i amb els que he compartirit i segueixo compartint gran part del meu temps, i que així sigui per molts d’anys. Albert, Ari, Cris, Eva, Fres, Joan, Laia R., Noé, Pau R., Pau S., Pollo, Txus, Xavi, Xevi i Yolanda.

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Resum

L'augment de diòxid de carboni (CO₂) a l'atmosfera amenaça els ecosistemes i la nostra forma de vida tal com la coneixem. La gran majoria de les emissions de CO₂ són degudes a la crema de combustibles fòssils. Per tant, existeix una necessitat de desenvolupar tecnologies eficients per disminuir les emissions de CO₂ i produir (bio)combustibles neutres de forma sostenible. Avui en dia, s’utilitza el que es coneix com a captura i emmagatzematge de carboni per mitigar les emissions de CO₂ a l'atmosfera. Per altra banda, també s’investiguuen diferents mètodes (químics, fotoquímics, electroquímics, biològics o inorgànics) de transformació de CO₂. En aquest sentit, els sistemes bioelectroquímics (BES) representen un nou enfocament prometedor, a través dels quals el CO₂ pot ser reduït a diferents compostos utilitzant electricitat provinent de fonts renovables, en un procés conegut com electrosíntesi microbiana (MES). La MES té lloc al biocàtode d’un BES, on els microorganismes creixen de forma autotròfica utilitzant CO₂ com a acceptor d'electrons i els electrons en forma d'electricitat com a donador d'electrons. Durant el procés es poden produir diferents compostos depenent de les possibilitats metabòliques dels microorganismes presents en el sistema. Degut a que la MES és una tecnologia incipient, aquesta tesi té com a objectiu utilitzar cultius mixtes seleccionats de forma natural per avaluar la producció de diferents compostos a partir de CO₂ com a única font de carboni.

En primer lloc es va demostrar que l'hidrogen pot ser generat en el biocàtode com un donador d'electrons alternatiu, per augmentar la disponibilitat de poder reductor. Ja que els microorganismes autòtrofs poden dur a terme la reducció de CO₂ a través de la via Wood-Ljungdahl, la producció d'hidrogen in-situ representa un avantatge en comparació amb la producció, tractament i transport externs. Encara que els elèctrodes de carboni permeten la producció d'hidrògen a potencials de reducció molt baixos, la presència de microorganismes no només va afavorir la seva producció a potencials de càtode més elevats, sinó que també es va produir amb un menor requeriment d’energia. Sent l’hidrògen el principal intermedi per a la reducció de CO₂ en BES, es va procedir a estudiar la producció d’altres compostos.
Es va demostrar la producció de metà, essent \textit{Methanobacterium} sp. el principal responsable de la seva producció a través de metanogènesi hidrogenotrófica. En aquesta tesi la producció bioelectroquímica de metà es va dur a terme en batch i en contínua a potencials catòdics < -0.6 V vs SHE, i va augmentar quan es va disminuir el temps de residència hidràulic (TRH) del biocàtode. En aquesta tesi es va assolir la velocitat de producció de metà més alta en termes volumétrics (100 mmol CH\textsubscript{4} L\textsuperscript{-1} d\textsuperscript{-1}) fins a la data. Aquest valor representa només la meitat de la velocitat de producció de CO\textsubscript{2} estimada en la digestió anaeròbia. Per tant, l’ús d’aquesta tecnologia podria ser una alternativa a les tècniques de millora de biogàs convencionals per a la producció de biometà, el qual està sent investigat.

La producció de compostos líquids com ara VFA, també es va investigar. La producció continuada d’acetat es va dur a terme a un potencial catòdic de -0,6 V vs SHE, i la velocitat de producció va augmentar quan el pH es va controlar a un valor lleugerament àcid (pH de 5,8). No obstant això, l’operació en continu del sistema no permetia la producció d’efluents amb concentracions elevades. L’operació en batch va permetre augmentar la concentració d’acetat, i a més es van obtenir butirat i alcohols com a productes finals de MES. Es va investigar el paper que juga la disponibilitat dels principals substrats, revelant que la disponibilitat d’hidrògen juga un paper clau en la producció de productes més reduïts (butirat i alcohols). L’operació del biocàtode en condicions de limitació de CO\textsubscript{2}, va afavorir l’augment de la pressió parcial d’hidrogen (P\textsubscript{H2}) al biocàtode i va desencadenar la producció de compostos amb un grau de reducció més elevat. Així, la limitació de CO\textsubscript{2} va resultar en la producció selectiva de butirat. Llavors, l’extracció selectiva i concentració de butirat es van investigar a través de l’extracció amb membra, obtenint una fase d’extracció altament concentrada en butirat.

En conclusió, els resultats presentats en aquesta tesi recolzen el potencial tecnològic dels BES per convertir-se en una alternativa per a la producció de diferents compostos a partir de CO\textsubscript{2}.  

xx
Abstract

The increasing level of carbon dioxide (CO₂) in the atmosphere is threatening ecosystems and humankind. The vast majority of CO₂ emissions are due to the burning of fossil fuels. Therefore, efficient technologies need to be developed to decrease CO₂ emissions and to sustainably produce carbon-neutral (bio)fuels. Nowadays, carbon capture and storage is applied to mitigate CO₂ emissions in the atmosphere, pumping it into geological storages. At the same time, different CO₂ transformation technologies, such as chemical, photochemical, electrochemical, biological or inorganic, are being investigated. In this sense, bioelectrochemical systems (BES) represents a novel promising approach, by which CO₂ can be reduced to target compounds using renewable electricity, in a process known as microbial electrosynthesis (MES). MES takes place in the biocathode of a BES, where microorganisms grow autotrophically using CO₂ as electron acceptor and electrons in form of electricity as electron donor. During the process different compounds can be produced depending on the metabolic possibilities of the microorganisms present in the system. With MES being a nascent technology, this thesis aims to use naturally selected mixed cultures to evaluate the production of different compounds from CO₂ as the only carbon source.

First of all, it was demonstrated that hydrogen can be generated in the biocathode as an alternative electron donor, to increase the availability of reducing power. Since autotrophic microorganisms are supposed to carry out CO₂ reduction through the Wood-Ljungdahl pathway, the in-situ production of hydrogen represents an advantage compared to external production, treatment and transport. Although plain carbon-like electrodes can drive hydrogen production at very low cathode potentials, the presence of microorganism not only favoured its production at higher cathode potentials, but it was also produced with a lower energy requirement. Once hydrogen is produced in the biocathode, it can be used as intermediate to drive the production of other compounds.

Methane production was demonstrated, with Methanobacterium sp. being the main responsible for its production through hydrogenotrophic methanogenesis. In this thesis bioelectrochemical methane production took place in batch and
continuous operation at poised cathode potentials \(<-0.6 \text{ V vs SHE}\), and increased when the hydraulic retention time (HRT) of the biocathode was decreased. In this thesis maximum ever reported volumetric methane production rate from CO\(_2\) in a BES of 100 mmol CH\(_4\) L\(^{-1}\) d\(^{-1}\) was achieved. This value represents only the half of the estimated CO\(_2\) production rate during anaerobic digestion. Thus, the use of this technology could be an alternative to conventional biogas upgrading techniques for the production of biomethane, which is currently being investigated.

The production of liquid products such as VFA, was also investigated. Continuous acetate production took place in the biocathode of a BES at a cathode potential of -0.6 V vs SHE, and the production rate increased when the pH was controlled at a slightly acidic value (i.e. 5.8). However, the continuous operation of the system did not allow for the production of highly concentrated effluents. The operation was changed to fed-batch to increase the titers obtained. Thus, increased concentration of acetate was observed, and butyrate and alcohols were also obtained as concomitant end-products of MES. The role of the availability of the main substrates was investigated, revealing that the hydrogen availability is playing a key role in the production of more reduced products (butyrate and alcohols). The operation of the biocathode under CO\(_2\) limited conditions favoured the build up of hydrogen partial pressure (P\(_{H_2}\)) and triggered the production of compounds with a higher degree of reduction. Thus, limiting CO\(_2\) resulted in the selective production of butyrate. Then, selective extraction and concentration of butyrate was approached through membrane liquid extraction, obtaining an extraction phase highly concentrated in butyrate.

In conclusion, the results presented in this thesis support that BES have the potential to become and alternative technology for the production of different target compounds from CO\(_2\).
Resumen

El aumento de dióxido de carbono (CO₂) en la atmósfera amenaza los ecosistemas y nuestra forma de vida tal como la conocemos. La gran mayoría de las emisiones de CO₂ son debidas a la quema de combustibles fósiles. Por tanto, existe una necesidad de desarrollar tecnologías eficientes para disminuir las emisiones de CO₂ y producir (bio)combustibles neutros de forma sostenible. Hoy en día, se utiliza lo que se conoce como captura y almacenamiento de carbono para mitigar las emisiones de CO₂ a la atmósfera. Por otra parte, también se investigan diferentes métodos (químicos, fotoquímicos, electroquímicos, biológicos o inorgánicos) de transformación de CO₂. En este sentido, los sistemas Bioelectroquímicos (BES) representan un nuevo enfoque prometedor, a través de los cuales el CO₂ puede ser reducido a diferentes compuestos utilizando electricidad proveniente de fuentes renovables, en un proceso conocido como electrosíntesis microbiana (MES). La MES tiene lugar en el biocátodo de un BES, donde los microorganismos crecen de forma autotrófica utilizando CO₂ como acceptor de electrones y los electrones en forma de electricidad como donador de electrones. Durante el proceso se pueden producir diferentes compuestos dependiendo de las posibilidades metabólicas de los microorganismos presentes en el sistema. Debido a que la MES es una tecnología incipiente, esta tesis tiene como objetivo utilizar cultivos mixtos seleccionados de forma natural para evaluar la producción de diferentes compuestos a partir de CO₂ como única fuente de carbono.

En primer lugar se demostró que el hidrógeno puede ser generado en el biocátodo como un donador de electrones alternativo, para aumentar la disponibilidad de poder reductor. Ya que los microorganismos autótrofos pueden llevar a cabo la reducción de CO₂ a través de la vía Wood-Ljungdahl, la producción de hidrógeno in-situ representa una ventaja en comparación con la producción, tratamiento y transporte externos. Aunque los electrodos de carbono permiten la producción de hidrógeno a potenciales de reducción muy bajos, la presencia de microorganismos no sólo favoreció su producción a potenciales de cátodo más elevados, sino que también se produjo con un menor requerimiento de energía. Siendo el hidrógeno el
principal intermedio para la reducción de CO₂ en BES, se procedió a estudiar la producción de otros compuestos.

Se demostró la producción de metano, siendo *Methanobacterium* sp. el principal responsable de su producción a través de metanogénesis hidrogenotrófica. En esta tesis la producción bioelectroquímica de metano se llevó a cabo en batch y en continuo a potenciales catódicos < -0.6 V vs SHE, y aumentó cuando se disminuyó el tiempo de residencia hidráulico (TRH) del biocátodo. En esta tesis se alcanzó la velocidad de producción de metano más alta en términos volumétricos (100 mmol CH₄ L⁻¹ d⁻¹) hasta la fecha. Este valor representa sólo la mitad de la velocidad de producción de CO₂ estimada en la digestión anaerobia. Por tanto, el uso de esta tecnología podría ser una alternativa a las técnicas de mejora de biogás convencionales para la producción de biometano, lo cual está siendo investigado.

La producción de compuestos líquidos como VFA, también se investigó. La producción continua de acetato se llevó a cabo a un potencial catódico de -0,6 V vs SHE, y la velocidad de producción aumentó cuando el pH se controló a un valor ligeramente ácido (pH de 5,8). Sin embargo, la operación en continuo del sistema no permitía la producción de efluentes con concentraciones elevadas. La operación en batch permitió aumentar la concentración de acetato, y además se obtuvieron butirato y alcoholes como productos finales de MES. Se investigó el papel que juega la disponibilidad de los principales sustratos, revelando que la disponibilidad de hidrógeno juega un papel clave en la producción de productos más reducidos (butirato y alcoholes). La operación del biocátodo en condiciones de limitación de CO₂, favoreció el aumento de la presión parcial de hidrógeno (P_H₂), y desencadenó la producción de compuestos con un grado de reducción más elevado. Así, la limitación de CO₂ permitió la producción selectiva de butirato. Entonces, su extracción selectiva y concentración fue investigada a través de la extracción con membrana, obteniendo una fase de extracción altamente concentrada en butirato.

En conclusión, los resultados presentados en esta tesis apoyan el potencial tecnológico de los BES para convertirse en una alternativa para la producción de diferentes compuestos a partir de CO₂.
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Chapter 1. Introduction
1.1. Background

Antropogenic carbon dioxide (CO₂) emissions have caused the atmospheric concentration to rise from 280 ppm in the pre-industrial period to more than 400 ppm today (IPCC, 2013). This increasing level of CO₂ in the atmosphere is threatening ecosystems worldwide (Sala et al., 2000). Although CO₂ is not considered a pollutant itself, it is a greenhouse gas (GHG) known to be the responsible of many environmental issues. Not only does it increase earth temperature and cause global warming, but also cause ocean acidification (Doney et al., 2009). Different strategies could be applied to mitigate CO₂ emissions, which include (i) reducing global energy use from fossil fuels, (ii) sequestering emissions and (iii) developing carbon-neutral fuels, (Lal, 2008). Up to date, political efforts have been carried out by governments in order to reduce CO₂ emissions. However, constant breaches of the established protocols by the signing parts demonstrated that politics are not effective when CO₂ emissions are concerned. Williams et al., stated that GHG reduction will depend substantially on technologies that are yet to be commercialised (Williams et al., 2012). Therefore, technological solutions need to be developed to mitigate the global warming.

Technical solutions have been focused on CO₂ sequestering, such as carbon capture and storage (CCS), which is already applied in different parts of the world to decrease CO₂ emissions from power plants or industrial processes. This technology is based on the collection, compression, transport and storage of the CO₂ in geological deposits, such as depleted oil and gas fields, deep coal seams, and saline formations. Although the process is already applied, it is not economically feasible, and research is attempting to reduce capture costs. Other drawbacks include transport of the CO₂ from emission to storage sites. Besides, the identification of the short-term and long-term leakage pathways need to be studied more in depth, as it could represent a serious risk for the environment and human health (Larsen and Petersen, 2007).
On the other hand, chemical and biological CO$_2$ transformation are being investigated to produce a wide range of chemicals (Figure 1.1). Renewable energy harvesting, such as wind or solar, are necessary for the development of carbon neutral chemicals and fuels, since they do not imply GHG emissions. Solar energy could become our primary energy source if researchers were able to attain its full potential (Lewis and Nocera, 2006). However, the production of liquid fuels and commodity chemicals would still rely on fossil fuels.

![Figure 1.1. Range of products produced from CO$_2$.](image)

Many technologies are at the research and development stage, such as chemical, photochemical, electrochemical, biological, reforming, and inorganic transformations, which use CO$_2$ for the generation of so-called carbon-neutral fuels (Mikkelsen et al., 2010). From the applicability point of view, these technologies offer great potential because they allow for (i) the mitigation of CO$_2$ emissions, (ii) the transformation of CO$_2$ into valuable compounds, and (iii) the storage of electrical energy in liquid or gas compounds. The conversion of CO$_2$ and renewable energy into stable liquid or gas products makes them easier to store, transport, or use when renewable energy harvesting is limited. However, current CO$_2$ mitigation and conversion techniques present limiting factors, as they require extremely large surface and volumes, energy intense processing steps and/or chemicals and expensive catalysts (Haszeldine, 2009).
In biological CO$_2$ mitigation, CO$_2$ is biologically converted to organic compounds. CO$_2$-fixing microorganisms include algae, cyanobacteria, β-proteobacteria, Clostridia and Archaea, which can use different metabolic pathways to reduce CO$_2$ to organic compounds (Jajesniak et al., 2014). The most ancient carbon fixation pathways are photosynthesis and the Wood-Ljungdahl for homoacetogenic bacteria or acetogens, also referred to as the reductive acetyl-CoA pathway (Martin, 2012). The most interesting advantage of biological CO$_2$ sequestration is that CO$_2$ is converted into biomass and commercially valuable products, including bio-diesel, biofuels and other commodity products with high added value. Biological carbon fixation is drawing an increasing interest from the scientific community. These processes imply the use of energy, which usually comes from natural resources. Therefore, the energy efficiency is a key parameter that will directly affect the feasibility of the process.

1.1.1. Energy harvesting from natural resources

Photosynthetic autotrophic growth is the process by which plants convert CO$_2$ and water into biomass, using sunlight as energy source. This process is known as photosynthesis, and its efficiency in energy conversion is around 0.2 – 2.0% (Moore et al., 1995). The energy efficiency of photosynthesis by different organisms is shown in Table 1.1, together with the energy conversion efficiency of different renewable energy harvesting processes.

<table>
<thead>
<tr>
<th>Process</th>
<th>Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional photosynthesis</td>
<td>0.2 – 2.0 average by plants</td>
<td>(Moore et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>7.0 – 8.0 sugarcane</td>
<td>(Schenk et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>3.0 – 7.0 microalgae for biofuel production</td>
<td></td>
</tr>
<tr>
<td>Solar panels</td>
<td>12.0 – 18.0 average market</td>
<td>(SunPower datasheet, 2013)</td>
</tr>
<tr>
<td></td>
<td>21.5 SunPower X-Series solar panels</td>
<td></td>
</tr>
<tr>
<td>Wind turbines</td>
<td>44.0 – 46.0</td>
<td>(Tony Burton et al., 2001)</td>
</tr>
<tr>
<td>Hydro power</td>
<td>85.0 – 95.0</td>
<td>(Bxhorn, 2016)</td>
</tr>
</tbody>
</table>
During conventional photosynthesis, CO₂ is converted into biomass, which can be then used with different purposes such as the production of 1st generation biofuels. However, the production of first generation biofuels is limited by the competition for land and water used for food production (Sims et al., 2010). In this sense, photosynthetic CO₂ fixation with microalgae for the production of biofuel represents a promising field with a high potential (Chisti, 2007). However, the use of large surface areas or the high energy consumption required for photo-bioreactors represent the main drawbacks. An interesting alternative is the use of acetogenic bacteria, which can use the Wood-Ljungdahl pathway to drive the reduction of CO₂. This process could be driven in bioelectrochemical system (BES), using renewable electricity. Recently, the bioelectrochemical transformation of CO₂ into valuable compounds using electricity as reducing power source to produce carbon-neutral commodities and fuels was defined as microbial electrosynthesis (MES) (Nevin et al., 2010), which represents an interesting sustainable alternative to other existing processes. If high electricity conversion efficiencies are achieved during MES, the use of renewable electricity, such as solar, wind, and especially hydro power to drive the process, could exceed conventional photosynthesis in terms of energy efficiency.

1.2. Bioelectrochemical systems

BES are an emerging technology based on the ability of some bacteria to exchange electrons with a solid state electrode. The existence of bacteria that were able to extracellular electron transfer was first reported in 1910 (Potter, 1910). However, it was only after 100 years later that applications started to be identified and developed at the research level (Arends and Verstraete, 2012; Schröder, 2011). The scope of BES ranges from bioelectricity generation to other more complex processes such as bioremediation, fermentation, and chemical production.
BES usually consist of an anode and a cathode separated by an ion exchange membrane (Figure 1.2). In the anode, oxidation processes deliver electrons to the electrode and release protons to the medium. Protons diffuse to the cathode compartment through the membrane, while electrons flow through the electric circuit. In the cathode, protons and electrons are used to carry out reduction processes. Anode and cathode are usually referred to as bioanodes and biocathodes, when microorganisms are used to drive the respective reactions. BES can be used for different purposes, depending on the reactions occurring in each compartment (Rabaey et al., 2009). Arends and Verstraete (2012) classified BES into three different concepts depending on its application, which were the energy, product, and sustainability concepts.

![Figure 1.2](image-url)  

**Figure 1.2.** Basic schematic representation of bioelectrochemical systems (BES). Different BES configurations can be applied depending on the aim they are setup for. Anions or cations will migrate through an anion or cation exchange membrane, respectively. Chemically or biologically catalysed oxidations and reductions occur in the anode and cathode compartments, respectively. Depending on the thermodynamics of the overall reactions, energy would be produced or supplied to the BES. Adapted from Rabaey and Rozendal, 2010.
1.2.1. Thermodynamics of bioelectrochemical systems

The thermodynamics, and therefore the operation of the BES is determined by the redox potential of the reactions occurring in the anode and the cathode. Anode and cathode half-cell potentials are reported with respect to the standard hydrogen potential (SHE), which at standard conditions has a potential of zero. To calculate the theoretical half-cell potentials equation 1.1 is used (Logan, 2008).

\[
E_{An} = E_{An}^0 - \frac{RT}{nF} \ln \left( \frac{[\text{products}]}{[\text{reactants}]} \right) \quad (\text{Eq. 1.1})
\]

Where \( E_{An} \) is the half-cell potential of the anode (V), \( E_{An}^0 \) is the half-cell potential (V) at standard conditions, \( R \) is the universal gas constant (8.31 J mol\(^{-1}\) K\(^{-1}\)), \( T \) is the temperature (K), \( n \) represents the number of electrons involved in the overall process, and \( F \) is the Faraday’s constant (95485 C mol e\(^{-1}\)). The quotient is the ratio of the concentration of the products divided by the reactants, raised to their respective stoichiometric coefficients \( (p \text{ and } r) \). The cathode potential (\( E_{Cat} \)) can be calculated with the same equation.

The overall cell potential (\( E_{cell} \)) will determine the process spontaneity, which is calculated according to equations 1.2 and 1.3.

\[
E_{cell} = E_{cathode} - E_{anode} \quad (\text{Eq. 1.2})
\]

\[
\Delta G = -n \cdot F \cdot E_{cell} \quad (\text{Eq. 1.3})
\]

Where \( E_{cell} \) is the cell voltage (V), \( E_{Cat} \) and \( E_{An} \) are the half-cell potentials (V) for cathode and anode, respectively. \( \Delta G \) corresponds to the Gibbs free energy (J) of the overall process.

If the resulting Gibbs free energy is a negative value, the process will take place spontaneously, and energy will be produced in the form of electricity, which is the case of the so-called microbial fuel cells (MFC). Otherwise, it means that the process will not take place spontaneously; therefore, energy will be needed to drive the process. These kind of systems are known as Microbial electrolysis cells (MEC).
(Logan and Rabaey, 2012). Table 1.2 shows some of the most typical reactions carried out in BES so far. The spontaneity of the whole process relies on the cell potential of the whole process. The Gibbs free energy is affected by the experimental conditions, such as pH, temperature, pressure, internal resistances, etc. Thus, reactions that are theoretically spontaneous could become non spontaneous due to different factors. This is the reason why BES are usually operated at a poised cathode or anode potentials (potentiostatic mode), or with a fixed current supply (galvanostatic mode), to carry out the processes of interest.

### Table 1.2. Thermodynamic spontaneity calculation of different processes in BES, under standard conditions \( (T = 298 \text{ K}; P = 1 \text{ atm}; \text{pH} = 7) \). Data taken from Rabaey and Rozendal, 2010; Thauer et al., 1977.

<table>
<thead>
<tr>
<th>Anode reaction</th>
<th>( E^0_{\text{an}} )</th>
<th>Cathode reaction</th>
<th>( E^0_{\text{cat}} )</th>
<th>( E_{\text{cell}} ) electrons involved</th>
<th>( \Delta G^0 ) (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degradation of acetate</td>
<td>0.28</td>
<td>Reduction of oxygen</td>
<td>0.82</td>
<td>0.54</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrate reduction to nitrite</td>
<td>0.42</td>
<td>0.14</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of H(^+) to H(_2)</td>
<td>-0.41</td>
<td>-0.69</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of CO(_2) to methane</td>
<td>-0.24</td>
<td>-0.52</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of CO(_2) to acetate</td>
<td>-0.28</td>
<td>-0.56</td>
<td>8</td>
</tr>
<tr>
<td>Water oxidation</td>
<td>0.82</td>
<td>Nitrate reduction to nitrite</td>
<td>0.42</td>
<td>-0.40</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of H(^+) to H(_2)</td>
<td>-0.41</td>
<td>-1.23</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of CO(_2) to methane</td>
<td>-0.24</td>
<td>-1.06</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of CO(_2) to acetate</td>
<td>-0.28</td>
<td>-1.10</td>
<td>8</td>
</tr>
</tbody>
</table>

#### 1.2.2. Bioelectrochemical systems applications

Traditionally BES were applied to produce electricity from wastewater treatment in a thermodynamically spontaneous process using devices known as MFC (Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Rabaey et al., 2003). The use of BES for bioelectricity generation was based on the biodegradation of the organic matter in the anode, and abiotic cathode reductions, such as oxygen, which is the most suitable electron acceptor for MFC (Logan et al., 2006). Thus, bioanodes have been extensively used to produce electricity from different types of wastewater (Sleutels
et al., 2012), such as domestic wastewater (Ahn and Logan, 2013; Rabaey et al., 2005b), industrial and agriculture wastewater (Cheng et al., 2007; Vilajeliu-Pons et al., 2015), and landfill leachate (Puig et al., 2011).

In the recent years, other applications of BES were identified, such as the production of chemicals and commodities (Logan and Rabaey, 2012). These compounds are usually obtained by reduction processes that take place in the cathode chamber, either in the absence or presence of microorganisms (biocathode). The overall cell potential may become negative, and therefore the process would be thermodynamically unfavourable. Thus, an extra energy input would be required to drive the process (i.e. MEC). Up to date, many options have been explored. Among other applications, anodic acetate oxidation coupled to abiotic cathodic hydrogen evolution (Ambler and Logan, 2011; Carmona-Martínez et al., 2015; Liu et al., 2005; Selembo et al., 2010) has been the most investigated so far. Regarding cathodic hydrogen production, expensive catalysts, such as precious metals (i.e. platinum or niquel) are required (He and Angenent, 2006). Therefore, the lack of efficient, sustainable, and inexpensive catalysts quickly guided the research of alternative cathode catalysts. In this sense, microorganisms were found a good candidate to replace conventional cathode materials due to its low cost, self-regeneration capability, and its metabolism diversity, which could be used to remove unwanted compounds or produce useful compounds (He and Angenent, 2006).

1.3. Biocathodes

Biocathodes take advantage of the ability of some microorganisms to grow under cathodic conditions and to drive the metabolism of interest. Microorganisms present in biocathode can interact with the electrode surface either directly or indirectly (see section 1.4.1). Under cathodic conditions, reductive reactions are carried out, which lead to a wide range of possibilities regarding removal and production of target compounds (Logan and Rabaey, 2012). Thus, between the
years 2004 and 2016, biocathodes publications quickly increased up to more than 400 in the last year (Figure 1.3).

![Figure 1.3](image)

The first time that a biocathode was used in a bioelectrochemical system was to improve oxygen reduction for electricity production in an air cathode MFC (Clauwaert et al., 2007). Afterwards, biocathodes started to be applied in more fields of research, offering promising opportunities for (i) the removal of contaminants, and (ii) the production of target commodity compounds.

Regarding the removal of contaminants present in contaminated waters, biocathodes have been utilised for nitrate (Pous et al., 2015, 2013; Virdis et al., 2010, 2008), perchlorate (Butler et al., 2010; Li et al., 2015; Mieseler et al., 2013), and sulphate removal (Coma et al., 2013), among others.

In the field of biological production, biocathodes have been used to enhance hydrogen production (Cheng and Logan, 2007; Jeremiasse et al., 2010; Rozendal et al., 2008) using protons as electron acceptor. However, other electron acceptors, such as CO₂, could be used allowing the production of a wide range of compounds.
CO₂ can be reduced in the biocathode of a BES through MES, to produce commodity compounds such as methane, volatile fatty acids (VFA) or alcohols (Nevin et al., 2010; Rabaey and Rozendal, 2010).

1.4. Microbial electrosynthesis

Up to date, methane production from CO₂ has been demonstrated using a BES (Cheng et al., 2009), which was then investigated more in depth to understand the effect of the cathode potential (Villano et al., 2010) and other operational parameters (Van Eerten-jansen et al., 2012), as well as the production mechanism (Van Eerten-Jansen et al., 2015), and the microorganisms involved in the process (Van Eerten-jansen et al., 2013).

In 2010, Nevin and colleagues demonstrated for the first time that acetate could be produced by a pure culture of *Sporomusa ovata* through MES using the cathode electrode as electron donor (Nevin et al., 2010). Later on, the same group also demonstrated the ability of other pure cultures of acetogenic microorganisms to drive the process (Nevin et al., 2011).

It was not since 2012 that the possibility of using mixed microbial cultures to perform MES was demonstrated (Marshall et al., 2012). The use of mixed cultures resulted in the concomitant production of different compounds, such as hydrogen, acetate and methane at a cathode potential of -0.59 V vs SHE. Despite hydrogen was not the final product, it is used as intermediate, so its presence is difficult to avoid. Otherwise, methane is considered an undesirable end product since methanogenic microorganisms compete with acetogenic microorganisms for electrons. Therefore methanogenesis inhibitors have been usually added to bioelectrochemical systems. When a methanogenesis inhibitor was added to the reactors, acetate and hydrogen production increased, while methane production was not observed (Marshall et al., 2012). One year later, the same authors demonstrated the improved performance of the microbial community in the long term, after being adapted to the conditions of the BES (Marshall et al., 2013).
Although both studies showed a similar reactor microbiome the later was likely better adapted to BES conditions.

Similarly, some other studies also reported the production of acetate through MES (Bajracharya et al., 2015; Jiang et al., 2013; Jourdin et al., 2014; Patil et al., 2015a; Xafenias and Mapelli, 2014). Jiang and co-workers (2013) showed the importance of the cathode potential. This parameter not only affected the production rate, but also the final product spectrum. Xafenias and Mapelli (2014) investigated the effect of the reactor configuration and the cathode potential on the final product, and reported higher acetate and lower methane production rates at lower cathode potentials, similarly to Jiang and coworkers (2013), who produced 129.23 mL d\(^{-1}\) of methane and 94.73 mg d\(^{-1}\) of acetate at -1.15 V. Jourdin et al. (2014), investigated the use of reticulated vitreous carbon modified with carbon nanotubes to enhance the production of acetate through MES (10.7 mM d\(^{-1}\)). Bajracharya et al. (2015), studied the performance of mixed culture and *Clostridium ljungdahlii* in a graphite felt and stainless steel cathode (2.6 mM d\(^{-1}\)). Finally, Patil et al. (2015a), showed the performance and reproducibility of the results for an enriched community performing MES (2 mM d\(^{-1}\)).

### 1.4.1. Electron transfer mechanisms

Up to date, different electron transfer mechanisms have been reported in biocathodes. These mechanisms are shown in Figure 1.4. Microorganisms can interact with electrons via direct electron transfer (DET) or mediated electron transfer (MET). In biocathode applications microorganisms can perform DET using membrane proteins, such as c-type cytochromes or hydrogenases (Rosenbaum et al., 2011), but they can also exchange electrons via physical cellular structures, known as nanowires (Gorby et al., 2006). Direct or indirect interspecies syntropy can also be established between two different species, MIET and DIET (Rotaru et al., 2014b).
Figure 1.4. Electron transfer mechanisms established between the microorganisms and an electrode surface. Adapted from Harnisch and Rabaey, 2012.

MET mechanisms involve the production of intermediate soluble redox compounds, which can be used by the microorganisms to interact with the electrode surface (Schröder, 2007). Phenanzines and flavins are some of the key examples of soluble mediators (Marsili et al., 2008; Rabaey et al., 2005a). Hydrogen can also be produced on the electrode surface, either biologically or purely electrochemically, and used as electron donor by microorganisms suspended in the solution.

Syntrophic relationships, such as interspecies DET and interspecies MET have been also proposed for biocathode applications with mixed cultures, being the first one more energetically conservative due to the fact that electrons are directly transferred between microorganisms, avoiding the production of intermediates (Derek R. Lovley, 2011; Stams and Plugge, 2009). So far, there is only one study that demonstrated the DIET between two pure cultures (Rotaru et al., 2014a).

1.4.2. Product spectrum

A whole range of compounds can theoretically be produced by MES (Rabaey and Rozendal, 2010), which are summarised in Table 1.3. Although the main product
obtained up to date is acetate, other compounds can be produced from CO₂ as well, such as longer VFA or alcohols.

The most energetically efficient known pathway to reduce CO₂ to organic compounds is the Wood-Ljungdahl pathway (Figure 1.5). Acetogenic microorganisms use this metabolic route to reduce CO₂ using H₂ as electron donor (Fast and Papoutsakis, 2012). Acetyl-CoA is the central intermediate of the Wood-Ljungdahl pathway and it is used as a building block for the production of a variety of commodities (Lovley and Nevin, 2013). *Clostridium ljungdahlii* has been used as model homoacetogen. This microorganism has the potential to produce a variety of organic acids, as well as fuels and other commodities via MES (Leang et al., 2013). Two studies successfully modified genetically *C. ljungdahlii* to produce acetone (Berzin et al., 2012) and butanol (Köpke et al., 2010).

**Table 1.3. Products that can be theoretically obtained from CO₂ by MES. Adapted from Rabaey and Rozendal, 2010.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Electrons consumed</th>
<th>Standard electrode potential (E’₀) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>8</td>
<td>-0.24</td>
</tr>
<tr>
<td>Acetate</td>
<td>8</td>
<td>-0.28</td>
</tr>
<tr>
<td>Butanol</td>
<td>24</td>
<td>-0.30</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12</td>
<td>-0.31</td>
</tr>
<tr>
<td>PHB</td>
<td>18</td>
<td>-0.31</td>
</tr>
<tr>
<td>Formate</td>
<td>2</td>
<td>-0.41</td>
</tr>
</tbody>
</table>
Chapter 1

Figure 1.5. Simplified schematic representation of the Wood-Ljungdahl pathway, used to reduce $\text{CO}_2$ using hydrogen as electron donor. Adapted from Fast and Papoutsakis, 2012.

Although pure cultures can be genetically modified to produce the compounds of interest, mixed cultures can also be an alternative. Agler et al. (2011), reviewed the different pathways for the microbial production with mixed cultures. This study shows the importance of short chain volatile fatty acids, such as acetate, as intermediate compound for the production of more valuable compounds, such as butyrate, ethanol or even higher carbon compounds through secondary fermentation processes. Other studies also demonstrated that long chain carboxylates, such as caproate, could be produced when acetate is used as a feedstock either in conventional anaerobic fermenters (Steinbusch et al., 2011), or
in BES (Van Eerten-Jansen et al., 2013). Despite acetate is nowadays the main product of MES, the presented studies suggested the possibility for its further transformation to more valuable compounds.

1.4.3. Limitations of microbial electrosynthesis from CO₂

Many limiting steps have to be considered regarding the utilisation of CO₂ as carbon source. Research efforts have to be performed to improve the efficiency of CO₂ capture, transformation, and utilisation in the nearby future (Figure 1.6).

![Figure 1.6. Schematic representation of the basic steps in the CO₂ recycling.](image)

The first step to be considered is the transport of the substrate. Although CO₂ is available in excess and can be used to provide buffer capacity (Rabaey et al., 2011), it has a relatively low solubility in water (33.7 mol L⁻¹ at 1 atm and 25 °C), and its concentration in air is around 0.04 % vol (Sander, 1999). The first step for the utilisation of CO₂ is already a challenge, since its low concentration in the atmosphere already represents a limiting step as far as atmospheric CO₂ is concerned. However, CO₂ is largely produced in industry, so flue gases or other gases highly enriched in CO₂ could be used instead, before releasing them to the atmosphere. Transformation usually requires CO₂ dissolved in water when it implies
the use of microorganisms. Since its solubility is low, more efforts are needed to increase its availability for further transformation.

In the case of CO$_2$ transformation in BES, some further limiting steps can be identified (Figure 1.7), which are ohmic losses due to electrode material (A), the interaction between the electrode and the microorganisms (B), the availability of CO$_2$ in the solution due to mass transfer limitation (C), the interaction between the microorganism and the substrate (D), titers and production rates that can be obtained (E), and the product extraction and purification (F). Moreover, product microbial inhibition could surge as a result of the production of certain compounds at high concentrations (i.e. VFA) (Jones and Woods, 1986). All this steps will affect CO$_2$ transformation efficiency, energy efficiency, product spectrum and selectivity, and downstream processing for the purification and concentration of the products of interest.

Figure 1.7. Schematic representation of the main limiting steps for CO$_2$ transformation in BES. Ohmic losses (A), electrode – microorganisms interaction (B), mass transfer limitation (C), microorganism – substrate interaction (D), product range (E), and product extraction and purification (F).
Chapter 1

The CO$_2$ transformation efficiency corresponds to the ratio between the carbon recovered in products and the carbon introduced into the system. Part of the carbon is diverted to biomass growth, whereas another part could be lost due to the nature of CO$_2$. The energy efficiency of the process is calculated by comparing the energy contained in the products to the energy spend in the process. It has to be considered that some energy losses are present (i.e. heat losses), so it is important to identify these losses and minimise them.

As it has been mentioned in the previous section (Table 1.3), a wide range of compounds can be theoretically produced from CO$_2$ in BES. However, up to date, only methane and acetate have been obtained as main gas and liquid products from CO$_2$ using naturally selected microorganisms. There is still a long way to walk in terms of MES technologies. It has been just 6 years from the first proof of concept using pure cultures (Nevin et al., 2010), and only 4 years since MES was demonstrated with mixed cultures (Marshall et al., 2012). Undoubtedly, the range of the final product obtained through MES is nowadays the main bottleneck of the technology, as the primary products obtained so far (acetate and methane) have a low economic value. In this sense, it is suggested that the modification of certain parameters could trigger the production of other compounds. Similarly to what has been reported in other fermentation processes, such as syngas or acetone-butanol fermentation (Ganigué et al., 2015), the hydrogen partial pressure, therefore the reducing power availability, could play a key role in controlling not only the specific productivity (Demler and Weuster-Botz, 2011), but also the final product (Yerushalmi et al., 1985a). In BES, the hydrogen partial pressure can be increased \textit{in-situ} by regulating the amount of electricity provided to the system.
Figure 1.8. Timeline with some of the most important achievements in microbial electrosynthesis from CO₂.

Regarding the practical implementation of MES, not only product selectivity is crucial, but also its purification to obtain a high purity compound, which could be directly used for industrial purposes. In this sense some studies already dealt with the electrochemical extraction of acetate from the fermentation broth, either in a separate process unit (Andersen et al., 2014), or in the same reactor during MES (Gildemyn et al., 2015). It is expected that the range of products from MES would be increased in the next years. Therefore, specific downstream or in-situ extraction processes for the recovery of the different products of interest should be developed.

Accordingly, widening the range of products that can be obtained through MES is essential for the future development of MES and its coupling with different technologies and processes. The elucidation of the key parameters governing and enhancing the transformation of CO₂ into different compounds should be one of the main goals for current researchers in this field. Furthermore, the identification of the main actors of the process, such as microorganisms involved, and reaction pathways could also help controlling and improving MES.
Chapter 2. Objectives
Chapter 2

This thesis is focused on the research of biocathodes for the bioproduction of valuable compounds from CO\textsubscript{2} in BES. When this thesis started in 2012, little was known about the feasibility to convert CO\textsubscript{2} into commodity chemicals using BES (chapter 1). Therefore the main objective of this thesis was:

- To explore the operation of mixed culture biocathodes to achieve and enhance the production of different target compounds through MES, and identify the main microorganisms and the production mechanisms involved.

Biocathodes are an exciting promising approach to drive catalytic CO\textsubscript{2} reductions. However, the biocathode electron transfer mechanisms are not well understood. Although little is known about the ability of microorganisms to use electrons, it is well known that carboxydrotrophic microorganisms can use hydrogen as reducing power source through the Wood-Ljungdahl pathway. One advantage of using BES is that H\textsubscript{2} can be produced in situ to overcome solubility and mass transfer limitations, and therefore directly supply H\textsubscript{2} to the CO\textsubscript{2} consuming microorganisms. To achieve the principal goal of this thesis, specific objectives were defined:

- To assess the cathode potential as a parameter to control the amount of reducing power provided to the biocathode in the form of H\textsubscript{2}, and the influence of the presence of microorganisms in this step of the process.
- To elucidate the bioelectrochemical processes, and the reaction mechanisms governing the production of methane in the biocathode.
- To identify basic operational parameters enhancing the production of methane for its further scaling-up and development of potential niche applications.
- To study the influence of the operational conditions enhancing the productivity and selectivity of different organic compounds from CO\textsubscript{2} during MES.
- To identify the microorganisms involved in the different steps of the process and the reaction pathways governing the production of different compounds.
Chapter 2

The chapters of the thesis are outlined (Figure 2.1) according to the objectives in various chapters:

Figure 2.1. Schematic representation of the outline of the present thesis. This thesis explored the production of different compounds using electricity and carbon dioxide as reducing power and carbon sources, respectively in a biocathode of a BES. The biological and abiotic production of hydrogen was assessed in chapter 4, the microbial community present in the biocathode was also identified. Methane production in different conditions was studied in chapters 5 and 6, as well as the electron transfer, microbial community and reaction mechanism of the biocathode (Chapter 5). The production of liquid compounds was also studied. Continuous production of acetate and the effect of the pH in the biocathode was studied in chapter 7, while the production of longer VFA and alcohols and the reaction mechanisms were studied in chapters 8 and 9.
Chapter 3. Methodology
3.1. Bioelectrochemical systems set-ups

Four different BES configurations were used during the experiments: i) flat plate reactor (Figure 3.1), ii) microcosm reactor (Figure 3.2), iii) h-shape reactor (Figure 3.3), and iv) tubular reactor (Figure 3.4).

3.1.1. Flat plate BES

A two-chambered BES consisted of two methacrylate rectangular frames (200×200×20 mm) separated by a cation exchange membrane (CMI-7000, Membranes International Inc., USA), to allow proton migration from the anode to the cathode, and to avoid product losses during the operation. The anode and cathode chambers were filled with granular graphite (model 00514, diameter 1.5–5 mm, EnViro-cell, Germany), to increase electrode surface area, and to sustain biofilm growth in the cathode chamber. The net volumes of the anode (NAC), and cathode (NCC) compartment decreased due to the graphite replenishment, and relied on the reactor size used in each study (volumes between 0.4 and 0.5 L). Graphite rods (130×5 mm, Mersen Ibérica, Spain) were inserted in the graphite beds and connected to the potentiostat. The electrodes were previously washed for at least 1 h with 1 M HCl and then in 1 M NaOH to remove possible metal and organic contamination. An internal recirculation loop (105 L d⁻¹) was placed in each compartment to maintain well-mixed conditions, and to minimize concentration gradients.
The advantage of this reactor configuration is that it offers a high versatility in terms of operation. In the studies shown in chapters 4, 6 and 7, the reactor was operated in continuous, while in the study shown in chapter 5, both batch and continuous operation were tested to study the reactor performance. Specific conditions of each study can be found in the corresponding chapters.

### 3.1.2. Microcosm reactor

It was the simplest configuration used to perform the electrochemical analyses presented in chapter 5. The microcosm reactor consist of a single chamber BES constructed in a 20-mL glass tube (Figure 3.2) according to Pous et al. (2014). A graphite rod and platinum wire were used as working electrode (WE) and counter electrode, respectively, whereas an Ag/AgCl was used as reference electrode (RE).
3.1.3. H-shape reactor

It is a typical reactor setup, which was used in chapter 8 (Figure 3.3) to conduct batch experiments to prove the production of liquid products different than acetate. It consisted in two chambers constructed using two 120-mL glass bottles separated by a cation exchange membrane (CMI- 7000, Membranes International Inc., USA). Commercial carbon cloth (NuVant’s ELATs LT2400W, FuelCellsEtc, USA), with an area of 9 cm² and an area to volume ratio of 0.075 cm²mL⁻¹, was used as a WE (cathode). An Ag/AgCl (+0.197 V vs. SHE, model RE-5B, BASI, United Kingdom) was also placed in the cathodic chamber as a RE, whereas a titanium rod (Ti plus 50 g m⁻² Pt, Magneto, The Netherlands) served as a CE in the anodic compartment. The cathode compartment had two butyl-rubber sampling ports. Finally, the cells were wrapped with a coil of plastic tubing connected to a thermostatic bath to control the operational temperature. In this case, the reactor was operated in batch.
3.1.4. **Tubular reactor**

It was used in the experiments performed in chapter 9 (Figure 3.4). A two-chambered tubular BES was constructed using PVC tubing. The BES consisted of concentric cathode (inner) and anode (outer) compartments separated by a tubular cation exchange membrane (CMI-1875T tubular membrane, Membranes international, US). Commercial carbon cloth (NuVant’s ELAT, LT2400W, FuelCellsEtc, US) with an area of 320 cm² was used as cathode electrode, which was connected to a platinum wire current collector using carbon paint. Ti-MMO was used as anode electrode (NMT electrodes, South Africa), and an Ag/AgCl electrode (+0.197 V vs. SHE, model RE-5B, BASI, UK) was placed in the cathode chamber and used as RE. External buffer tanks were connected to the anode and cathode to allow for the recirculation of liquid (5.8 L h⁻¹), and therefore stirring and sampling of the liquid and gas phase.

![Figure 3.3. Schematic representation (A) and picture (B) of the h-shape reactor design.](image)
3.2. Experimental procedure

In all the studies, CO₂ was the main carbon source, and it was fed in the BES by saturation of liquid synthetic medium with CO₂ gas. Different inoculum were used, selected from different sources, such as wastewater sludge, anaerobic digestion sludge, syngas fermentation broth, or previously working BES. The operational conditions applied in each study were also different in terms of applied cathode potential, batch/continuous flow, etc. The specific characteristics regarding all the parameters used in each study can be found in the corresponding chapters.

Samples from the liquid and gas phase were periodically taken, depending on the aim of each study. The different chemical analyses that were performed are explained in section 3.3. These analyses allowed for the calculation of the production rates, and in the case of chapter 9, also the CO₂ consumption (section 3.4). The BES were operated in chronoamperometry (CA), and other electrochemical techniques were occasionally performed (section 3.5). Microbial analyses of the microorganisms present in the system were also performed when necessary (see section 3.6).
Table 3.1. Summary of the reactor type, inoculum and operation conditions used in each study performed during this thesis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Reactor</th>
<th>Inoculum</th>
<th>Carbon source – electron acceptor</th>
<th>Operation</th>
<th>Cathode potential (V vs SHE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 4</td>
<td>Flat plate</td>
<td>Wastewater sludge and previous MFC</td>
<td>CO₂</td>
<td>Continuous</td>
<td>From -0.4 to -1.8</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Flat plate</td>
<td>Anaerobic digestion sludge</td>
<td>Biogas (CO₂)</td>
<td>Batch and continuous</td>
<td>-0.8</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Flat plate</td>
<td>Previous BES used in chapter 5</td>
<td>CO₂</td>
<td>Continuous</td>
<td>-0.7 and -0.8</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>Flat plate</td>
<td>Anaerobic digestion and retention basin sludge</td>
<td>CO₂</td>
<td>Continuous</td>
<td>-0.6</td>
</tr>
<tr>
<td>Chapter 8</td>
<td>H-shape</td>
<td>Syngas fermenting lab-scale reactor</td>
<td>CO₂</td>
<td>Batch</td>
<td>-0.8</td>
</tr>
<tr>
<td>Chapter 9</td>
<td>Tubular</td>
<td>Syngas fermenting lab-scale reactor</td>
<td>CO₂</td>
<td>Batch</td>
<td>-0.7 and -0.8</td>
</tr>
</tbody>
</table>

3.3. Chemical analyses

Depending on the aim of the experiment, different analyses were performed. Chemical oxygen demand (COD), and sulphates (S-SO₄²⁻) measurements were performed according to the recommendations of the American Public Health Association (APHA) for standard wastewater (APHA, 2005).

Measurements for pH and conductivity were performed with a pH-meter (pH-meter basic 20+, Crison, Spain) and an electric conductivity meter (EC-meter basic 30+, Crison, Spain). For pH in-line measurements a sensor (model 5303, Crison, Spain) was placed in the cathode recirculation loop to measure the pH with a transmitter.
Chapter 3

(MultiMeter MM44, Crison, Spain) connected to a memograph (Graphic data manager RSG40, Memograph M, Endress+Hauser, Switzerland).

The optical density of the cathode was regularly measured to control the microorganism growth and therefore the proper system operation. Measurements were performed with a spectrophotometer at a wavelength of 600 nm.

Gas chromatographic techniques were used to analyse the gas composition and VFA and alcohol concentrations in the liquid phase. Different equipment was used during this thesis. The liquid samples were analysed with two different gas chromatographs (GC). In the study presented in chapter 4 a Varian CP-3800 equipped with FactorFour™ CP8860 column and a Flame Ionization Detector (FID) was used to detect Volatile Fatty Acids (VFA) and alcohols; whereas the gas samples were analysed with an Agilent 7820A GC System equipped with Washed Molecular Sieve 5A and Porapak® Q columns and a Thermal Conductivity Detector (TCD).

In the subsequent studies the production of organic compounds (VFA and alcohols) in the liquid phase was measured with an Agilent 7890A GC equipped with a DB-FFAP column and a FID. Samples were acidified with ortho-phosphoric acid (85%, Scharlau, Spain) and an internal standard (crotonic acid) was added before the analysis to ensure the results obtained. The composition of the gas phase was analysed in a second channel of the GC equipped with an HP-Molesieve column and a thermal conductivity detector TCD.

3.4. Calculation of the production rates

3.4.1. Gas products distribution

The amount of gaseous products was calculated from the volume of gas produced, which was measured with gas trap placed in the effluent of the reactors. The measurements of the volume produced, together with the pressure, and the composition of the gas, allowed for the calculation of the moles produced of each compound, through the ideal gas law.
When necessary the concentration of the gas compounds, such as CO₂, H₂ or CH₄, in the liquid phase were calculated according to Henry’s law (Equation 3.1).

\[
[i]_{\text{liq}} = K_{h,i} \cdot P_i \quad \text{(Equation 3.1)}
\]

Where, \(i\) refers to the compound itself; \([i]_{\text{liq}}\) is the concentration (mol L⁻¹) of the compound in the liquid phase; \(K_{h,i}\) is the Henry’s constant (mol L⁻¹ atm⁻¹) of the compound at the experimental temperature; and \(P_i\) (atm) is the partial pressure of the compound in the gas phase. \(P_i\) is calculated from the total pressure and the molar fraction of \(i\) in the gas phase, according to equation 3.2.

\[
P_i = P_t \cdot y_i \quad \text{(Equation 3.2)}
\]

Where \(P_t\) is the total pressure (atm) measured in the reactor; and \(y_i\) is the molar fraction of the compound \(i\) in the gas phase.

The \(K_{h,i}\) was calculated as function of the temperature according to the experimental conditions through equation 3.3.

\[
K_{h,i} = K_{\theta,i} \cdot \exp \left[ -\frac{\Delta H_{\text{sol}}}{R} \left( \frac{1}{T} - \frac{1}{T^\theta} \right) \right] \quad \text{(Equation 3.3)}
\]

Where, \(K_{\theta,i}\) is the Henry’s constant of \(i\) at the standard temperature \((T^\theta = 298.15 \text{ K})\); and \(\Delta H_{\text{sol}}\) is the enthalpy of dissolution of \(i\). Some Henry’s constants and enthalpies of dissolution of the most typical compounds measured in this thesis are shown in table 3.2.

| Table 3.2. ΔH_{sol}/R and Henry’s constant standard values of different gas compounds, used for calculation in Equation 3.3. |
|---------------------------------|------|-----------------|
| **Compound** | ΔH_{sol}/R (K) | \(K_{\theta,i}\) (mol L⁻¹ atm⁻¹) (Sander, 1999) |
| CO₂ | 2400 | 3.4 \times 10⁻² |
| H₂ | 500 | 7.8 \times 10⁻⁴ |
| CH₄ | 1586 | 1.4 \times 10⁻³ |
| CO | 1300 | 9.5 \times 10⁻⁴ |
3.4.2. Gas production rate

Different methods have been applied to calculate the gas production rates, depending on the reactor design and mode of operation used. In the study presented in chapter 4, the volumetric production rate of hydrogen \((Q_{H2}; \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ NCC} \cdot \text{d}^{-1})\) was calculated according to equation 3.4.

\[
Q_{H2} = \frac{\int_{t_0}^{t} C_{H2} V_g dt}{V_{NCC}} \quad \text{(Equation 3.4)}
\]

Where \(V_g\) is the gas volume (m\(^3\)) sampled over a period of time (days), \(C_{H2}\) is the composition (v/v) of hydrogen in the gas, and \(V_{NCC}\) is the net cathode compartment volume (m\(^3\)).

In the studies presented in chapters 5 and 6, the methane molar production rate was calculated using the slope obtained from plotting the moles of methane harvested over time, and normalising this value according to the estimated electrode surface of the reactor. The moles of methane were calculated from the volume and concentration of methane in the gas phase \((P_{CH4})\) according to equation 3.2 and the ideal gas law.

Finally, in chapter 9, the consumption and production of the different gas compounds were calculated from the pressure and composition of the gas phase according to equations 3.1, 3.2 and 3.3.

3.4.3. Liquid phase composition

The liquid products were analysed in terms of product concentration in moles per litre. Since different compounds can be synthesised from CO\(_2\), the concentrations of those compounds were expressed as the concentration of carbon (C) in the molecule. Thus, in most cases the moles of carbon per litre is reported (mM C). The moles of carbon were calculated for each compound according to its molecular weight and the number of carbon atoms contained on its structure, as shown in equation 3.5.
\[ \text{mMC} = \frac{C_i n_{C_i}}{M_i} \] (Equation 3.5)

Where \( C_i \) is the concentration of the product \( i \) in the liquid phase (in mg L\(^{-1}\)), \( n_{C_i} \) is the number of carbon atoms contained in the molecular structure of \( i \), and \( M_i \) is the molecular weight of \( i \) (in mg mmole\(^{-1}\)).

### 3.4.4. Organic carbon production rate

Different operation modes were applied during the experiments performed. In the results presented in chapter 7, the BES was working in continuous flow, while the BES used in chapters 8 and 9, was operated in batch.

In the case of batch operation the cumulated moles of products were presented as function of time, and the production rate was calculated through the linear regression of experimental results. Regarding continuous operation, the measured concentrations should be corrected according to the influent flow, and normalised to liquid volume as indicated in equation 3.6. In both cases, the production rates are expressed as mM C d\(^{-1}\).

\[ \text{mMC d}^{-1} = \frac{\text{mMC} Q}{V} \] (Equation 3.6)

Where, \( \text{mMC} \) is the concentration of carbon in the products, calculated as indicated in equation 3.5, \( Q \) (L d\(^{-1}\)) is the influent flow, and \( V \) (L) is the volume of the reactor.

### 3.5. Electrochemical analyses

Different potentiostat (BioLogic, France) models were used (model SP-50 and model VSP; Figure 3.6) with a three-electrode configuration to poise the cathode potential. The WE was the cathode electrode, the RE was an Ag/AgCl (RE; +197mV vs standard hydrogen electrode [SHE], model RE-5B, BASI, United Kingdom) placed in the cathode chamber for potentiostatic control, and the counter electrode was placed in the anode chamber. On a regular basis the BES was operated in chronoamperometry (CA) mode at different cathode potentials. The current
demand, cell potential, power consumed, among other parameters were monitored at 5-minute interval. All of the voltages were reported with respect to SHE.

Additionally, electrochemical analyses were performed to understand the BES in terms of its electroactive capabilities (Harnisch and Freguia, 2012). The vast majority of these techniques are based on the scanning of the voltage and the analysis of the response in the current. Some of the most used analysis are cyclic voltammetry (CV), linear sweep voltammetry (LSV), or differential pulse voltammetry (DPV) were performed when necessary (see corresponding chapters for more details).

![Different models of BioLogic potentiostat used in this thesis. In the upper part of the image two different SP-50 models, in the lower part a VSP model.](image)

### 3.5.1. Coulombic and energy efficiencies

The calculation of the coulombic efficiency (CE) and the energy efficiency ($\eta_E$) are based on the comparison of the energy consumed, and the energy contained in the form of final products; therefore these values are reported as a percentage. These equation were recently summarised by Patil and colleagues (2015b). The equation used for the CE calculation is shown in equation 3.7.

$$
CE = \frac{F \sum_i M_{p,i} \Delta e_i}{\int I \, dt} \quad \text{(Equation 3.7)}
$$
Where $F$ is Faraday’s constant; $M_{p,i}$ are the moles of product ($i$); $\Delta e_i$ is the difference in degree of reduction between the substrate and the product (i.e. the number of electrons consumed per mol of product); and $\int I \, dt$ is the integration of the current supplied over time.

The energy efficiency of the process was calculated from the relationship between the energy contained in the products and the input energy calculated from the cell potential according to equation 3.8.

$$\eta_E = \frac{\Delta G_i \cdot M_{p,i}}{E_{cell} \int I \, dt} \quad \text{(Equation 3.8)}$$

Where $\Delta G_i$ is the Gibbs free energy content in the product; and $E_{cell}$ is the cell potential, which is calculated according to the half cell potentials using equation 1.2.

3.6. Microbial community analyses

3.6.1. Microscopy observation techniques

In the studies presented in chapters 4 and 5 qualitative microbial analyses, such as scanning electron microscopy (SEM) were performed in samples extracted from the biocathode. The samples were immersed in 2.5% (w/v) glutaraldehyde in a 0.1 M cacodylate buffer at pH 7.4 for a period of 4 hours. Next, the samples were washed and dehydrated in an ethanol series. Washes were done with cacodylate buffer and with water, both per duplicate. Dehydration with graded ethanol followed temperature steps of 50, 75, 80, 90, 95 and 3x100 °C in periods of 20 minutes. The fixed samples were dried with a critical-point drier (model 122 K-850 CPD, Emitech, Germany) and sputtered-coated with a 40 nm gold layer. The coated samples were examined with a SEM (model DSM-960; Zeiss, Germany) at 20 kV and images were captured digitally.
3.6.2. PCR-DGGE analyses

In the studies presented in chapters 4 and 5, molecular microbial community analyses, such as PCR-DGGE were performed. DNA was extracted using the Fast DNA® SPIN Kit for soil (MP Biomedicals, US) according to the manufacturer's instructions. DNA quantification was assessed with a nanodrop spectrophotometer (Thermo Scientific, US) characterized by 260/280 and 260/230 nm absorbance ratio in order to be comparable with quantitative real-time PCR (qPCR). Bacterial diversity and microbial community structure of the cathode biofilm was analysed for 454 pyrosequencing (Research and Testing Laboratory, Lubbock, US). The 16S rRNA gene was analysed by primers 341F-907R for bacteria (Muyzer et al., 1993), and 341F-958R for archaea (DeLong, 1992). Sequences obtained were analysed using MOTHUR software (v.1.22.1) at 97% similarity (Schloss et al., 2009). Sequences shorter than 250 bp and longer than 600 bp were excluded. Chimeric sequences were discarded by executing UCHIME (Edgar et al., 2011). These sequences were then clustered into OTUs using the UPARSE algorithm (Edgar, 2013). Taxonomic classification was done by RDP classifier (Cole et al., 2009). The most abundant sequences of bacteria and archaea were compared to BLAST supported by NCBI in order to obtain the closest similarity related to genbank database sequence. Alignments and identity matrix comparisons of deposited sequences were performed in BioEdit (v. 7.0) (Hall, 1999). Post-data analysis was done by KRONA (Ondov et al., 2011). Additionally, 16S rRNA gene was also quantified by qPCR to estimate the total amount of bacteria and archaea. All reactions were performed in a 7500 Real Time PCR system (Applied Bio- systems, US) using the SYBRH Green PCR Mastermix. Primers and thermal cycling conditions for bacteria (341F-534R) and archaea (364af-A934b) 16S rRNA were used (Kemnitz et al., 2007; López-Gutiérrez et al., 2004). Results were analysed using SDS software (Applied Biosystems, US). Standard curves were obtained using serial dilutions from102 to 107 copies of linearised plasmids containing the respective functional genes. Controls without templates gave null or negligible values.
Chapter 4. Assessment of biotic and abiotic graphite cathodes for hydrogen production in microbial electrolysis cells

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Assessment of biotic and abiotic graphite cathodes for hydrogen production in microbial electrolysis cells

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ABSTRACT

Hydrogen represents a promising clean fuel for future applications. The biocathode of a two-chambered microbial electrolysis cell (biotic MEC) was studied and compared with an abiotic cathode (abiotic MEC) in order to assess the influence of naturally selected microorganisms for hydrogen production in a wide range of cathode potentials (from −400 to −1800 mV vs SHE). Hydrogen production in both MECs increased when cathode potential was decreased. Microorganisms present in the biotic MEC were identified as Hoeflea sp. and Aquiflexum sp. Supplied energy was utilized more efficiently in the biotic MEC than in the abiotic, obtaining higher hydrogen production respect to energy consumption. At −1000 mV biotic MEC produced 0.89 ± 0.10 m3 H2 d−1 m−3NCC (Net Cathodic Compartment) at a minimum operational cost of 3.2 USD kg−1 H2. This cost is lower than the estimated market value for hydrogen (6 USD kg−1 H2).

1. Introduction

Hydrogen is a sustainable energy carrier, which releases water as the only product when it is burnt and it can be produced from a variety of sources. Nowadays, most of produced hydrogen comes from large-scale processes such as gasification, pyrolysis, thermochemical water splitting, steam reforming and electrolysis [1]. These processes use fossil fuels, consume large amounts of energy or both. For this reason they contribute significantly to global warming, mainly due to carbon dioxide emissions and large electric consumption, especially when it comes from non-renewable sources. Even though these methods are energy intensive, at present they are the only way of supplying large amounts of H2 for industrial application.

One of the most promising technologies for a future sustainable production of hydrogen is the use of microbial electrolysis cells (MEC). Theoretically, a relatively low amount of voltage (−414 mV), which could be produced from renewable sources, is required to drive the process. But in practice this voltage is substantially increased due to overpotentials of the system [2].

In the conventional MEC configuration, microorganisms are used in the anode chamber to recover energy contained in organic matter. This oxidation generates protons and...
electrons, which are transferred to an electrochemical cathode containing a metal catalyst (i.e. platinum, nickel or stainless steel) enhancing hydrogen production [3–8]. However, the use of metal catalysts requires high capital and operational costs and they have to be constantly replaced, mainly due to corrosion or deactivation problems. An alternative approach is the use of microorganisms as a biological catalyst in the cathode chamber. Electrotrophic microorganisms are able to accept electrons directly or indirectly and may use them to reduce protons to hydrogen, as described in Equation (1).

\[2H^+ + 2e^- \rightarrow H_2, \quad E'_0 = -414 \text{ mV}\]  

(1)

Some recent studies have been focused on hydrogen production and its coproduction using microorganisms in the biocathode of a MEC [9–14]. Table 1 summarizes some literature studies using MECs. Generally, higher volumetric hydrogen productions were achieved using a metal catalyst in an abiotic cathode. Cheng and Logan [3], who obtained 17.80 m³ H₂ m⁻³ MEC d⁻¹, demonstrated that Platinum (Pt) showed a good performance. But the highest production rate was achieved by Jeremiasse et al. [8] using Nickel (Ni) foam as cathode catalyst. They produced hydrogen at a maximum rate of 50.00 m³ H₂ m⁻³ MEC d⁻¹, which decreased during operation due to anode and cathode overpotentials.

Hydrogen production rates by microorganisms in a biocathode are usually one order of magnitude lower than those obtained by metal catalysts [9–11,13,14]. The highest volumetric hydrogen production rate obtained by Jeremiasse et al. [12] with a biocathode was 2.20 m³ H₂ m⁻³ Net Cathode Compartment (NCC) d⁻¹, being the only example in which a biocathode yielded hydrogen production rates in the range of those obtained by metal catalysts.

On the other hand, in terms of cathodic hydrogen recovery (i.e. electrons from the anode recovered in the form of hydrogen at the cathode), up to date biocathodes are found to reach lower values than metal catalyzed cathodes. Using a biocathode, Rozendal et al. [9] reached a maximum cathodic hydrogen recovery of 57%, which was much higher than the control electrode used in the same study (25%). Meanwhile values up to 93% were obtained using nickel [8], 84% using stainless steel [7] and 96% using platinum as catalyst [4]. However, other authors noted [15] that the use of biocathodes could reduce costs of construction and operation of the system. Moreover it could overcome most of the problems related to the use of metal catalysts such as corrosion or deactivation.

There are still some gaps in the knowledge about how biocathodes work and which microorganisms are involved in hydrogen production. A better understanding of microorganisms and its metabolic pathways could improve hydrogen production rate and energy recovery in the near future. Thus it could make MEC biocathodes a promising cost-effective production platform for hydrogen gas [10].

Although optimistic results have been obtained in recent studies, further efforts are needed to improve MECs for hydrogen production and make this an economically feasible process [8,12]. In this study, a biotic and an abiotic (pure electrochemical reactions) MEC for hydrogen production were thoroughly studied. Measurements of hydrogen production were conducted to compare both systems in a wide range of cathode potentials. Although Rozendal et al. [10] already compared a naturally selected culture (biocathode) with a control (abiotic), they did it at an unique cathode potential of −700 mV vs SHE. As far as we know, this is the first time that a naturally selected culture in a biocathode and an abiotic cathode are compared in a wide range of cathode potentials.

<table>
<thead>
<tr>
<th>Operational mode</th>
<th>Cathode material</th>
<th>Cathode potential (mV vs SHE)</th>
<th>Applied potential (mV)</th>
<th>Q₀₁ (m³ H₂ m⁻³ cathode liquid volume day⁻¹)</th>
<th>Coulombic efficiency, CE (%)</th>
<th>Cathodic hydrogen recovery CE (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>500</td>
<td>0.24⁺</td>
<td>n/a</td>
<td>21</td>
<td>[11]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>700</td>
<td>2.20⁹</td>
<td>n/a</td>
<td>50 ± 2.3</td>
<td>[12]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>590</td>
<td>0.29⁹</td>
<td>54</td>
<td>n/a</td>
<td>[13]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>710</td>
<td>0.04</td>
<td>92 ± 6.3</td>
<td>57 ± 0.1</td>
<td>[2]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>700</td>
<td>0.63</td>
<td>n/a</td>
<td>49</td>
<td>[3]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Biocathode</td>
<td>n/a</td>
<td>750</td>
<td>0.01³</td>
<td>~ 80</td>
<td>n/a</td>
<td>[14]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Carbon cloth with Pt</td>
<td>n/a</td>
<td>800</td>
<td>3.12 ± 0.02⁶</td>
<td>96.8 ± 1.4</td>
<td>96 ± 1.1</td>
<td>[6]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Carbon cloth with Pt</td>
<td>n/a</td>
<td>1000</td>
<td>17.80⁹</td>
<td>n/a</td>
<td>93</td>
<td>[5]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Stainless steel type 30460 mesh</td>
<td>n/a</td>
<td>900</td>
<td>1.40 ± 0.13⁶</td>
<td>87 ± 5</td>
<td>n/a</td>
<td>[8]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>Stainless steel brush cathodes type 304</td>
<td>n/a</td>
<td>600</td>
<td>1.70 ± 0.1⁷</td>
<td>n/a</td>
<td>84</td>
<td>[9]</td>
</tr>
<tr>
<td>Single-chamber MEC</td>
<td>60 mg Ni in 267 µL Nafion on carbon cloth</td>
<td>n/a</td>
<td>600</td>
<td>1.30 ± 0.3⁵</td>
<td>92.7 ± 15.8</td>
<td>79 ± 10</td>
<td>[7]</td>
</tr>
<tr>
<td>Two-chambered MEC</td>
<td>Ni foam</td>
<td>n/a</td>
<td>1000</td>
<td>50.00⁹</td>
<td>n/a</td>
<td>90</td>
<td>[10]</td>
</tr>
</tbody>
</table>

a Referred to MEC total liquid volume.
b Calculated from given data; n/a: non-available.
(from ~400 to ~1800 mV vs SHE) for hydrogen production. Due to relative lack of information about which microorganisms are involved in hydrogen production, we identified dominant members of the naturally selected communities in the biocathode using high-throughput molecular methods.

2. Materials and methods

2.1. Experimental setup

A two-chambered MEC was constructed using a previously described design [16]. The MEC consisted of an anode and a cathode placed on opposite sides of a single methacrylate rectangular chamber. The anode and cathode chambers were filled with granular graphite (model 00514, diameter 1.5–5 mm, EnViro-cell, Germany), which decreased the volumes to 400 mL anodic compartment (NAC) and 390 mL NCC, respectively. The electrodes were previously washed in 1 M HCl and 1 M NaOH to remove possible metal and organic contamination. Two thinner graphite electrodes (130 × 4 mm [anode] and 130 × 4 mm [cathode], Sofacial, Spain) were introduced on each chamber. A cation exchange membrane (CMI-7000, Membranes International Inc., USA) was placed between the anode and cathode frames. Synthetic water was continuously fed at 1.75 and 1.51 L d⁻¹ at the steady state in anode and cathode, respectively. An internal recirculation loop (105 L d⁻¹) was placed in each compartment to maintain well-mixed conditions, to avoid concentration gradients and clogging of the granular matrix. A methacrylate cylinder was placed in the cathode effluent as a gas trap, in order to quantify and analyze gas production. The system was in a thermostatically controlled room at 22 ± 1 °C and at atmospheric pressure. To determine the influence of biological activity, an abiotic MEC with similar characteristics was constructed, resulting in an NCC of 400 mL, and operated as a control.

The cathode potential was monitored with an Ag/AgCl reference electrode (~197 mV vs Standard Hydrogen Electrode, model RE-5B, BASI, United Kingdom). All voltages are reported with respect to standard hydrogen electrode (SHE).

2.2. Influent characteristics

Bicarbonate was used as a carbon source in the cathode to promote the growth of autotrophic microorganisms. In the anode, bicarbonate improves the availability and transport of protons. Anode and cathode feed of both biotic and abiotic MEC consisted of nitrogen-purged synthetic medium with no added organic carbon sources, and had the following characteristics: 4 g L⁻¹ NaHCO₃, 10 mL L⁻¹ buffer (10 g L⁻¹ NH₄Cl, 60 g L⁻¹ Na₂HPO₄, 0.15 g L⁻¹ CaCl₂, 2.5 g L⁻¹ MgSO₄ · 7H₂O; 5 g L⁻¹ NaCl, 30 g L⁻¹ KH₂PO₄) and 0.1 mL L⁻¹ microelements solution (1 g L⁻¹ EDTA, 1 g L⁻¹ FeSO₄·7H₂O, 70 mg L⁻¹ MnCl₂·4H₂O, 100 mg L⁻¹ CuCl₂·2H₂O, 6 mg L⁻¹ H₂BO₃, 130 mg L⁻¹ CaCl₂·6H₂O, 2 mg L⁻¹ CuCl₂·2H₂O, 24 mg L⁻¹ NiCl₂·6H₂O, 36 mg L⁻¹ Na₂MoO₄·2H₂O, 238 mg L⁻¹ CoCl₂·6H₂O (adapted from Ref. [17])). The medium had a pH and conductivity around 8.0 and 5 mS cm⁻¹, respectively.

2.3. MEC start up and operation

The cathode of the biotic MEC was inoculated and operated in a recirculation loop for 4 days. The inoculum was a mixture of two different effluents coming from i) an urban wastewater treatment plant treating organic matter, nitrogen and phosphorus biologically, and ii) the effluent from a parent Microbial Fuel Cell (MFC) treating wastewater, with simultaneous nitrification-denitrification at the cathode. Microbial communities of complex diversity, including members of the Actinobacteriaceae, Bacteroidetes, Proteobacteria, Firmicutes, Chloroflexiaceae and Deinococcaceae groups, were present at samples used as inoculum (results not published).

During inoculation process a vigorous recirculation loop (150 L d⁻¹) was applied to generate stress conditions for the microorganisms and to force them to fix at the electrode surface. After inoculation, biotic MEC was started up in Open Circuit Voltage (OCV) and continuously fed with synthetic medium. When the biotic MEC reached a steady voltage value, different tests were done by gradually decreasing the cathode potential from ~400 to ~1800 mV. Once cathode potential was poised, samples were taken after the system reached the steady state. Steady state conditions were assumed when current demand and voltage were maintained at constant values, approximately 3–4 days after posing cathode potential. Based on NCC, HRT was about 6.24 and 7.65 h in biotic and abiotic MEC, respectively. Abiotic MEC was not inoculated, both anode and cathode compartments consisted of previously treated graphite electrodes.

MECs were operated in three-electrode configuration, where Working Electrode (WE) was the cathode electrode, Reference Electrode (RE) was an Ag/AgCl (described before) placed in the cathode chamber and Counter Electrode (CE) was the anode electrode. Cathode potential was poised, and current demand was monitored with a potentiostat (BioLogic, Model SP50, France). All the experiments were duplicated.

2.4. Analyses and calculations

Samples for the determination of chemical oxygen demand (COD) were taken on each experiment and analyzed with standard wastewater methods according to [18]. Chromatographic techniques were used to analyze volatile organic compounds with a Varian CP-3800 equipped with FactorFour™ CP8860 column and a Flame Ionization Detector (FID) in order to detect Volatile Fatty Acids (VFA; Acetate, Propionate, Butyrate) and Alcohols (Ethanol, Methanol, Propanol and Butanol).

Produced gas was trapped in a methacrylate chamber and sampled with a glass syringe. Gas samples were analyzed to detect hydrogen, carbon dioxide, methane, oxygen and nitrogen (H₂, CO₂, CH₄, O₂, N₂) with an Agilent 7820A GC System equipped with Washed Molecular Sieve 5A and Porapak® Q columns and a Thermal Conductivity Detector (TCD). Gas production calculations were given with respect to experimental conditions (Temperature 22 ± 1 °C; atmospheric pressure).

Gas production performance was characterized by calculating volumetric hydrogen production rate (Qₕ₂),
m^3 H_2 m^-3 NCC d^-1) normalized to cathode liquid volume, which is given by the following equation:

\[ Q_{H_2} = \frac{\int t \cdot C_{H_2} \cdot V_{gas} dt}{V_{NCC}} \]  

(2)

Where \( V_{gas} \) is the gas volume (m^3) sampled over a period of time (days), \( C_{H_2} \) is the concentration (% by volume) of hydrogen in the gas, and \( V_{NCC} \) is the net cathode compartment volume (m^3).

Cathodic hydrogen recovery (\( r_{cat} \), %) was used to evaluate hydrogen production efficiency of MECs and calculated as previously described by Logan and coworkers [19]:

\[ r_{cat} = \frac{n_{H_2}}{n_{CE}} \times 100 \]  

(3)

Where \( n_{H_2} \) is the amount (moles) of hydrogen experimentally recovered at the cathode, and \( n_{CE} \) is the amount that theoretically could have been produced based on the measured current. \( n_{CE} \) is given by the following equation [19]:

\[ n_{CE} = \frac{\int t \cdot I_{cat} dt}{2F} \]  

(4)

Where \( I \) is the measured intensity (A), \( dt \) is the time interval over which data are collected; \( F \) is Faraday’s constant (96,485 C mol^-1 of electrons); and 2 as conversion number of moles of electrons into hydrogen.

Economic feasibility of the MECs was evaluated in terms of hydrogen production versus energy consumed over time, based on measured voltage and intensity. For this purpose, estimated operational costs per kg of hydrogen produced were calculated in order to facilitate comparison with existing data and other authors. Hydrogen operational costs were calculated from energy consumed and they were based on average current prices of electric energy in the US [20].

Cyclic voltammetry (CV) were performed using a potentiostat (model SP50, BioLogic, France). A three-electrode configuration was used for CV tests, which was the same that it was operated. Four cycles were done from -200 mV to -1800 mV by imposing a linear scanning potential rate of 0.1 mV s^-1. To represent the results, the average of the four cycles was calculated. CV experiments were done to distinguish between biotic and abiotic MEC performances. An additional deionized water medium was prepared for biotic MEC to determine the effect of turnover (presence of substrate) and non-turnover (substrate depletion) conditions [21].

2.5. Biofilm characterization

To assess the composition of the cathode microbial community, graphite granules samples were extracted from the cathode at the first and last days of operation. Biofilm was dislodged from graphite surface by an ultrasonic bath (P-Selecta, Spain) for one cycle of 1 min followed by 2 min of centrifugation at 4000 rpm. Pellets were mixed and pooled in a single sample. Nucleic acids were extracted using the Fast DNA® SPIN Kit for soil (MP Biomedicals, US) according to the manufacturer’s instructions. The 16S rRNA gen was amplified by PCR using universal primers 357F [22] and 907R [23], PCR products were analyzed by denaturing gradient gel electrophoresis (DGGE) according to method described by Ref. [24]. A denaturing gradient of 35–65% of urea-formamide with 6% acrilamide at 60 °C and a voltage of 160 V was applied during 14 h. Analysis of gel images was done with the GelComparII v.6.1 software. Intense and differential DGGE bands were excised, purified and reamplified by PCR using the above mentioned primers and conditions. Reamplified bands were sequenced in reverse direction using the 907R primer (Macrogen, Holland).

Scanning Electron Microscopy (SEM) analyses were performed at the end of the operational period. Graphite samples from the biotic and abiotic MEC were extracted to compare the electrode surface. The samples were immersed in 2.5% (w/v) glutaraldehyde in a 0.1 M cacodylate buffer at pH 7.4 for a period of 4 h. Next, the samples were washed and dehydrated in an ethanol series. Washes were done with cacodylate buffer and with water, both per duplicate. Dehydration with graded ethanol followed temperature steps of 50, 75, 80, 90, 95 and 3 × 100 °C in periods of 20 min. The fixed samples were dried with a critical-point drier (model K-850 CPD, Emitech, Alemany) and sputtered-coated with a 40 nm gold layer. The coated samples were examined with a SEM (model DSM-960; Zeiss, Germany) at 20 kV and images were captured digitally.

Energy-dispersive X-ray spectroscopy (EDX; QUANTAX Microanalysis System) was also performed in the abiotic MEC graphite samples in order to identify the compounds deposited on the surface. Analyzed samples were not pretreated. Digital images of both SEM and EDX analysis were collected and processed by ESPRIT 1.9 BRUKER program (AXS Microanalysis GmbH, Berlin, Germany).

3. Results and discussion

3.1. Influence of cathode potential on hydrogen production

The influence of cathode potential on the hydrogen production rate was assessed by setting cathode potential from -400 to -1800 mV. All experiments were done comparing both biotic and abiotic MECs. Table 2 shows hydrogen production rate and the current demand at the different cathode potentials. In both MECs, current demand was directly related to hydrogen production. During biotic experiments, current demand increased very slowly, compared with the higher increase in the abiotic MEC. The low increasing rate of current demand was in agreement with the low hydrogen production rate observed in the biotic MEC. When both systems were fixed at cathode potential between -400 and -900 mV no gas formation was observed. In this range no variations were observed on the pH and the conductivity of the effluent.

When biotic MEC was poised at cathode potentials lower than -900 mV, gas was produced. The produced gas was enriched in H_2 (83–87%) and N_2 (13–17%), no carbon dioxide and methane were detected. In contrast to results obtained by Rozendal et al. [10], methane was not detected in the produced gas although bicarbonate was the only carbon source. This fact could indicate that methanogens were not active.
microorganisms in the MEC. Due to operational conditions, acetoclastic neither hydrogenotrophic methanogenesis were likely [25].

From cathode potentials between −900 and −1800 mV, the volumetric hydrogen production in the biotic MEC increased almost linearly ($R^2 = 0.957$) from 0.10 ± 0.01 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$ to 11.60 ± 1.10 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$. Above −1400 mV, volumetric hydrogen production rate was below 10.00 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$ which is the value estimated to be the minimum volumetric hydrogen production rate required for practical applications [9]. Below −1600 mV, volumetric hydrogen production rate increased to values above 10.00 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$ (Table 2).

The abiotic MEC produced gas at cathode potentials below −1000 mV. At such potentials (from −1000 to −1800 mV) abiotic gas was also enriched in hydrogen (81–93% H$_2$) being nitrogen the remaining fraction. As observed in the biotic MEC, no other compounds were detected in the gas composition. Abiotic hydrogen production also increased linearly ($R^2 = 0.977$) with cathode potential, from 0.50 ± 0.02 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$ at −1000 mV to 57.50 ± 4.00 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$ at −1800 mV. At cathode potentials equal or lower than −1200 mV, hydrogen production rate was above 10.00 m$^3$ H$_2$ m$^{-3}$ NCC d$^{-1}$.

When gas production started to increase, pH and conductivity in both systems rose up too. In the cathode of the biotic MEC operating at −1800 mV, pH and conductivity reached values of 10.8 and 8.5 mS cm$^{-1}$, respectively. A similar case was observed with abiotic MEC, with a resulting pH of 11.9 and a conductivity of 10.2 mS cm$^{-1}$ at the same cathode potential. The increases on conductivity of the cathode were probably due to transport of ion species other than protons and hydroxyl ions (i.e. Na$^+$, K$^+$, NH$_4^+$) through the membrane [26], which was confirmed by the anode conductivity decrease. No detectable variations on COD were observed and VFA and alcohols were not detected in the aqueous phase in any of the experiments.

### 3.2. Electrochemical characterization

Fig. 1 shows CV tests of biotic and abiotic MEC. CVs corroborated what was observed previously in Table 2. Hydrogen production was directly related to current demand, which increased much higher in the abiotic MEC than in the biotic at cathode potentials below −1000 mV. At poised cathode potentials lower than −1000 mV, intensity demand of the abiotic MEC was greater than the biotic MEC. In both biotic and abiotic CVs an oxidation peak could be observed at −530 and −560 mV vs SHE, respectively. According to Nernst equation, the shift on cathode potentials could be caused by small differences on pH between biotic and abiotic MEC. These oxidation peaks are associated to hydrogen oxidation [27]. In the case of abiotic MEC, hydrogen oxidation peak was much higher than in the biotic MEC because a higher quantity of hydrogen was produced. Under non-turnover conditions, no oxidation peak was observed due to low conductivity of the water, which limits electron transfer through the medium [16]. Only the hydrogen produced at the electrode surface could be detected. The high conductivity of the medium favored electron transfer through the medium and allowed high hydrogen production rates.

### 3.3. Identification of cathode microbial community

The composition of the microbial community in the biofilm of the biotic MEC after 45 days of operation was rather simple according to results obtained by PCR-DGGE (Figure S1). The
DGGE band pattern clearly differentiated from that of the parent MFC used as inoculum (Figure S1), thus indicating that microbial species were specifically enriched during the operational period. Three main bands of different intensities were detected. Obtained sequences showed the highest similarity to Hoeflea sp. (97%), Aquiflexum sp. (92%) and an unknown member of the Actinobacteria (Table S1). Aquiflexum sp. and Hoeflea sp. are both bacteria frequently observed in marine habitats with a broad tolerance to differences in salinity and alkalinity. Aquiflexum is described as a fermentative bacteria, which could produce hydrogen through fermentation [28,29]. According to this general description, it remains unclear whether the observed Aquiflexum phylotype could participate in H₂ production in the biocathode at the used conditions, since no organic matter was added and neither organic acids nor alcohols were produced. Hoeflea sp. has no described relationship with hydrogen production, but high conductivities measured on the cathode are optimal for their growth [30]. Finally, Actinobacteriaceae are heterotrophic bacteria playing an important role in the decomposition of organic matter or decaying biomass.

Microbial population had been reduced to Actinobacteriaceae, Bacteroidetes and Proteobacteria at biotic MEC cathode, but no correspondence was obtained for any of the observed bands. These changes on the microbial community were selected from the system, which had restricted operational conditions. No organic matter was present in the MEC influent, the only carbon source was bicarbonate and the cathode was poised at very low cathode potentials. Conductivity and pH at the cathode were always higher than 5 mS cm⁻¹ and 8.0, respectively. Although these results provide the first attempt to characterize the microbial community structure of a hydrogen producing biocathode, further analyses, such as culture-dependent methods or additional molecular methods, are required to verify microbial identification and determine H₂ producing activity by microorganisms.

3.4. Biotic and abiotic cathode morphology

At the end of the experimental period, graphite granules samples from the cathodes of both biotic and abiotic MEC were extracted and analyzed by Scanning Electron Microscopy (SEM). In Fig. 3 the obtained SEM images of abiotic (A) and biotic (B) cathodes are shown.

In the image of the abiotic MEC a large quantity of crystals over the graphite surface could be observed. The elemental composition analysis showed that mostly oxygen, calcium and carbon, but also sodium, phosphorus, magnesium and aluminum were present in different proportions in the abiotic graphite surface. On the other hand, large quantities of microorganisms were attached to the cathode of the biotic MEC forming a biofilm. Most microorganisms were forming large aggregations. The dominant morphology among the observed cells was large rods, although some spirochetes were also observed. The surface of the biotic cathode was covered by different substances: (1) mineral precipitation coming from the feed components (with high concentration of sodium bicarbonate) and (2) Exopolymeric Substances (EPS), secreted by some microorganisms to remain attached at the electrode. The mineral precipitation over the graphite surface in both biotic and abiotic MEC could lead to higher energy consumption due to overpotentials of the system.
3.5. Energy recovery

In most tests, energy required to achieve cathodic potentials in the abiotic MEC was higher than that required for biotic MEC. Hydrogen production rate versus energy consumed is represented in Fig. 2. For both biotic and abiotic MEC, the relationship between both parameters was found to be linear, obtaining an R² of 0.982 and 0.977 for biotic and abiotic MEC, respectively. Net cathode compartment volume was applied to correct the linear relation obtained, the slope observed for biotic and abiotic MEC was 0.116 m³ H₂ kWh⁻¹ and 0.064 m³ H₂ kWh⁻¹, respectively. By using microorganisms as cathode catalyst, the biotic MEC achieved hydrogen production values which represent almost half consumption of energy. Although hydrogen production rates in the abiotic MEC were higher than that in the biotic MEC (Table 2), energy consumption in the biotic MEC was much lower. Hydrogen was produced in a more efficiently way in the biotic MEC, obtaining productions as high as 0.365 m³ H₂ kWh⁻¹ at −1000 mV, while the highest production observed in the abiotic MEC was 0.071 m³ H₂ kWh⁻¹ at −1200 mV. The pH difference between the anode and the cathode of abiotic MEC was higher than biotic MEC. As shown by the Nernst equation, the resulting membrane pH gradient causes a potential loss of 60 mV per pH unit. Therefore, the energy demand of the abiotic MEC was higher than the biotic MEC to overcome this loss.

The results of the present study, in terms of energy recovery and efficiency are shown in Table 3, and compared to other existing technologies for hydrogen production [31]. Efficiency of the biotic and abiotic MEC was calculated from cathodic hydrogen recovery, applied current was the only electron source.

The average efficiency (η) obtained was 113% and 67% in the biotic and abiotic MEC, respectively. However maximum values observed were of 175% and 96% at −1000 mV and −1800 mV in the biotic and abiotic MEC, respectively. Those high efficiencies could only be explained by parallel reactions in the MECs. In general, energy requirements for hydrogen production in the biotic MEC were considerably lower compared to the abiotic. As other researchers already demonstrated, electrons were utilized more efficiently in the biotic MEC [10].

At best operation conditions, energy requirements of the biotic MEC (33.2 kWh kg⁻¹) are below that of partial oxidation of heavy oil, coal gasification and grid electrolysis of water, and close to Steam methane reforming (22.4 kWh kg⁻¹), which is a well-established hydrogen production technology.

3.6. Perspectives of hydrogen production in a biotic cathode

Although the MEC systems were not optimized for hydrogen production, an analysis of the results obtained can be made to assess the viability of its use and compared with other studies using MECs.

Although hydrogen production rate in the biotic MEC was lower than that obtained for the abiotic, the first one showed a better performance in terms of energy requirements. Best conditions in terms of energy requirement and therefore operational costs are found at a cathode potential of −1000 mV for the biotic MEC and −1200 mV for the abiotic, with estimated production costs of 3.20 USD kg⁻¹ H₂ and 16.44 USD kg⁻¹ H₂ respectively.

Estimated operational production costs of hydrogen on the biotic MEC was below estimated market value of hydrogen (6 USD kg⁻¹ H₂) [32] and also in the range of the US Department of Energy threshold cost of hydrogen for 2020, which was estimated in 2–4 USD kg⁻¹ H₂ [33], while the estimated production cost with abiotic MEC was much higher.

The lowest operational costs for hydrogen production of this study was slightly higher than 3.01 USD kg⁻¹ H₂ found by Cusick et al. [34] for a MEC treating domestic wastewater at the anode and producing hydrogen at the cathode using a Pt catalyst.

In this study conditions for methanogenic development were avoided. Organic matter was not present on the system so acetoclastic methanogenesis cannot occur. Although bicarbonate was used in the cathode, the pH was always higher than 8, avoiding the presence of CO₂ and therefore hydrogenotrophic methanogenesis.

Finally, other studies suggest that other modifications on parameters of the biotic MEC can be made to optimize the system [34,35]. This could lead to its potential economic viability in future implementation. By introducing wastewater treatment at the anode chamber, the degradation of organic matter could reduce operational costs by reducing electric consumption of the system. In a recent study, Villano et al. [35] demonstrated the feasibility of a MEC in which degradation of low strength wastewaters could occur at the anode chamber. 600 mg L⁻¹ of acetate were consumed by microorganisms present at the anode, generating a current of 110 mA. This current was used at the cathode chamber to biologically produce hydrogen and methane. In the biotic system of the present study a current of 18 mA was necessary to produce hydrogen at −1000 mV. If organic matter could be used in the anode chamber to avoid electric consumption, operational

<table>
<thead>
<tr>
<th>Table 3 – Key parameters of the results obtained in the present study and other existing hydrogen producing technologies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotic MEC (this study)</td>
</tr>
<tr>
<td>Efficiency, based on energy input (%)</td>
</tr>
<tr>
<td>Energy consumption (kWh kg⁻¹ H₂)</td>
</tr>
</tbody>
</table>

* Data calculated from Ref. [28].
cost would be considerably reduced. Even higher hydrogen production rates could be achieved without increasing operational costs. Further investigations would be necessary to determine the operational conditions and the viability of this experimental modification.

4. Conclusions

Hydrogen had a linear relationship with cathode potential in biotic and abiotic MECs within a range of −900 to −1800 mV. CV tests corroborated that higher hydrogen production rates could be achieved by decreasing cathode potential. At poised cathode potential of −1600 mV hydrogen production rate rose to values above 10 m³ H₂ m⁻³ NCC d⁻¹, which is estimated to be the minimum production for practical applications. Microorganisms present in the cathode of the biotic MEC were identified as *Hoeflea* sp. and *Aquiflexum* sp.

The results of the present study point out that biotic MEC shown a better performance in terms of hydrogen production per kWh consumed and therefore, lower estimated operational costs, which are below hydrogen market value and hydrogen threshold cost for 2020.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2013.11.017.

REFERENCES


Supplementary information

Assessment of biotic and abiotic graphite cathodes for hydrogen production in microbial electrolysis cells.

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Figure S1. Acrylamide gel image of the PCR-DGGE band pattern of the microbial community in the cathode of the biotic MEC and the parent MFC. Marked bands were excised, re-amplified by PCR and sequenced.

Table S1. PCR-DGGE sequences from bands of cathode compartment of biotic MES.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Most probable identified species a</th>
<th>GenBank accession number</th>
<th>Band number</th>
<th>% Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes</td>
<td>Hoeflea marina</td>
<td>NR_043007</td>
<td>1</td>
<td>97</td>
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<tr>
<td>Proteobacteria</td>
<td>Aquiflexum balticum</td>
<td>NR_025634</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>Actinobacteriaceae</td>
<td>Micromonospora echinosusca</td>
<td>NR_044891</td>
<td>3</td>
<td>82</td>
</tr>
</tbody>
</table>

a Identification was determined through NCBI’s BLAST searches using reference genomic sequences (http://www.ncbi.nlm.nih.gov)

Figure S2. EDX image and results of the graphite samples from the abiotic MEC cathode.
Chapter 5. Deciphering the electron transfer mechanisms for biogas upgrading to biomethane within a mixed culture biocathode

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Deciphering the electron transfer mechanisms for biogas upgrading to biomethane within a mixed culture biocathode†

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Biogas upgrading is an expanding field dealing with the increase in methane content of the biogas to produce biomethane. Biomethane has a high calorific content and can be used as a vehicle fuel or directly injected into the gas grid. Bioelectrochemical systems (BES) could become an alternative for biogas upgrading, by which the yield of the process in terms of carbon utilisation could be increased. The simulated effluent from a water scrubbing-like unit was used to feed a BES. The BES was operated with the biocathode poised at −800 mV vs. SHE to drive the reduction of the CO₂ fraction of the biogas into methane. The BES was operated in batch mode to characterise methane production and under continuous flow to demonstrate its long-term viability. The maximum methane production rate obtained during batch tests was 5.12 ± 0.16 mmol m⁻² per day with a coulombic efficiency (CE) of 75.3 ± 5.2%. The production rate increased to 15.35 mmol m⁻² per day (CE of 68.9 ± 0.8%) during the continuous operation. Microbial community analyses and cyclic voltammograms showed that the main mechanism for methane production in the biocathode was hydrogenotrophic methanogenesis by Methanobacterium sp., and that electromethanogenesis occurred to a minor extent. The presence of other microorganisms in the biocathode, such as Methylomicrobiaceae, revealed the presence of side reactions, such as oxygen diffusion from the anode compartment, which decreased the efficiency of the BES. The results of the present work offer the first experimental report on the application of BES in the field of biogas upgrading processes.

Introduction

Anaerobic digestion (AD) is a widespread process to produce biogas through the valorisation of solid and liquid organic wastes. Biogas consists of a mixture of mainly methane (CH₄; 35–65%) and carbon dioxide (CO₂; 15–50%) with trace amounts of other compounds (i.e. hydrogen sulphide (H₂S), volatile organic compounds, siloxanes and water), which can be used in cogeneration units to obtain energy in the form of heat and electricity.¹

The term biomethane has been used to describe methane-rich biogas (95% v/v) from AD of organic wastes, which could be directly used as a vehicle fuel or injected into the gas grid.² Biogas upgrading technologies imply (i) the removal of harmful trace components, and (ii) the upturn of the methane content to increase the calorific capacity of the biogas.³ According to the International Energy Agency (IEA), water scrubbing is the most used technique for biogas upgrading, accounting for about 40% of the total upgrading plants.⁴ This technology consists of an absorption tower where CO₂ is absorbed in water and biomethane is obtained. The absorbed CO₂ is released in the atmosphere in a subsequent stripping tower,⁵ what often implies the consumption of energy. The transformation of the CO₂ contained in the effluent of the absorption tower into methane could be a useful strategy to increase the biomethane production and could contribute to reduce the CO₂ emissions. In this light, bioelectrochemical systems (BES) can be a promising technology to drive the reduction of dissolved CO₂ into methane. Some authors already proposed the coupling of BES with AD in single reactors to increase the yield of the whole process.⁶⁻⁷ This work proposes a new promising application for BES, which is the replacement of the stripping tower of a water scrubbing unit for biogas upgrading. The possibility of coupling water scrubbing and BES opens the door to an innovative hybrid process. The bioelectrochemical conversion of CO₂ to methane is known as electromethanogenesis, and was first presented by Cheng and coworkers.⁸ Other researchers studied the methane production mechanism at different cathode potentials, and the

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ra09039c
performance at the long term operation. Accordingly, methane production can take place in a biocathode mainly by two mechanisms: (i) hydrogenotrophic methanogenesis (eqn (1)), using hydrogen as electrochemical mediator, which can be produced in situ either bioelectrochemically or electrochemically, and (ii) using directly electrons as reducing power source (eqn (2)).

\[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \]  

(1)

\[ \text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}; E_0 = -240 \text{ mV vs. SHE} \]  

(2)

Methane production using hydrogen as intermediate was shown to be the predominant mechanism in methane producing biocathodes poised at low cathode potentials (i.e. \(-750 \text{ mV vs. SHE}\)). Nevertheless, Eerten-Jansen et al., also suggested the production of methane through other intermediates, such as acetate or formate. The conversion of CO2 to methane through direct electron transfer was suggested by Cheng et al., 2009 and Fu et al., 2015, who used a pure culture of *Methanobacterium palustre* and thermophilic methanogens, respectively. Finally, both direct and hydrogen mediated electron transfer took place in the biocathodes studied by Siegert and co-workers. Syntrophic relationships, such as interspecies hydrogen transfer and direct interspecies electron transfer (DIET) have been also proposed for the production of methane with mixed cultures, being the last one more energetically conservative due to the fact that electrons are directly transferred between microorganisms, avoiding the production of intermediates. So far, there is only one study that demonstrated the DIET between two pure cultures. Since mixed cultures have been usually utilised for the production of methane, the mechanism by which it is produced in a biocathode may differ among different studies. Thus, methane production mechanism in biocathodes is still unclear, so that more studies regarding the microorganisms involved and its electrochemical interaction with the electrode surface are needed to understand and optimise the whole process.

The present work proposes the coupling of water scrubbing with BES for biogas upgrading. The methane production and the electrochemical performance of the BES were studied. Electrochemical and microbiological analyses were carried out to understand how the microbial community dealt with the reducing power, which microorganisms were involved in the electron transfer, and which intermediates and final products affected the bioelectrochemical performance of the BES.

**Experimental**

**BES construction**

A two-chambered BES was constructed using a previous described design by Batlle-Vilanova et al. A schematic representation of the BES design and the equipment is presented in the ESI (Fig. S1†). In the present study, after the graphite replenishment of the compartments, the net anode compartment (NAC) and the net cathode compartment (NCC) volumes were 410 mL and 420 mL, respectively. The resulting cathode electrode surface was 0.57 m² (see ESI, Section S2†). The system was thermostatically controlled at 34.7 ± 1.1 °C and under atmospheric pressure conditions.

The BES was operated in a three-electrode configuration with a potentiostat (BioLogic, model VSP, France), which controlled the cathode potential and monitored the current demand. The biocathode was used as working electrode (WE) and the anode as counter electrode, the reference electrode (RE) was an Ag/AgCl (+197 mV vs. Standard Hydrogen Electrode [SHE], model RE-5B, BASI, United Kingdom) placed in the cathode chamber. All voltages within this study are reported with respect to SHE.

**BES start-up**

The operational period of the BES was divided into different stages depending on the mode of operation (Fig. S2†). The cathode was inoculated in closed circuit voltage (CCV) mode at −600 mV. As inoculum, 100 mL of diluted effluent from an anaerobic digester were used. The inoculum was continuously recirculated at a high flow of 150 L per day for 6 days to generate stress conditions to the microorganisms and to force them to get attached to the electrode surface. The anode was not inoculated and water was used as electron donor (H2O/O2, E0 = 820 mV). Synthetic medium was used in both anode and cathode compartments, which was prepared based on ATCC1754 growth medium, and was already described elsewhere.

**Batch operation**

Thirteen batch tests were consecutively performed in the biocathode under the same conditions, unless otherwise stated. Test 6 was performed in open circuit voltage (OCV), without applying any voltage, to check whether the biocathode was able to produce methane by non-electrochemical mechanisms. Batch tests lasted between 4 and 10 days. Before each batch test, the biocathode and the anode were washed with synthetic medium with 3 times the net volume of each chamber. The biocathode was connected to a methacrylate chamber filled with the same medium to collect the gas produced.

Gas samples were taken regularly to quantify the methane production, and liquid samples to check the concomitant production of other valuable compounds in the liquid phase (i.e. volatile fatty acids [VFA] and alcohols). A pH sensor (model 5303, Crison, Spain) was placed in the cathode recirculation...
loop to measure the pH with a transmitter (MultiMeter MM44, Crison, Spain) connected to a memograph (Graphic data manager RSG40, Memograph M, Endress+Hauser, Switzerland).

After the batch tests the BES was operated in continuous mode, under the same conditions than during the start-up period (Fig. S2†), to validate the operational stability of the BES in the long-term.

Analyses and calculations

Liquid and gas analyses. The composition of the gas phase was analysed with an Agilent 7890A (Agilent Technologies, US) gas chromatograph (GC) equipped with an HP-Molesieve column and a thermal conductivity detector (TCD) to detect hydrogen, oxygen, nitrogen, methane, carbon monoxide and CO2.26 VFA and alcohols in the liquid phase were analysed in a second channel of the same GC equipped with a DB-FFAP column and a flame ionisation detector (FID).28 Sulphate concentration (SO4\(^{2-}\)) was analysed according to the standard methods for the examination of water and wastewater.21 A presence/absence analysis of H2S in the gas phase was conducted with a CP-3800 GC (Varian, US) equipped with a pulsed flame photometric detector (PFPD) and a GS-GasPro column (Agilent Technologies, US).

All of the production rates are given in mmol of carbon per m\(^2\) of electrode surface per day (mmol m\(^{-2}\) per day).

Calculation of coulombic and energy efficiency. The efficiency on electron removal or supply by a BES through an electrical circuit has been traditionally referred to as coulombic efficiency (CE),23 and it is calculated as shown in eqn (3):24

\[
CE(\%) = \frac{8F \times n_{\text{CH}} \times I(t) \times dt}{t_0 \times t_1} \times 100
\]

where, 8 are the number of electrons consumed per mole of methane produced, F is Faraday's constant (96 485 C mol\(^{-1}\)), \(n_{\text{CH}}\) are the moles of methane produced between \(t_0\) and \(t_1\), and \(I(t)\) is the current (A) integrated over time (from \(t_0\) to \(t_1\) in seconds).

The energetic efficiency of the process (\(\eta_E\)) was calculated according to eqn (4):20,24

\[
\eta_E(\%) = \frac{-\Delta G_{\text{CH}} \times n_{\text{CH}} \times E_{\text{cell}} \times I(t) \times dt}{t_0 \times t_1} \times 100
\]

where \(\Delta G_{\text{CH}}\) is the Gibbs free energy of methane oxidation (\(-890.4\) kJ mol\(^{-1}\)),27 and \(E_{\text{cell}}\) is the voltage applied to the cell (V).

Cyclic voltammetry analyses. Electrochemical analyses were conducted in a single-chamber BES according to Pous et al., 2015.26 Three different tests were prepared with fresh medium for (i) abiotic conditions, (ii) biocathode microorganisms, and (iii) biocathode microorganisms after medium exchange. All the cyclic voltammograms (CV) were performed under turnover conditions. Before the CV performed with microorganisms from the biocathode, a chronoaeropmetry at \(-800\) mV, was performed during 120 h previous to the CV to favour the microorganisms' growth. The last CV was performed 30 minutes after the medium exchange. Platinum wire, graphite rod (9.74 cm\(^2\)), and Ag/AgCl were used as counter electrode, WE and RE, respectively. The cathode was poised at \(-800\) mV. When current demand was observed, the CVs were performed. The scan window was from 0 to \(-800\) mV, and the scan rate 10 mV s\(^{-1}\). Three cycles were performed in each CV and data from the last cycle is shown.

Biocathodic community analyses. At day 188, between batch test 2 and 3, the BES was opened and about 26 g of granular graphite from different parts of the biocathode were extracted and integrated to assess the microbial community composition.

DNA was extracted using the Fast DNA® SPIN Kit for soil (MP Biomedicals, US) according to the manufacturer’s instructions. DNA quantification was assessed with a nanodrop spectrophotometer (Thermo Scientific, US) characterized by 260/280 and 260/230 nm absorbance ratio in order to be comparable with quantitative real-time PCR (qPCR).

Bacterial diversity and microbial community structure of the cathode biofilm was analysed for 454 pyrosequencing (Research and Testing Laboratory, Lubbock, US). The 16S rRNA gene was analysed by primers 341F-907R for bacteria,22 and 341F-958R for archaea.28 Sequences obtained were analysed using MOTHUR software (v. 1.22.1) at 97% similarity.26 Sequences shorter than 250 bp and longer than 600 bp were excluded. Chimeric sequences were discarded by executing UCHIME.26 The most abundant sequences of bacteria and archaea were compared to BLAST supported by NCBI in order to obtain the closest similarity related to genbank database sequence. Alignments and identity matrix comparisons of deposited sequences were performed in BioEdit (v. 7.0).21 Post-data analysis was done by KRONA.24

Additionally, 16S rRNA gene was also quantified by qPCR to estimate the total amount of bacteria and archaea. All reactions were performed in a 7500 Real Time PCR system (Applied Biosystems, US) using the SYBR® Green PCR Mastermix. Primers and thermal cycling conditions for bacteria (341F-534R) and archaea (364af-A934b) 16S rRNA were used.25,26 Results were analysed using SDS software (Applied Biosystems, US). Standard curves were obtained using serial dilutions from 10\(^2\) to 10\(^7\) copies of linearised plasmids containing the respective functional genes. Controls without templates gave null or negligible values.

Results

Inoculation and start-up periods

According to the results observed by Marshall et al., the biocathode was poised at \(-600\) mV, because they demonstrated that the concomitant production of acetate, methane and hydrogen occurred at that potential.25 A negligible current demand was observed in the biocathode along the first part of the start-up period (Fig. S3†). At day 64, the NaHCO\(_3\) added to the medium was removed, causing a slightly decrease of the medium pH (from 6.7 ± 0.5 to 5.4 ± 0.2), which favoured inorganic carbon speciation towards CO\(_2\). Up to day 75, neither gas nor organic
compounds production was observed in the biocathode. The average current demand from day 64 to day 75 was around 1.1 ± 0.2 mA m⁻². At day 75 the cathode potential was decreased to −800 mV to increase the amount of reducing power supplied to the biocathode. From that point on, the current demand increased and methane was detected as the main product. At day 159, when the current demand stabilised around 28.6 ± 0.6 mA m⁻², batch tests were performed in the biocathode to characterise and quantify the methane production.

Performance of the biocathode during operation

After the start-up period, different consecutive batch tests were performed in the biocathode under the same conditions. The tests were ordered chronologically and identified with a number. The results obtained for the different tests are shown in Table 1. Methane production rate, CE and ηₑ for each test were obtained from the linear plot between the mmol of methane produced over time. The most representative tests are presented in the ESI (Fig. S4†).

Analyses of the liquid phase revealed that compounds such as VFA and alcohols were not produced in the biocathode. Nevertheless, sulphate concentration in the influent was 3.6 mg L⁻¹, and it was not detected in the effluent, so sulphates were completely consumed in the biocathode. Qualitative analyses demonstrated the presence of H₂S in the off gas. Methane was detected in the gas phase with a volumetric concentration of 65–85%, being the rest CO₂ (15–35%) and trace amount of oxygen (1–8%) and H₂S.

The overall performance of the biocathode from tests 1 to 5 was very similar. The pH in those tests was around 6. The current demand ranged from 2.4 to 40.8 mA m⁻², and the production rate from 1.36 to 1.89 mmol m⁻² per day was obtained with associated CEs from 39.0 ± 1.6 to 51.7 ± 4.5%. Test 6 was performed in OCV mode. A 20-fold lower amount of methane was produced and the pH decreased 1.4 units compared to the previous test, which was caused by the lack of electrochemical activity in the biocathode. The BES was negatively affected after the OCV test.

Subsequent tests (i.e. 7 to 9) showed a lower performance in terms of current demand and methane production rate. Biogas was directly flushed to the biocathode once per day during test 10 and 11 to favour substrate availability, displace oxygen diffused to the cathode, and promote the growth of methanogenic microorganisms. The current demand was monitored as a parameter indicating the activity of the biocathode. By applying this strategy the system recovered, and after 2 batch tests (i.e. test 12) the performance was similar to the previous tests. Test 12 showed similar performance to tests 1 to 5 (Table 1). In the subsequent and last test, the activity increased, obtaining a higher current demand of 70.7 ± 6.6 mA m⁻² and a production rate of 5.12 ± 0.16 mmol m⁻² per day, with a CE of 75.3 ± 5.2%, which coincided with the higher pH (i.e. 7.1) of the biocathode. Comparative analysis (Fig. S5†) showed that methane production rate was linearly related (r² = 0.99) to the current demand of the biocathode. The higher the current demand was, the higher the methane production rate. No evidence of dependence between the production rate and other parameters (i.e. pH and CE) was observed in this study (Fig. S5†). Although it was not investigated in the present study, previous studies suggested that other parameters, such as temperature, inoculum source or electrode materials could affect methane production.16,38,39 The ηₑ during the firsts tests (i.e. test 1 to test 5) were similar, ranging from 21.2 ± 1.8 to 31.7 ± 2.1%, and increased to 39.7 ± 3.6% in the last batch test (i.e. test 13).

After batch tests, the BES was operated in continuous flow under the same conditions than the start-up period (Fig. S2†). Fig. 1 shows the evolution of the current demand and the production rate during this period. The average methane production rate during the first days was 3.99 ± 0.06 mmol m⁻² per day, which increased and stabilised at 15.35 mmol m⁻² per day after 43 days of continuous operation. The results regarding the continuous operation are included in Table 1 (test 14) and correspond to the results obtained at the end of the period, when current demand and production rate were stable. The CE and the ηₑ obtained during the continuous operation were 68.9 ± 0.8% and 39.7 ± 1.3%, respectively, which were close to

Table 1 Tests with biogas as the sole carbon source. OCV: open cell voltage; n/a: not available

<table>
<thead>
<tr>
<th>Test</th>
<th>Operation mode</th>
<th>Current demand (mA m⁻²)</th>
<th>pH</th>
<th>r²</th>
<th>Production rate (mmol CH₄ m⁻² per day)</th>
<th>CE (%)</th>
<th>ηₑ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Batch</td>
<td>32.0 ± 2.4</td>
<td>6.1 ± 0.1</td>
<td>0.998</td>
<td>1.63 ± 0.01</td>
<td>45.3 ± 1.9</td>
<td>26.4 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>Batch</td>
<td>40.8 ± 3.5</td>
<td>5.9 ± 0.3</td>
<td>0.979</td>
<td>1.66 ± 0.05</td>
<td>39.0 ± 1.6</td>
<td>21.2 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>Batch</td>
<td>31.1 ± 6.8</td>
<td>6.3 ± 0.1</td>
<td>0.996</td>
<td>1.36 ± 0.02</td>
<td>47.9 ± 5.8</td>
<td>23.7 ± 5.8</td>
</tr>
<tr>
<td>4</td>
<td>Batch</td>
<td>32.8 ± 5.2</td>
<td>6.1 ± 0.2</td>
<td>0.981</td>
<td>1.89 ± 0.05</td>
<td>45.9 ± 3.6</td>
<td>30.3 ± 4.5</td>
</tr>
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<td>27.3 ± 2.4</td>
<td>6.0 ± 0.1</td>
<td>0.999</td>
<td>1.67 ± 0.02</td>
<td>51.7 ± 4.5</td>
<td>31.7 ± 2.1</td>
</tr>
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<td>0.891</td>
<td>0.09 ± 0.02</td>
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<td>0.14 ± 0.01</td>
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<td>25.1 ± 4.7</td>
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<td>n/a</td>
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<td>68.9 ± 0.8</td>
<td>26.4 ± 3.9</td>
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the last batch test, whilst the current demand and the methane production rate were triplicated.

**Biocathode microbial community**

Preliminary observations using scanning electron microscopy suggested that the biocathode consisted of a dense microbial community (Fig. S6†). Results of the specific microbial community analysis through pyrosequencing for archaea and bacteria are shown in Fig. 2. More detailed results of the pyrosequencing analyses are shown in Fig. S7†. Archaea and bacteria community analysis showed abundances of $3.36 \times 10^6$ DNA copies per g of graphite and $2.77 \times 10^6$ DNA copies per g of graphite, respectively, which means that the biocathode was composed by 55% of archaea and 45% of bacteria. *Methanobacterium* sp. dominated the archaea community (Fig. 2A). *Methanobacterium subterraneum* composed 10% of the *Methanobacterium* genus, which was identified with a similarity of 99% among other non-identified species.

Bacteria community presented higher diversity of phylums than archaea (Fig. 2B). *Proteobacteria* composed 51% of the sample, more concretely *Alphaproteobacteria* (32%). The dominant genus of the *Alphaproteobacteria* was identified as *Methylcystis* sp. (20%). The rest of the community was divided in several phylums, highlighting the presence of *Firmicutes* (*Clostridium* sp. 12.0%) and *Bacteroidetes* (*Anaerophaga* sp. 5.6%).

**Electrochemical characterisation**

CVs were performed in microcosms using the effluent of the biocathode to figure out which was the predominant methane production mechanism. The results of the CVs and its first derivative are presented in Fig. 3. Abiotic CVs showed a plain shape, demonstrating that redox active species were not present in the synthetic medium. Current density was low, and...
hydrogen reductive wave appeared at very negative potentials, such as \(-640\) mV. After a growth phase of 120 hours, the CVs performed with microorganism from the biocathode showed a higher current density. One cathodic catalytic wave was observed at a midpoint potential of \(-190\) mV. Similarly, another catalytic wave, typically related to hydrogen production, was also observed, which started at a potential of \(-440\) mV. In the CV performed after the medium exchange the reductive wave allocated at \(-190\) mV disappeared, whereas hydrogen reductive wave remained.

**Discussion**

**Methane production**

The methane production rate was directly related to the current demand of the biocathode. The low quantity of methane detected during the OCV test confirmed that methane production mostly relied on the electricity provided to the biocathode, and previous studies demonstrated that methane was not abiotically produced at such cathode potential. Thus, methane production in the biocathode was bioelectrochemically driven.

The lower current demand and methane production after the OCV test were likely caused by the lack of available reducing power for a period of about 70 hours. Thus, non-electrochemical microorganisms present in the biocathode, such as *Methylocystis* sp. were favoured over methanogens during the OCV test. Another hypothesis was that the lack of activity caused the pH inside the biocathode being similar to the influent pH, which was close to 5. At such low pH values, the methanogenic metabolism could be inhibited, so that, after the OCV test it took time to recover the previous performance. Otherwise, the increased activity of the last batch test could have been caused by a higher pH of the biocathode. While previous tests had not significant differences of pH, being its value around 6, in test 13 it increased to 7.1, likely due to the higher current demand and the increase of the bioelectrochemical activity. Although substrate availability is lower at high pH values, some microorganisms from *Methanobacterium* sp. have been reported to grow better at pH higher than 6.5, with optimum growth values even higher than 7. Thus, the pH conditions were more favourable for the methanogens. The effect of the pH in the electromethanogenic biocathode cannot be contrasted to other studies because it has not been reported yet.

The results regarding the continuous operation (i.e. test 14) were similar to the last batch tests in terms of pH, CE and \(\eta_r\). However, the current demand and the methane production rate, were triplicated, likely because the higher substrate availability caused by the continuous CO₂ supply in the form of a saturated solution.

**Microbial activity of the biocathode**

The presence of only one genus of archaea responds to the specific conditions applied to the biocathode. *Methanobacterium* genus was described as an hydrogenotrophic methanogen by Kotelnikova and co-workers. This genus was responsible for methane production in the studied biocathode. Previous studies already reported the dominance of *Methanobacterium* sp. in methane-producing biocathodes, with high similarities to *Methanobacterium palustre*. Both, direct and hydrogen mediated production, were observed in biocathodes dominated by *Methanobacterium* sp.

In contrast, the bacterial community was highly-diversified. The most abundant species which could play a role in the biocathode were examined. *Methylocystis* sp. is especially common near environments where methane is produced. The presence of methane in the biocathode favoured this microorganism. It has been described in previous articles that *Methylocystis* sp. is able to transform methane to CO₂ under aerobic conditions. Its activity could advantage the archaea activity, removing the oxygen diffused from the anode to the biocathode, and creating an anaerobic environment. However, this reaction decreased the efficiency of the process due to the methane consumption, as suggested by other authors.

*Firmicutes* members, such as *Clostridium* sp. have several environmental roles. Hydrogen production from organic compounds was demonstrated by six mesophilic *Clostridia*. Recently, *Firmicutes* were identified in an autotrophic hydrogen producing biocathode.

*Anaerophaga* sp. was identified in consortium of sulphate-reducing bacteria in biofilms used to analyse the corrosion behavior. It was detected also in electricity producing bioanodes and its activity decreased in the long term because of the lack of dissolved electron acceptors, such as sulphate.

**Bioelectrochemical methane production**

According to the results obtained in the present study, the main reactions that took place inside the biocathode were represented in Fig. 4. Given the operational conditions, the relatively high methane production rates, and the biocathode microbial community composition, hydrogen was most likely used as intermediate for methane production. Liquid phase analyses did not reveal the presence of acetate, even at very low concentrations (<5 mg L⁻¹), and formate production is unlikely at the poised cathode potential. Although hydrogen was not detected in the gas phase, previous studies demonstrated that it was produced not only electrochemically, but also by microorganisms of the biocathode community, such as *Clostridium* sp., that were able to catalyse its production. The CVs presented in this study supported the evolution of the hydrogen reductive wave towards lower current densities. Hydrogen catalytic wave increased in the CVs in presence of microorganisms, and remained after the medium exchange. Thus, it was suggested the ability of some microorganisms present in the biocathode, such as *Clostridium* sp., to attach to the electrode surface and catalyse hydrogen production at low cathode potentials. Hydrogenotrophic methanogens, such as *Methanobacterium* sp., have been reported to grow very rapidly in microbial electrolysis cells. Interspecies hydrogen transfer occurred between *Clostridium* sp. and *Methanobacterium* sp., which combined the hydrogen produced with CO₂ to obtain methane, according to
eqn (1). Although this was likely the predominant methane production mechanism, the combination of CO2 with protons and electrons according to eqn (2), could not be discarded as it likely occurred to a minor extent. In this sense, it was not clear whether the cathodic catalytic wave observed at –190 mV could be related to methanogens or sulphate-reducing bacteria, because both reactions occurred at similar theoretical potentials.54,55 According to Su et al., 2012 the presence of the cathodic catalytic wave at the potential of –190 mV could be related to sulphate reduction.56 Otherwise, Fu et al., 2015 demonstrated similar behaviour of a biocathode methanogenic community.15 In that study, the cathodic catalytic wave remained after the medium exchange, contrarily, in the present study the cathodic catalytic wave disappeared, which suggested that the methanogens of the present study were not attached to the electrode surface and likely used a mediator to deal with reducing power.

The relatively low CE observed during the operation of the BES was caused by the presence of cross-over reactions (Fig. 4). Some of these cross-over reactions were suggested and supported by the microbial community identification. The relatively high abundance of Methylloecysis sp. suggested the presence of oxygen in the cathode. Since water oxidation occurred in the anode chamber, the oxygen generated in this reaction can diffuse to the cathode through the membrane.29 In the cathode it was partly electrochemically reduced to water, due to the reducing conditions, and partly used by Methylloecysis sp. to consume methane, which decreased the CE (see ESI, Table S1†). The presence of sulphate in the influent promoted the growth of sulphate reducing bacteria. This was suggested by the presence of Anaerophaga sp. which reduced SO42− in the liquid phase to H2S. Since sulphate reducers and methanogens are direct competitors for hydrogen, the activity of the methanogens, and therefore the CE, decreased due to the presence of this microorganisms in the biocathode (Table S1†).31 Although oxygen was the main electron sink, sulphate reduction was another cross-over reaction that decreased the CE of the process. However, the effect of sulphate reduction on the CE compared to oxygen was 100 and 10 times lower during batch and continuous operation, respectively (Table S1†). Another potential electron sink is the production of hydrogen as intermediate. At such low cathode potential, hydrogen was used as mediator to produce methane through hydrogenotrophic methanogenesis.8,11 Gas quantification, especially when hydrogen is present, is not accurate as it may diffuse out of the reactor through the connectors, the tubes, or the membrane, therefore part of the CE losses could have been caused by these reasons.23–24

Perspectives

The present study demonstrated that the main methane production mechanism of the studied BES was hydrogen mediated. Hydrogenotrophic methanogens, such as Methanobacterium sp. drove methane production. It was suggested that syntrophic interactions between bacteria and archaea species present in the microbial community sustained the development of the biocathode and allowed for the production of methane. However, some of these interactions decreased the efficiency of the process. Understanding of the microbial community involved in methane production in BES and its interaction with the cathode electrode could lead to the development of practical applications for BES as a biogas upgrading process.

Nowadays, the methane content of the biogas can be increased through water scrubbing, releasing CO2 to the atmosphere in a stripping unit according to Fig. 5A. However, this study has shown the potential of BES in the field of biogas upgrading. The replacement of the stripping unit by a BES according to Fig. 5B could increase the yield of the process in terms of carbon utilisation, and therefore reduce the CO2 emissions, especially when renewable energy is used to drive the process. Typically, the effluent from an absorption tower for biogas upgrading has a CO2 and methane content in the solution of around 96% and 4%, respectively, when this effluent was treated with the BES, an additional amount of biogas was produced, which was composed by 65–85% CH4, 15–35% CO2 and 1–8% O2. The present study demonstrated the robustness of the operation at long term (i.e. more than 420 days) of a BES using CO2 absorbed from biogas and producing methane. The methane production rate obtained during continuous operation was 15.35 mmol m−2 per day with a CE of 68.9 ± 0.8%, which is three times higher compared to the best results obtained during batch tests. A recent study by Siegert and co-workers suggested that the production rate could be even more increased by choosing a good combination of anode and cathode materials.16

The energy efficiency of the process was found in this study as the key bottleneck for scalability and applicability of BES for biogas upgrading. The kWh recovered in the form of methane represented 39.7 ± 1.3% of the kW h supplied to the BES. Accordingly, the actual energy efficiency could restrict the potential application of this technology to the energy storage
during off-peak energy production periods or renewable energy harvesting and storage in the form of methane, which is easier handled than electricity and can be distributed through the existing gas grid. Regarding the energy efficiency, the oxidative conditions of the anode could be used to carry out profitable reactions to increasing the overall energy efficiency and the value of the process. In a study carried out by Luo and colleagues, the authors demonstrated the production of methane obtaining the electrons from a salinity gradient in the anode by reverse electrodialysis. This process avoided part of the energy consumption and the presence of oxygen, which allowed for the production of nearly pure methane in the biocathode, with relatively high CE. Thus, avoiding oxygen in the anode could be a useful strategy to increase the efficiency in future applications.

Finally, an exhaustive cost-benefit analysis needs to be performed to discern whether the application of the technology proposed in the present study would be economically feasible to replace a stripping tower.

Conclusions

This study demonstrated the production of methane using the simulated effluent of a biogas scrubbing-like unit as the sole carbon source in a BES. Methane obtained from the biocathode increased linearly with time and was directly dependent on the current demand of the BES. The viability and robustness of the system at long term was demonstrated, obtaining the maximum production rate of 15.35 mmol m\(^{-2}\) per day operating the BES in continuous flow. The microbial community from the biocathode was identified, being the archaea Methanobacterium the dominant genus. The reactions that took place in the biocathode were elucidated. The methane production mechanism was found to be mainly hydrogenotrophic methanogenesis using hydrogen that was bioelectrochemically produced in the biocathode. Cross-over reactions, such as oxygen and sulphate reduction, and methane oxidation were found to decrease the CE of the process.

The results presented in this study highlight the application of BES in the field of biogas upgrading technologies, to increase the overall yield of the process and to reduce CO\(_2\) emissions. These results open the door to further applications such as energy storage or production of biomethane to inject into the gas grid or use as a vehicle fuel.

Acknowledgements

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References

Deciphering the electron transfer mechanisms for biogas upgrading to biomethane within bioelectrochemical systems

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Electronic supplementary information (ESI)

Summary

This supporting information material provides some extra information about the materials and methods and the results obtained in batch and continuous mode, as well as calculations on the CE losses, and a more detailed composition of the microbial community of the biocathode. Seven additional figures and one table are presented within this document, which was divided into five different sections.
Section S1. Schematic representation of the BES and the equipment.

Figure S1. Schematic representation of the BES design and the equipment used in the present study.

Section S2. Calculation of the cathode surface area.

Section S3. Operational conditions and start-up of the BES.

Figure S2. Mode of operation, cathode potential, and carbon source of the BES. Dashed lines at the beginning of each period indicate the day at which changes were applied.

Figure S3. Current demand of the biocathode during the start-up period.

Section S4. BES performance during batch test and continuous operation

Figure S4. Methane cumulated over time during batch tests 1, 6 and 13. Regression coefficient ($r^2$) and the continuous line correspond to the linear plot obtained.

Figure S5. Comparative analysis between the CE, current demand, pH and production rate obtained during batch tests.

Section S5. Microbial analyses

Figure S6. SEM image of the biocathode graphite surface.

Figure S7. Detailed results obtained for the pyrosequencing analyses of the biocathode microbial community.
Section S6. Calculation of the CE losses

Table S1. Calculation of the \( \text{CCR}_{\text{SO}_4^{2-}} \), \( \text{CCR}_{\text{O}_2} \) and \( \text{CE}_{\text{loss}} \) of the BES.
Section S1. Schematic representation of the BES and the equipment.

Figure S1. Schematic representation of the BES design and the equipment used in the present study.
Section S2. Calculation of the cathode surface area

To calculate the electrode surface of the cathode it was assumed that granular graphite was in form of spheres with a diameter of 4 mm (r=2mm).

The Area/m\(_{NCC}\)^3 ratio was calculated using the volume and area equations (equation and 2, respectively) of the sphere and using the net volume of the cathode chamber.

\[
\begin{align*}
(1) \quad V &= \frac{4}{3} \pi r^3 \\
(2) \quad A &= 4\pi r^2
\end{align*}
\]

Where, \(V\) is the volume of the sphere in m\(^3\), \(A\) is the area in m\(^2\), and \(r\) is the radius in m.

The volume of a sphere of granular graphite was 3.35·10\(^{-8}\) m\(^3\).

The number of spheres in the cathode can be subtracted from the ratio between the volume occupied by the spheres and the volume of each sphere.

The volume occupied by the spheres was 3.8·10\(^{-4}\) m\(^3\).

The number of spheres was 11340.

The area of a sphere of granular graphite was 5.03·10\(^{-5}\) m\(^2\).

The total area is obtained from multiplying the area of each sphere by the number of spheres. The total area was 0.57 m\(^2\).
Section S3. Operational conditions and start-up of the BES.

The BES was inoculated and started-up in continuous mode until an stable current demand and methane production were observed. Figure S1 show the different periods and the initial and end days for each of them. Figure S2 demonstrate the current demand of the biocathode during the start-up period (i.e. from day 0 to 159).

Figure S2. Mode of operation, cathode potential, and carbon source of the BES.

Dashed lines at the beginning of each period indicate the day at which changes were applied.
Figure S3. Current demand of the biocathode during the start-up period.
Section S4. BES performance during batch test and continuous operation

The amount of methane harvested over time in the most representative tests and the regression coefficient ($r^2$) obtained for each plot are presented in figure S3. A comparative analysis of the different parameters of the system was performed to discern whether some parameters of the BES were related, and are shown in figure S4. It was found that there was not a direct relationship, except for the current demand and the production rate ($r^2 = 0.990$).

**Figure S4.** Methane cumulated over time during batch tests 1, 6 and 13. Regression coefficient ($r^2$) and the continuous line correspond to the linear plot obtained.
Figure S5a. Comparative analysis between the CE, current demand, pH and production rate obtained during batch tests.
Figure S5b. Comparative analysis between the CE, current demand, pH and production rate obtained during batch tests.
**Section S5. Microbial analyses**

Qualitative microbial analyses, such as scanning electron microscopy (SEM), were performed in graphite samples extracted from the biocathode. The materials and methods, and SEM images from the biocathode samples are shown in figure S5. The complete results from the pyrosequencing analyses of the microbial community of the biocathode are shown in high resolution in figure S6.

**Scanning electron microscopy (SEM)**

SEM analysis was performed at the same time as microbial community composition analysis (before starting test 3). Graphite samples from the biotic and abiotic MEC were extracted to compare the electrode surface. The samples were immersed in 2.5% (w/v) glutaraldehyde in a 0.1 M cacodylate buffer at pH 7.4 for a period of 4 hours. Next, the samples were washed and dehydrated in an ethanol series. Washes were done with cacodylate buffer and with water, both per duplicate. Dehydration with graded ethanol followed temperature steps of 50, 75, 80, 90, 95 and 3×100 °C in periods of 20 minutes. The fixed samples were dried with a critical-point drier (model K-850 CPD, Emitech, Alemania) and sputtered-coated with a 40 nm gold layer. The coated samples were examined with a SEM (model DSM-960; Zeiss, Germany) at 20 kV and images were captured digitally. Energy-dispersive X-ray spectroscopy (EDX; QUANTAX Microanalysis System) was also performed in the abiotic MEC graphite samples in order to identify the compounds deposited on the surface. Analyzed samples were not pretreated. Digital images of both SEM and EDX analysis were collected and processed by ESPRIT 1.9 BRUKER program (AXS Microanalysis GmbH, Berlin, Germany).
**Figure S6.** Scanning electron microscopy (SEM) image of the biocathode graphite surface.
Figure S7a. Detailed results obtained for the pyrosequencing analyses of the biocathode microbial community.
Figure S7b. Detailed results obtained for the pyrosequencing analyses of the biocathode microbial community.
Section S6. Calculation of the CE losses

The coulombs consumed by cross-over reactions were quantified from the oxygen diffusion and the sulphate reduction in the biocathode, and compared to the coulombs consumed for methane production to estimate the CE losses according to equation 3.

\[
CE_{\text{loss}}(\%) = 100 \cdot \left( \frac{CCR_{O_2} + CCR_{SO_4^{2-}}}{CRR} \right)
\]

Where \( CCR \) is the coulombic consumption rate obtained from the coulombs consumed over time, \( CCR_{O_2} \) is the coulombic consumption rate due to oxygen oxidation and \( CCR_{SO_4^{2-}} \) is the coulombic consumption rate due to sulphate reduction.

\( CCR, CCR_{SO_4^{2-}} \) and \( CCR_{O_2} \) are expressed in Coulombs d\(^{-1}\). \( CCR_{CH_4} \) and \( CCR_{SO_4^{2-}} \) were calculated according to equation 4 and 5, respectively:

\[
(4) \quad CCR_{CH_4} = 8 \cdot m_{CH_4} \cdot F
\]

\[
(5) \quad CCR_{SO_4^{2-}} = 8 \cdot m_{SO_4^{2-}} \cdot F
\]

In equation 2, \( m_{CH_4} \) is the slope of the linear plot obtained from the moles of methane harvested over time (moles d\(^{-1}\)), 8 are the number of electrons consumed per mole of methane produced, and \( F \) is Faraday’s constant (96485 C mole of electrons\(^{-1}\)). In equation 3, \( m_{SO_4^{2-}} \) is the slope corresponding to the sulphate consumption rate (moles d\(^{-1}\)), and 8 are the number of electron consumed to reduce one mole of sulphate to hydrogen sulphide, according to equation 6.

\[
(6) \quad SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + H_2O
\]

Once in the cathode chamber, oxygen can be electrochemically reduced according to Equation 7, or used to biologically oxidize methane by *Methylocystis* sp. according to Equation 8.

\[
(7) \quad O_2 + 2H^+ + 2e^- \rightarrow H_2O
\]

\[
(8) \quad CH_4 + 2O_2 \rightarrow CO_2 + H_2O
\]

Therefore, \( CCR_{O_2} \) can be calculated by two different equations, one considering directly \( O_2 \) reduction (equation 9) and the other considering the methane consumed (equation 10).

\[
(9) \quad CCR_{O_2} = J_{O_2} \cdot A \cdot 2 \cdot F
\]

\[
(10) \quad CCR_{O_2} = \frac{J_{O_2} \cdot A \cdot 8 \cdot F}{2}
\]

Where in equation 9, \( J_{O_2} \) is the oxygen diffusion flux (mol\(_{O_2}\) m\(^{-2}\) d\(^{-1}\)), \( A \) is the membrane area (0.04 m\(^2\)), 2 is the moles of electron that are electrochemically consumed per
mole of oxygen, and F is Faraday’s constant. In equation 10, 8 correspond to the moles of electrons consumed per mole of methane produced, and 2 to the number of moles of oxygen necessary to oxidize 1 mole of methane (equation 8).

Oxygen diffusion flux ($J_{O2}$) was calculated according to other authors, from Equation 11.

$$J_{O2} = \frac{D_{O2} \cdot (C_{O2,an} - C_{O2,cat})}{\delta}$$

Where $D_{O2}$ is the diffusion coefficient of oxygen determined for a CMI-7000 cation exchange membrane (Membrane International Inc., USA) ($3.72 \times 10^{-5}$ m² d⁻¹). $C_{O2,an}$ and $C_{O2,cat}$ are the dissolved oxygen concentration in the anode and cathode compartments (mole O₂ m⁻³), respectively; and $\delta$ the thickness of the membrane (0.45 $\times$ 10⁻³ m). The resulting units for oxygen diffusion flux are mole O₂ m⁻² d⁻¹.

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<th>$CCR_{O2}$ Eq. 7 (C d⁻¹)</th>
<th>$CCR_{O2}$ Eq. 8 (C d⁻¹)</th>
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<td>356.8</td>
<td>33.5 - 66.9</td>
</tr>
<tr>
<td>8</td>
<td>25.1 ± 4.7</td>
<td>541.0</td>
<td>1.8</td>
<td>178.9</td>
<td>356.8</td>
<td>33.1 - 66.0</td>
</tr>
<tr>
<td>9</td>
<td>25.1 ± 2.3</td>
<td>342.5</td>
<td>1.8</td>
<td>178.9</td>
<td>356.8</td>
<td>52.2 - 104.2</td>
</tr>
<tr>
<td>10</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<td>n/a</td>
</tr>
<tr>
<td>11</td>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>12</td>
<td>54.2 ± 8.3</td>
<td>1330.2</td>
<td>1.8</td>
<td>178.9</td>
<td>356.8</td>
<td>13.4 - 26.8</td>
</tr>
<tr>
<td>13</td>
<td>75.3 ± 5.2</td>
<td>3133.3</td>
<td>1.8</td>
<td>178.9</td>
<td>356.8</td>
<td>5.7 - 11.4</td>
</tr>
<tr>
<td>14</td>
<td>68.9 ± 0.8</td>
<td>6390.8</td>
<td>23.2</td>
<td>178.9</td>
<td>356.8</td>
<td>3.2 - 5.6</td>
</tr>
</tbody>
</table>

In all cases, the major contributor to the CE losses was the oxygen that diffused from the anode to the cathode. The presence of *Methylocystis* sp. caused the CE of the BES to be lower than it would be considering only pure electrochemical O₂ reduction.

Chapter 6. Short hydraulic retention times enhance methane production rate in continuous flow bioelectrochemical systems

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Abstract:

Bioelectrochemical systems (BES) have been postulated as a platform technology to transform CO₂ to methane. Research efforts need to increase the outcome in terms of methane production rate and quality of the product for its application. This work focuses on the effect of the biocathode hydraulic retention time (HRT) on the methane production rate and quality. HRT from 19.8 to 1.9 h were tested at the cathode potentials of -0.8 and -0.7 V vs SHE. Methane production rates were higher at -0.8 V, and increased at shorter HRT. The maximum methane production rate of 74.1 ± 4.1 mmol m⁻² d⁻¹ was obtained at -0.8 V and 6.0 h HRT, with a coulombic efficiency of 89.7 ± 0.2 %, and a CO₂ conversion efficiency of 95.9 ± 4.3 %. Tests performed at -0.7 V vs SHE revealed that HRT shorter than 6.0 h decreased methane concentration in the gas (45.1 ± 10.1 % at 1.9 h HRT) and carbon conversion efficiency. At short HRT hydrogen production rate was higher than hydrogen utilisation rate by hydrogenotrophic methanogens. This could be due to the pH decrease or the very low HRT, which could cause partial inhibition of methanogenesis. Maximum methane production rate obtained in terms of reactor volume (2.54 m³ m⁻³ d⁻¹) was close to CO₂ production rate in AD (5 m³ m⁻³ d⁻¹). By using a BES for biogas upgrading, biomethane production could be increased from the current 0.55 to 0.95 Lₗₗbiomethane Lₗₗbiogas⁻¹.
Highlights:

Methane production rate increased at short HRT in continuous operation

Production rate of 100 mmol CH$_4$ L$^{-1}$ d$^{-1}$ was obtained at -0.8 V and 6h HRT.

HRT below 6 h negatively influenced the quality of the gas in terms of methane

Biomethane production yield could be increased by using BES for biogas upgrading

Keywords: Biocathode; Biogas upgrading; Electromethanogenesis; Hydrogenotrophic methanogens
Chapter 7. Continuous acetate production through microbial electrosynthesis from CO₂ with microbial mixed culture

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b Chemical Engineering Department, IQS-School of Engineering, Ramon Llull University, Via Augusta 390, Barcelona 08017, Spain.
Continuous acetate production through microbial electrosynthesis from \( \text{CO}_2 \) with microbial mixed culture

Pau Batlle-Vilanova, a Sebastià Puig, a* Rafael Gonzalez-Olmos, a, b Maria Dolors Balaguer a and Jesús Colprim a

Abstract

BACKGROUND: Microbial electrosynthesis represents a promising approach for renewable energy storage in which chemically stable compounds are produced using \( \text{CO}_2 \) as feedstock. This report describes the continuous production of acetate through microbial electrosynthesis from \( \text{CO}_2 \) and assesses how the production rates could be increased.

RESULTS: A continuous acetate production rate of 0.98 mmol C L\(^{-1}\) \( \text{NC}_{-1} \) d\(^{-1}\) was obtained using \( \text{CO}_2 \) as feedstock and with pH control around 5.8. These conditions increased substrate availability and favoured microbial electrosynthesis. Cyclic voltammograms demonstrated the electroautotrophic activity on the biocathode surface, which increased with pH control and caused current demand and acetate production rate to rise exponentially.

CONCLUSION: pH decrease was shown to be an effective strategy to increase substrate availability and enhance microbial electrosynthesis. By making microbial electrosynthesis a feasible technology, \( \text{CO}_2 \) could become an alternative feedstock for the carboxylate platform.

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Keywords: biocathode; carbon dioxide valorization; electroautotrophs; homoacetogenesis; valuable compounds

INTRODUCTION

The progressive awareness of fossil fuel depletion and global warming caused by increasing energy demand in developed areas around the world is currently unquestionable. In recent decades, this growing awareness has been guiding researchers all over the world to investigate the production of electricity and fuels from alternative sources. Many technologies are at the research and development stage, such as chemical, photochemical, electrochemical, biological, reforming, and inorganic transformations, which use carbon dioxide (\( \text{CO}_2 \)) for the generation of so-called carbon-neutral fuels.\(^1\) The main advantages of using \( \text{CO}_2 \) as carbon source are: (i) high availability; (ii) buffer capacity; (iii) independence from land use; and (iv) positive impact on greenhouse gas budget.\(^2\)

From the applicability point of view, these technologies offer great potential because they allow: (i) the mitigation of \( \text{CO}_2 \) emissions; and (ii) the transformation of \( \text{CO}_2 \) into valuable compounds. The possibility of coupling these technologies with renewable energy production is especially interesting from the point of view of off-peak energy storage and energy harvesting fluctuations.\(^3\) The conversion of electricity into stable liquid products makes them easier to store, transport, or use when renewable energy harvesting is limited. However, most of these technologies present limiting factors such as high consumption of hydrogen (hydrogenation),\(^4\) or the use of expensive catalysts and large amounts of electricity (chemical and electrochemical reduction processes).\(^3\)

Microbial electrosynthesis is a novel biotechnological process, which was first described by Nevin and colleagues as the electricity-driven reduction of \( \text{CO}_2 \) using microorganisms as electrocatalysts in a bioelectrochemical system (BES).\(^5\) Future research in this field could make microbial electrosynthesis a feasible technology platform for mitigating \( \text{CO}_2 \) emissions and storing renewable-harvested energy in chemically-stable products.\(^6\) Recent studies reported microbial electrosynthesis in the lab with electricity as the sole electron donor and \( \text{CO}_2 \) as the electron acceptor.\(^5,7-10\) In all these studies, \( \text{CO}_2 \) was consumed to produce acetate as the main product. Although acetate itself does not have a high value, it represents a useful intermediate for further processes, such as secondary fermentation, to produce compounds of higher value, including alcohols or long chain fatty acids. The processes by which these products can be obtained was described by Agler and co-workers as ‘the carboxylate platform’.\(^11\)

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The present study aims at continuous production of acetate from CO₂ through the use of a BES performing microbial electrosynthesis. There are some studies on microbial electrosynthesis in batch tests, but few in continuous mode, and none dealing with the importance of operational parameters to improve overall performance. In this study, the pH of the biocathode was modified to assess its influence on system performance in terms of production rate, thus taking microbial electrosynthesis a step forward in its practical implementation.

MATERIALS AND METHODS

Bioelectrochemical system setup

A two-chamber BES was constructed using a previously described design, and shown in Fig. 1. It consisted of two methacrylate frames (200 × 200 × 20 mm) separated by a cation exchange membrane (CMI-7000, Membranes International Inc., USA), to allow proton migration from the anode to the cathode, and to avoid product losses during the operation. The anode and cathode chambers were filled with granular graphite (model 00514, diameter 1.5–5 mm, EnViرو-cell, Germany), to increase electrode surface area, and to sustain biofilm growth in the cathode chamber. The net volumes decreased to 414 mL net anode electrode and 450 mL net cathode electrode volume, to sustain biofilm growth in the cathode chamber. The net volumes decreased to 416 mL net anode electrode and 450 mL net cathode electrode volume. The net volumes decreased to 416 mL net anode electrode and 450 mL net cathode electrode volume.

Inoculation procedure and bioelectrochemical system operation

The BES was operated in a three-electrode configuration. The working electrode (WE) was the cathode electrode, the reference electrode (RE) was an Ag/AgCl (+197 mV vs standard hydrogen electrode [SHE], model RE-5B, BASI, United Kingdom) placed in the cathode chamber and the counter electrode was placed at the anode chamber. All of the voltages were reported with respect to SHE. Cathode potential was poised chronoaamperometrically at −600 mV. This potential was selected because previous researchers demonstrated that it was sufficient to allow for acetate production from CO₂. The current demand was monitored with a potentiostat (BioLogic, Model VSP, France).

Analysis and calculations

Liquid and gas analysis

The production of organic compounds (volatile fatty acids (VFA) and alcohols) in the liquid phase was measured with an Agilent 7890A gas chromatograph (GC) equipped with a DB-FFAP column and a flame ionization detector (FID). Samples were acidified with ortho-phosphoric acid (85%, Scharlau, Spain) and an internal standard (crotonic acid) was added before the analysis to ensure the results obtained.

The production gas was trapped in a methacrylate chamber and sampled with a glass syringe. The composition of the gas phase was measured with an HP-Molesieve column and a thermal conductivity detector (TCD) to detect H₂, O₂, N₂, CH₄, CO, and CO₂.

All of the production rates are given in mmol per litre of NCC per day (mmol C L⁻¹ NCC⁻¹ d⁻¹).

Coulombic efficiency

The efficiency of electron removal or supply by a BES through an electrical circuit has been traditionally referred to as coulombic efficiency (CE). In this study the coulombs found in the product were divided by the coulombs consumed to calculate the CE according to Equation (1):

\[ \text{CE} = \frac{V_{\text{NCC}} F \sum n_i \cdot C_i \int_{t_0}^{t_j} 1 \, dt}{100} \]
Acetate production from CO₂ through microbial electrosynthesis

Figure 1. Schematic diagram and operation flow of the bioelectrochemical system.

Table 1. Identification of the periods and summary of the operational parameters applied

<table>
<thead>
<tr>
<th>Period</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>19 days</td>
<td>35 days</td>
<td>10 days</td>
<td>24 days</td>
</tr>
<tr>
<td>Influent</td>
<td>HCO₃⁻ + CO₂</td>
<td>HCO₃⁻ + CO₂ + CH₄ inhibitor</td>
<td>CO₂ + CH₄ inhibitor</td>
<td>CO₂ + CH₄ inhibitor</td>
</tr>
<tr>
<td>pH control</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>5.8 ± 0.2</td>
</tr>
</tbody>
</table>

where $V_{\text{NCC}}$ is the cathode liquid volume (L), $F$ is Faraday’s constant (96485 C mol e⁻¹), $i$ represents the compounds produced in the system (acetate or methane), $C_i$ is the concentration of the $i$ compound in the effluent (mol C–Ci L⁻¹), $n_i$ is the number of electrons consumed per mol C–Ci produced (mol e mol C–Ci⁻¹) (4 e⁻ for acetate production, and 8 e⁻ for methane production), and $I$ (A) is the current demand of the system, which was integrated for one HRTc ($t_j$) before each sampling.

where $V_{\text{NCC}}$ is the cathode liquid volume (L), $F$ is Faraday’s constant (96485 C mol e⁻¹), $i$ represents the compounds produced in the system (acetate or methane), $C_i$ is the concentration of the $i$ compound in the effluent (mol C–Ci L⁻¹), $n_i$ is the number of electrons consumed per mol C–Ci produced (mol e mol C–Ci⁻¹) (4 e⁻ for acetate production, and 8 e⁻ for methane production), and $I$ (A) is the current demand of the system, which was integrated for one HRTc ($t_j$) before each sampling.

RESULTS AND DISCUSSION

Evolution of the biocathode performing microbial electrosynthesis

Initially, the inoculum was neither electrochemically active nor able to fix CO₂ and convert it into valuable compounds. The system was initiated by feeding the mineral solution into the biocathode with the potential at −600 mV. Figure 2 presents the evolution of the pH, current demand and production rate for each period.

During period 1 gas production was observed with 68% and 32% of methane and CO₂, respectively. The average methane production rate was $0.60 ± 0.06$ mmol C L⁻¹ NCC⁻¹ d⁻¹. Other products, such as VFAs or alcohols were not detected in the liquid phase during this period. The pH was stable at $7.8 ± 0.1$, which was caused by the influent composition. The current demand of the system was low and stable at $1.9 ± 0.3$ A m⁻¹ NCC⁻¹. During this period, methane production was associated with high CE, which was 263.9 ± 9.3%.

The high CE indicated that other reactions delivering electrons occurred in the biocathode. In this sense, methane was likely produced mainly from biodegradation of the remaining organic matter present in the inoculum instead of electrothrophically.

Since this study intended the production of acetate from CO₂ through microbial electrosynthesis, the next step was to inhibit the
methanogenic activity by adding 2-bromoethanolsulfonate during influent preparation.\textsuperscript{7}

At the beginning of period 2 (day 19), methane production quickly disappeared after a lag-phase of 4 days, from this point on gas production was not observed and acetate was the only detected product in the liquid phase throughout the experiment. The pH gradually decreased from 7.5 ± 0.3 to 6.5 ± 0.1 due to acetate production (pKa = 4.76). From day 21 to day 28, a current demand of 2.0 ± 0.2 A m\textsubscript{NCC}\textsuperscript{-3} was observed, without change compared with period 1 (Fig. 2), and acetate production rate was 0.41 ± 0.07 mmol C L\textsubscript{NCC}\textsuperscript{-1} d\textsuperscript{-1}. From day 28, the acetate production rate decreased to 0.21 ± 0.00 mmol C L\textsubscript{NCC}\textsuperscript{-1} d\textsuperscript{-1} at the end of the period, while the current demand increased to 3.3 ± 0.4 A m\textsubscript{NCC}\textsuperscript{-3}. Similarly to what happened in the previous period, acetate production in the first half of period 2 (up to day 45) was associated with a CE of 94.0 ± 7.6%.

The pH during period 3 was 6.8 ± 0.2. As the removal of NaHCO\textsubscript{3} from the mineral medium did not cause the expected pH decrease, a pH control was applied to the biocathode during period 4. Previous studies reported that the pH range of many homoacetogenic bacteria was acidic-like;\textsuperscript{17} therefore, the pH was decreased to approximately 5.8 by pH control. That pH was within the optimum pH growth range of many homoacetogenic bacteria, so that the microbial community was not inhibited, whereas CO\textsubscript{2} ratio increased from 24% to 76% of the total inorganic carbon according to its pH equilibrium. Thus, it meant an increase of the CO\textsubscript{2} concentration, as well as a higher proton availability, with respect to period 3.

Bioelectrochemical production of acetate is theoretically based on Equation (2).\textsuperscript{18,19} By decreasing the pH of the biocathode the concentration of both CO\textsubscript{2} and protons increased so that acetate production became thermodynamically favourable. According to the Nernst equation, hydrogen electrolytic reduction could have become also thermodynamically favourable due to the higher proton availability, so that during period 4, acetate could have been produced by two mechanisms: (i) bioelectrochemically (Equation (2)); and (ii) through a hydrogen-mediated mechanism (Equations (3) and (4)).

\[
\text{CO}_2 + 7 \text{H}^+ + 8 \text{e}^- \rightarrow \text{CH}_3\text{COO}^- + 2 \text{H}_2\text{O}; \quad E'_0 = -280 \text{ mV} \quad (2)
\]

\[
2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{H}_2; \quad E'_0 = -410 \text{ mV} \quad (3)
\]

\[
\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2 \text{H}_2\text{O} \quad (4)
\]

In any case, the higher substrate availability caused higher activity from the autotrophic microorganisms.\textsuperscript{20} Some homoacetogenic bacteria, such as \textit{Morella thermoacetica} or \textit{Clostridium ljungdahlii} can obtain energy from the proton gradient between the inside and outside of the cell.\textsuperscript{21,22} Thus, another hypothesis was that the pH decrease and the ability of the microbial biocathode community to drive this metabolism could have caused an acetate production increase.

Period 4 started at day 64. From day 64 to day 72, the current demand remained stable at 5.9 ± 0.2 A m\textsubscript{NCC}\textsuperscript{-3}. Then it increased exponentially (R\textsuperscript{2} (ln(I)) = 0.89) and stabilized at 12.3 ± 0.8 A m\textsubscript{NCC}\textsuperscript{-3} at day 82. In the same way, the acetate production rate also increased from 0.27 ± 0.02 to 0.82 ± 0.12 mmol C L\textsubscript{NCC}\textsuperscript{-1} d\textsuperscript{-1}, obtaining the highest production rate of 0.98 mmol C L\textsubscript{NCC}\textsuperscript{-1} d\textsuperscript{-1}, which means a 363% increase, when compared with the production rate obtained at the beginning of the same period. The CE...
was 28.9 ± 6.1%, which was close to the CE obtained in period 3 and the second half of period 2. Similarly, Zaybak and co-workers observed that the behaviour of the system suggested that microbial production switched to microbial electrosynthesis.23 In this study, the current demand increase during period 2 and the CE decrease and its stabilization during the later periods suggested that the organic matter from the inoculum was completely consumed, and that the acetate was produced through microbial electrosynthesis. Thus, methanogenesis inhibition and the complete removal of the organic carbon sources in the biocathode during period 2 caused electroautotrophic activity.

The CE obtained in BES applications was usually below 100%, which indicated that electrons were lost in other processes, such as the concomitant production of non-identified products,10 BES losses, including imperfect catalysis on the electrode surface and ohmic losses due to the electrode and the electrical circuit,19 or biomass maintenance and growth. Another source of electron losses could be the diffusion of oxygen in the biocathode,14 which is especially common when water is used as electron donor in the anode.

These results suggested an evolution of the microbial electrosynthesis process towards higher production of acetate. The increase in acetate production could be related to either (i) a direct effect of pH on microbial metabolism, (ii) a higher substrate availability, or both.

**Electrotrophic activity of the biocathode**

CVs were performed in the biocathode to identify redox processes that occurred on the electrode surface, which could be associated with the electrotrophic activity of the microorganisms. Figure 3(A) shows the turnover CV of each period. Redox peaks could not be identified in any of the CVs performed during periods 1 and 2. Thus, the lack of redox processes occurring on the electrode surface indicated that electroautotrophic microorganisms were not active during those periods. This outcome was caused by either (i) a low ratio of biofilm covering the electrode surface; (ii) the presence of organic matter, which did not favour the electrochemical activity of the biofilm because of its higher affinity to organic matter as source of carbon and reducing power; or (iii) the presence of bicarbonate buffer in the media. Therefore, substrate was not available for electroautotrophic growth.

In the CVs obtained during periods 3 and 4, redox peaks were clearly identified, which was due to the lower rate of electron transfer compared with the scan rate of the CVs.25 The presence of redox peaks suggested the activity from electroautotrophic microorganism growing on the biocathode surface. Figure 3(B) shows the first derivative of the CVs performed during periods 3 and 4, from which the potentials where the maximum oxidation and reduction activity took place were identified. Electrochemical activity of the biofilm on the electrode surface was clearly observed, as a redox couple and a reduction peak were detected. The formal potential obtained for the redox couple was between −1 mV and +23 mV, and the maximum reducing activity occurred at a potential of −436 mV. The redox couple could be related to the presence of electrochemically active species that were close to the electrode surface (i.e., membrane proteins)26 or other compounds that were easily oxidized and reduced (i.e., flavoproteins). The reductive peak was likely related to the biological reduction of protons to hydrogen according to Equation (3) or to the reduction of CO₂ to acetate according to Equation (2). Although the theoretical potential for H₂ and CO₂ reduction into acetate are −410 mV and −280 mV, respectively, BES design, and especially ohmic losses could have caused a shift in the experimental potential.19 The reduction peak intensity substantially increased in period 4, caused by the higher current demand, and indicated major electrotrophic activity occurring on the electrode surface.

It could be concluded that the microorganisms present in the biocathode were not electrochemically active during periods 1 and 2, likely because of the presence of organic matter.24 When organic matter was completely removed from the biocathode, autotrophic activity started, and since the microorganism from the biocathode consumed electrons, redox processes taking place on the biocathode surface were observed in the CVs of periods 3 and 4. Figure 2 shows the decrease of production rate up to day 45; from that day production was stable and then increased in period 4.

Earlier studies demonstrated that microbial electrosynthesis can be started by adding synthetic organic matter, such as glucose or fructose.23 However, according to the results of this study, the presence of endogenous organic matter from the inoculum is enough to allow the microbial metabolism to switch from heterotrophic to electroautotrophic. It has also been demonstrated that the higher the substrate availability, the higher the electroautotrophic activity of the biocathode. Thus, substrate availability can increase by decreasing the pH. Although it was not clear whether the higher activity of the studied BES during period 4 was caused directly by the pH decrease or indirectly by the increase in substrate concentration, the results obtained suggested that the pH was a key parameter to enhance the process.

**Implications and perspectives of microbial electrosynthesis**

There are only a few studies regarding the feasibility of microbial electrosynthesis from CO₂ at the lab-scale. Although previous studies demonstrated that both pure and mixed microbial cultures are able to perform microbial electrosynthesis, the use
of mixed cultures is especially interesting. Their lower sensitivity to environmental changes makes them a promising source of microorganisms, rather than pure cultures, for future applications and scaling-up. Syntrophic interactions established between different species present in a mixed community can be beneficial to microbial electrosynthesis. In a BES, syntrophic relationships can be established from many points of view, such as electron transfer, by-product transformation, oxygen removal, etc. Table 2 summarizes some recent literature studies regarding microbial electrosynthesis from CO₂. The first proof of concept was reported by Nevin and colleagues using pure cultures of acetogenic microorganism, such as Sporomusa ovata or Clostridium ljungdahlii. Others reported microbial electrosynthesis with mixed cultures. In all reports acetate was the main product obtained. An important operational aspect when mixed cultures are aimed to perform microbial electrosynthesis is that the use of a methanogenic inhibitor is needed to avoid methanogenesis.

Jiang and co-workers showed the importance of the cathode potential. This operational parameter can be set at more negative values to not only increase current density and hydrogen production, but also methane and acetate production. Other studies reported higher production of acetate when CO₂ was flushed in intermittently and in continuous fed-batch experiments. In all studies hydrogen was detected in the gas phase, so that the acetate production mechanism was likely hydrogen-mediated (i.e. Equation (4)).

Hydrogen could be produced either purely electrochemically or bioelectrochemically. Electrochemical hydrogen production requires very low potentials, which is the case studied by Jiang et al., who produced acetate at a cathode potential < −950 mV. While in the studies by Marshall et al., hydrogen could have been bioelectrochemically produced by the microorganisms in a biocathode working at −590 mV. According to Equations (2) and (4), in both studies the substrate availability was high due to the production of hydrogen (i.e. Equation (3)), so that higher production rates were obtained. Marshall et al., achieved the highest production rate, likely due to higher substrate availability from CO₂ continuous flush and the bioelectrochemically adapted microbial community. The adaptation and maturity of the microbial community made it able to consume CO₂ and electrons or hydrogen at high rates.

In this study it was demonstrated that the application of an external pH control under acidic-like conditions can be used as a strategy to increase substrate availability, and therefore increase the production rate during microbial electrosynthesis. Although hydrogen was not detected in this study, it could have been bioelectrochemically produced and rapidly uptaken by other microorganisms to produce acetate, so that both mechanism of acetate production (i.e. Equations (2) and (4)) were possible, as happened in other studies. Further research should study which is the optimal pH during microbial electrosynthesis with different microbial communities and whether the use of a methanogenic inhibitor could be avoided by decreasing the pH to 5.5, without affecting or even increasing the acetate production rate during microbial electrosynthesis.

The recent advances that have been made regarding microbial electrosynthesis suggest that this process could be integrated into the carboxylate platform. The carboxylate platform was recently proposed for the conversion of organic waste to bioproducts through fermentative processes. Agler and co-workers showed that acetate plays a key role as a substrate in secondary fermentations; therefore, its continuous production is a key point. Other studies also demonstrated that long chain carboxylates, such as caproate, could be produced when acetate is used as a feedstock in conventional anaerobic fermenters, or in BES. Regarding the continuous production of acetate from CO₂, the carboxylate platform should be considered not only from the point of view of bioproduction from organic waste but also from CO₂ mitigation, contributing to the reduction of greenhouse gas emissions.

CONCLUSIONS

In this study acetate was continuously produced from CO₂ in a biocathode through microbial electrosynthesis at a maximum production rate of 0.98 mmol L⁻¹ d⁻¹ and a CE around 30%. The maximum reducing activity took place around −436 mV; the peak intensity increase corresponded with acetate production rate increase, which suggested higher electroautotrophic activity on the biocathode surface.

The results regarding acetate production rate and the CVs demonstrated that pH control at slightly acidic conditions improved microbial electrosynthesis. This fact was caused by a direct effect of low pH, the indirect increase of substrate availability, or both.

This work could guide further studies to reach higher production rates in microbial electrosynthesis cells. Thus, microbial electrosynthesis could become a competitive technology for the future capture and transformation of CO₂.

ACKNOWLEDGEMENTS

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Chapter 8. Microbial electrosynthesis of butyrate from carbon dioxide

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Microbial electrosynthesis of butyrate from carbon dioxide†

R. Ganigué,‡ S. Puig,†‡ P. Batlle-Vilanova, M. D. Balaguer and J. Colprim

This work proves for the first time the bioelectrochemical production of butyrate from CO₂ as a sole carbon source. The highest concentration of butyrate achieved was 20.2 mM, with a maximum butyrate production rate of 1.82 mMC d⁻¹. The electrochemical characterisation demonstrated that the CO₂ reduction to butyrate was hydrogen driven. Production of ethanol and butanol was also observed opening up the potential for biofuel production.

The depletion of fossil resources, their ever-increasing high price and the negative environmental impacts derived from their use are forcing the transition to more sustainable energy and chemical production models, based on renewable and carbon-neutral commodity chemicals and fuels. Carbon dioxide (CO₂) can be transformed into added-value products mainly by chemical transformations, photochemical, chemical and electrochemical reductions, biological conversions, reforming, and inorganic transformations. Current CO₂ mitigation and conversion techniques require extremely large surface and volumes, energy intense processing steps and/or chemicals and expensive catalysts. Microbial electrosynthesis has been recently postulated as a promising approach to transform CO₂ into value-added compounds. In such systems, carboxydotrophic microorganisms are harnessed to fixate CO₂ into products via the Wood–Ljungdahl pathway, using electrical current as a driving force. This concept was first proven by Nevin et al. (2010), who transformed CO₂ into acetate using pure cultures, up to a concentration of 2 mM. Two subsequent studies from Marshall and co-workers increased, using mixed cultures, the acetate titer to 28.5 mM and 175 mM, respectively.

To date, acetate has been the sole product of CO₂ reduction in BioElectrochemical Systems (BESs). However, the autotrophic production of acetate is not very attractive from the economic standpoint due to its low market price. In this light, several authors have attempted to upgrade it to higher value products. Sharma and co-workers investigated the biocatalysed reduction of acetic and butyric acid to bioalcohols and mid-chain fatty acids via direct electron transfer at −0.65 V vs. standard hydrogen electrode (SHE). They observed the transformation of those into a mixture of products, including 0.8 mM of methanol, 0.2 mM of ethanol, 0.4 mM of propanol, 0.6 mM of butanol and 0.2 mM of acetone, as well as lower amounts of propionic and caproic acids. In a similar way, Steinbusch et al. (2010) studied the bioelectrochemical ethanol production through mediated acetate reduction with methyl viologen. They reached 13.5 mM of alcohols as well as C₄ compounds. Finally, Eerten-Jansen et al. (2013) poised the cathode potential at −0.9 V vs. SHE to biologically reduce acetate, obtaining 6.8 mM of caproate and 3.0 mM of butyrate as main products.

Two experiments were performed to prove the bioelectrochemical production of butyrate from CO₂. These were conducted in 240 mL two-chambered H-type BES. A cathode made of commercial carbon cloth (NuVant’s ELAT LT2400W, FuelCellsEtc, USA), with an area of 9 cm² and an area to volume ratio of 0.075 cm² mL⁻¹, was used as a working electrode. An Ag/AgCl (+0.197 V vs. SHE, model RE-5B, BASI, United Kingdom) served as a counter electrode in the anodic compartment. Both chambers were filled with 120 mL of mineral medium similar to ATCC 1754 (Tanner et al., 1993), containing 2-bromoethanesulfonate to inhibit methanogenesis (see ESI, Table S1). These compartments were separated by a cationic exchange membrane (CMI-7000, Membranes International Inc., USA) and stirred to avoid mass transfer limitations. The cathode compartment had two butyl-rubber sampling ports. Finally, the cells were wrapped with a coil of plastic tubing connected to a thermostatic bath to control the operational temperature. Temperatures for first and...
second experiments were 35.5 ± 2.5 °C and 33.9 ± 1.1 °C, respectively.

The biocathode was poised at a potential of −0.8 V vs. SHE using a potentiostat (BioLogic, Model SP50, France), based on a three-electrode configuration. Software from the same producer (EC-Lab v10.37) was used to run simultaneous multitechnique electrochemistry routines, which included cyclic voltammetry (CV) and chronoamperometry (CA). The parameters for CV are as follows: scan rate: 1 mV s⁻¹; \( E_1 = -0.8 \) V vs. SHE; \( E_2 = 0.0 \) V vs. SHE. For CA, the parameters were \( E_{\text{cathode}}(E) = -0.8 \) V vs. SHE.

Initially, a control experiment was performed in 120 mL airtight serum bottles to prove that no bacterial growth or metabolite production occurred when reducing power was not provided. Bottles were filled with 100 mL of mineral medium and 5 mL of enriched carboxydotrophic mixed culture from a syngas fermenting lab-scale reactor capable of producing two-carbon (C2) and four-carbon (C4) organic acids and alcohol. The inoculum was dominated by species of the genus Clostridium (see ESI† Table S2). Bottles were regularly sparged with CO₂ up to a pressure of 2 atm, and incubated at 37 °C for a period of 60 days. During that period, no increase in optical density or concentration of products was observed.

Subsequently, a BES H-type system was inoculated using 5 mL of the same enriched carboxydotrophic mixed culture from a syngas fermenting lab-scale reactor, and operated as described. Pure CO₂ (Praxair, Spain) was bubbled regularly every 2–3 days for a period of 5 minutes to ensure substrate availability (at 33.9 °C, concentration at saturation of 26.9 mM of carbon (mMC) as CO₂). Liquid samples were taken periodically from both anodes and cathodes for the monitoring of liquid products concentration and pH. The volume withdrawn was substituted with fresh mineral medium. The concentration of organic acids and alcohols was analysed using an Agilent 7890A gas chromatograph (Agilent, USA) equipped with a DB-FFAP column and a flame ionisation detector. Gas samples were periodically drawn for the analysis of hydrogen, carbon dioxide, methane, oxygen and nitrogen (H₂, CO₂, CH₄, O₂, N₂) in the biocathode headspace. Samples were analysed using a second channel of the GC equipped with an HP-Molesieve column and a thermal conductivity detector (TCD).

Next, Fig. 1 depicts the total accumulated concentration of products, coulombs supplied, together with the product speciation and pH at the cathode during a 19 days experiment. The demand of electrons increased linearly from day 0 until the end of the experiment. A total of 7000 coulombs were supplied to the system, with a current demand of around 6.3 A m⁻² on day 17. At the end of the experiment the total concentration of products was 55 mMC. Microbial electro-synthesis commenced with the production of acetate, and its concentration increased almost linearly throughout the experiment, reaching a maximum concentration of 40 mMC. Butyrate was first observed on day 3, and during the following 9 days was produced at a rate of 1.49 mMC d⁻¹, up to a maximum concentration of 15 mMC. Butyrate is one of the end products of Clostridium carboxidivorans/Clostridium ragosinae, bacteria present in the inoculum, and could have been directly produced from CO₂ via the Wood–Ljungdahl pathway coupled to acetyl-CoA reduction. Under acidic conditions and excess of reducing power, butyrate production may be favoured over acetate by acetogens because of a slightly higher pH (4.82 vs. 4.76). To validate that butyrate can be produced from CO₂ in a BES, a second experiment was conducted with the same set-up and conditions. Fig. 2 shows that current demand exponentially increased throughout the experiment, with current densities increasing from 3 to 20 A m⁻². The concentration of total added-value products increased in parallel, reaching 104 mMC on day 34. The rate of carbon fixation into products was linear from day 7 to day 27, around 4.1 mMC d⁻¹. Initially, acetate was the only compound produced, and its maximum concentration – around 55 mMC – was reached on day 20. The evoluation of pH in the biocathode was linked to the production of organic acids, and it decreased from pH 7 on day 7 to pH 6.35 on day 20. At that point, butyrate was detected, together with ethanol. The simultaneous presence of both compounds in the fermentation broth opens up a new potential route for butyrate production. Clostridium kluyveri, which had been detected in the parent syngas fermentation reactor, can produce butyrate by the combination of acetate and ethanol, by the so-called chain elongation reactions. In this light, it is unclear which is the true mechanism governing butyrate production, or whether both metabolic routes occurred in parallel.

The production of butyrate subsequently led to the decrease of the concentration of acetate and the pH, which reached values of pH 4.63 on day 34. In parallel, the concentration of ethanol and butanol increased to 30.8 mMC and 7.3 mMC, respectively. It is hypothesized that the decrease of pH, coupled
to the high concentrations of organic acids, and the increase of available reducing power (as will be later discussed) led to the re-assimilation of acetate and butyrate and their conversion into ethanol and butanol. Homoacetogens obtain their energy by proton-gradient driven phosphorylation. If high concentrations of unionised organic acids penetrate the bacterial membrane, they can cause the proton gradient between inside and outside to collapse. The conversion of acids to solvents is one of the mechanisms that \textit{Clostridium} sp. utilise to prevent further pH decrease during fermentations. In this respect, ethanol production started when the concentration of acetate was 40 mM, corresponding to an unionised acetic acid concentration of 0.91 mM. As a consequence, pH increased from pH 6.32 to 6.45 from days 17 to 20. Although higher acetic acid concentrations and similar pH values were reached by Marshall \textit{et al.} (2013), no butyrate or ethanol production was reported. This could be explained by two reasons: (i) the lower amount of reducing power supplied in that study, which could have limited solventogenesis; and (ii) differences in the bacterial community.

When comparing the performance of the BES in the present study, to the syngas fermentation experiments performed by Sánchez and co-workers using the same biocatalyst, overall production of C4 compounds was fairly similar (around 30% of the total products), although more alcohols were produced in the syngas study. Besides, the concentration of butyrate was fairly similar (around 30% of the total products), and co-workers using the same biocatalyst, overall production of C4 from CO\textsubscript{2} was 32% CO and 32% H\textsubscript{2}, being the mass transfer of these compounds the key bottleneck of syngas fermentation technology. In this light, MES has the potential to circumvent this barrier by the \textit{in situ} supply of reducing power, either in the form of electrons or H\textsubscript{2}.

Turnover CVs were periodically carried out to characterise electrochemically both BESs. Fig. 3 presents two representative CVs performed under turnover conditions, one corresponding to the control (abiotic) test and the second one when the biofilm had fully developed, producing organic acids (acetate and butyrate) and bioalcohols (ethanol and butanol) from CO\textsubscript{2}. A sudden increase of the current demand was observed on the abiotic test at a cathode potential of −0.65 V vs. SHE. This CV shape is typically linked to the catalytic production of H\textsubscript{2} using non-precious metals (i.e. carbon cloth) as electrodes. During the production of organic acids and alcohols, hydrogen production started before the control CV, at −0.55 V vs. SHE. The production of H\textsubscript{2} at higher cathode potential and the higher current indicated that its production was partially biocatalysed, decreasing the important energy losses associated to purely electrolytic reduction. In this respect, it is important to bear in mind that proton availability plays a critical role in H\textsubscript{2} production, and hence the reducing power availability for bacterial utilization. The lower the pH, the higher the H\textsubscript{2} production, and electron availability. This phenomenon explains the exponential increase of the coulombs supplied and current density during the second experiment, linked to the decrease in media pH. Besides, the higher reducing power availability likely favoured the production of more reduced end-products than acetate, such as butyrate, ethanol or butanol.

The coulombic efficiencies of the experiments were calculated based on the current demand and final concentration of products, and were 28% and 32%, respectively. Two main electron sinks of the system were hypothesized: (i) protons were reduced to hydrogen at the electrode surface, consuming electrons (Fig. 3). In this light, part of the electrons supplied may have been lost as un-utilized hydrogen, diffusing out of the cathode through the connectors and the membrane, or stripped during the periodic CO\textsubscript{2} flushing. These phenomena have been extensively described in the literature; and
(ii) the oxidation of water was likely the main reaction in the anode, which led to the production of O2. Oxygen can permeate to the cathode through the cationic exchange membrane, being subsequently reduced to water again or used for oxidizing organic compounds, with the associated consumption of electrons.18 Gas sample analysis showed low H2 concentrations in the headspace (0–1% v/v). On the contrary, high dissolved oxygen (DO) concentrations (8 mgO2 L−1) were measured in the anode compartment at the end of the experiment, revealing this second potential sink as the main contributor to electron loses from the system.

Finally, it is important to consider that separation and recovery of fermentation products, even from highly specific pure cultures, can account for over 60% of the total production costs.19 To date, a number of technologies have been developed for the separation of organic acids from fermentation broths (both online and offline), including liquid:liquid extraction and electrodialysis.10 Recently, Andersen and co-workers developed a processing pipeline to transform carboxylates into fine chemicals by combining Membrane Electrolysis (ME) and Biphasic Esterification (BE).20 This is of special interest because MES of butyrate from CO2 could be coupled to this concept, with organic acids produced in the cathode being extracted and concentrated in the anode, prior to esterification.

This study demonstrates for the first time the bioelectrochemical CO2 transformation to butyrate. The electrochemical characterisation demonstrated that the CO2 reduction to butyrate was hydrogen driven. Production of ethanol and butanol was also observed at low pH values and high concentrations of undissociated organic acids, opening up the potential for the bioelectrochemical production of biofuels from CO2 as a sole carbon source. Future work should aim to increase product titers and coulombic efficiency of the system by minimising the main contributor to electron loses from the system.

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Notes and references

Microbial electrosynthesis of butyrate from carbon dioxide

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Summary:

- Microbial community analysis methods
- S1. Mineral medium composition.
- S2. Most probable sequence identification of DGGE bands. Similarity values and closest match to sequences of identified bacteria in GenBank reference RNA database are indicated. Accession numbers appear in parentheses.

Number of pages: 4
**Microbial community analysis methods**

DNA was extracted from cell pellets using the FastDNA® SPIN kit for soils (MP, Biomedicals) following the manufacturer’s instructions. Partial 16S rRNA gene fragments were obtained by PCR amplification using the bacterial universal primers 357F and 907R. Reaction mixtures and PCR amplification conditions have been described previously (Lane, 1991). A 44 base pair GC clamp sequence was added to the 5’ end of 357F primer for separation of PCR products by denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla, 1998). All chemicals and Taq polymerase used in PCR amplifications were provided by Qiagen (Qiagen Ltd. Sussex, UK). PCR amplifications were performed in a 9700GeneAmp thermal cycler (Applied Biosystems, Foster City, CA). When necessary, different PCR products of the same sample were pooled and concentrated to an appropriate concentration of 100 ng/µL. Twenty-five µL of concentrated 16S rRNA PCR products were loaded on 6% (vol/vol) acrylamide-bis-acrylamide gels with a 40 to 65 % urea-formamide denaturing gradient (Bäckman et al., 2003). DGGE was performed in a Ingeny phorU system (INGENY, The Netherlands) as described previously (Prat et al., 2009). DGGE gel was run for 17 h at 160V and stained for 30 min with Sybr® Gold (Molecular Probes Europe, Invitrogen Corporation, UK), for visualization under UV excitation. The more intense bands of every position in the gel were excised using a sterile scalpel. The DNA fragments were recovered by elution in Tris/HCl buffer (10 mM at pH 7.4) at 65°C during 2 hours and re-amplified as described above. Sequencing in both directions of 16S rRNA gene fragments obtained from re-amplification of excised DGGE bands, was performed by the Macrogen service (Macrogen, Korea). Sequences were examined for the presence of chimeras using the Uchime algorithm (Edgar et al., 2011), manually refined using the Bioedit v7.0 package and aligned using the ClustalW software. Aligned sequences were analyzed with the
BLASTn® program at the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/) and bacterial species identified as closer similarities to known sequences using the nucleotide collection database. Partial 16S rRNA gene sequences were submitted to GenBank public database with accession numbers from KM489062 to KM489069.

References


**Table S1. Mineral medium composition.**

<table>
<thead>
<tr>
<th>Minerals Component</th>
<th>Trace elements Component</th>
<th>Vitamins Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$ 1 g L$^{-1}$</td>
<td>Nitrilotriacetic acid 20.0 mg·L$^{-1}$</td>
<td>Biotin 20.0 µg·L$^{-1}$</td>
</tr>
<tr>
<td>NaCl 1</td>
<td>MnSO$_4$·H$_2$O 10.0</td>
<td>Folic acid 20.0</td>
</tr>
<tr>
<td>NH$_4$Cl 0.25</td>
<td>Fe(SO$_4$)$_2$(NH$_4$)$_2$·6H$_2$O 8.0</td>
<td>Pyridoxine hydrochloride 100.0</td>
</tr>
<tr>
<td>MgOH 0.05</td>
<td>CoCl$_2$·6H$_2$O 2.0</td>
<td>Thiamine hydrochloride 50.0</td>
</tr>
<tr>
<td>KCl 0.1</td>
<td>ZnSO$_4$·7H$_2$O 0.002</td>
<td>Riboflavin 50.0</td>
</tr>
<tr>
<td>CaCl$_2$ 0.03</td>
<td>CuCl$_2$·2H$_2$O 0.2</td>
<td>Nicotinic acid 50.0</td>
</tr>
<tr>
<td>BrCH$_2$CH$_2$SO$_3$Na 6.3</td>
<td>NiCl$_2$·2H$_2$O 0.2</td>
<td>DL- calcium pantothenate 50.0</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$·2H$_2$O 0.2</td>
<td></td>
<td>Vitamin B12 1.0</td>
</tr>
<tr>
<td>Na$_2$SeO$_4$ 0.2</td>
<td></td>
<td>p-aminobenzoic acid 50.0</td>
</tr>
<tr>
<td>Na$_2$WO$_4$ 0.2</td>
<td></td>
<td>Lipoic acid (Thioctic acid) 50.0</td>
</tr>
</tbody>
</table>

**Table S2. Most probable sequence identification of DGGE bands.** Similarity values and closest match to sequences of identified bacteria in GenBank reference RNA database are indicated. Accession numbers appear in parentheses.

<table>
<thead>
<tr>
<th>DGGE band</th>
<th>Closest Bacterial species</th>
<th>Identities (%)</th>
</tr>
</thead>
</table>
| 1 | *Clostridium carboxidivorans* P7 (NR_104768.1)  
*Clostridium scatologenes* K29 (AB610570)  
*Clostridium drakei* FP (NR_114863.1) | 100 |
| 2 | *Clostridium ljungdahlii* DSM13528 (NR_074161.1)  
*Clostridium ragsdalei* (DQ020022)  
*Clostridium autoethanogenum* DSM10061 (CP006763.1) | 100 |
| 3 | Uncultured *Firmicutes* clone (GU559846.1) | 94 |
Chapter 9. Selective microbial electrosynthesis and extraction of butyrate from carbon dioxide

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Abstract:

The present work studies the conditions for the selective microbial electrosynthesis (MES) of butyrate from carbon dioxide (CO₂). Three batch tests were performed within 70 days of operation in a tubular bioelectrochemical system (BES), at the cathode potential of -0.8 V vs SHE. CO₂ supply was limited to build up hydrogen partial pressure (P₇₆₂) and trigger the production of compounds with a higher degree of reduction. Acetate and butyrate were detected in test 1, at concentrations of 96.3 mM and 29.5 mM, respectively. In test 2, CO₂ supply was limited and P₇₆₂ increased and maintained above 1 atm, which resulted in a switch of the product spectrum. Butyrate became the predominant product of MES, with a concentration of 59.7 mM versus 20.3 mM of acetate. Finally, 87.5 mM of butyrate and 34.7 mM of acetate were obtained in test 3. Analyses of the gas phase demonstrated a positive slope of the production rate of butyrate towards high P₇₆₂ and low P₀₂. Microbial analyses showed a high relative abundance of Megasphaera sp. (>39 %) in the biocathode bulk community, which was putatively responsible for butyrate production through chain elongation. Selective extraction and concentration of butyrate was performed by membrane liquid extraction. Starting from a simulated production broth containing 17.9 mM of acetate and 46.8 mM of butyrate, butyrate was concentrated 5.4 times. A concentration solution with 15.4 mM of acetate and 252.4 mM (ratio butyrate/acetate of 16.4) of butyrate was obtained. The results open the door to MES to become a production platform of C4 compounds from CO₂.
Graphical abstract

Keywords: Bioproduction; carbon capture and utilisation; hydrogen partial pressure
Chapter 10. Discussion
10.1. Production of valuable compounds in a two-chamber BES

Production of commodity chemicals through MES using CO₂ as the only carbon source represents a new bioproduction concept described previously by Nevin and colleagues (2010 and 2011). Although some researchers had already reported the production of hydrogen and methane, the production of liquid compounds had been proven only using pure cultures of *Sporomusa ovata*, *Moorella thermoacetica*, and different *Clostridium* spp (Nevin et al., 2011, 2010). In those studies, acetate was the main product, and the highest amount obtained was almost 1 mmole in 6 days, with *Sporomusa ovata*. One of the main benefits of applying BES for the production of valuable chemical from CO₂ and renewable energy is to reduce GHG emissions and to move a step forward towards a biobased economy.

Originally the term MES was used to describe the bioelectrochemical reduction of CO₂ using renewable electricity in the biocathode of a BES. In the recent years, other concepts have been introduced within this term, such as electrochemical fermentations or electro-fermentation to produce chemical compounds from organic matter in a BES (Schievano et al., 2016). Within this thesis, MES is always used to describe the bioelectrochemical reduction of CO₂. Table 10.1 presents the different studies on microbial electrosynthesis of the valuable compounds reported in this thesis.
### Table 10.1. Summary of the studies for hydrogen, methane, and VFA and alcohols production from CO₂ as the only carbon source in two-chamber BES with a biocathode.

<table>
<thead>
<tr>
<th>Reactor operation</th>
<th>Microorganism source</th>
<th>Cathode material</th>
<th>Membrane</th>
<th>Cathode potential (V vs SHE)</th>
<th>Electron acceptor</th>
<th>End product</th>
<th>Production rate (units)</th>
<th>CE (%)</th>
<th>Enriched microorganisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>Effluent from another biocathode Ref. (Rozendal et al., 2008)</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>&lt;-0.6</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.2 L/L d 8 mmol/L d</td>
<td>21</td>
<td>n/a</td>
<td>(Jeremiasse et al., 2010)</td>
</tr>
<tr>
<td>Continuous</td>
<td>Mixture of effluents of biocathode operated for more than 30 with Bicarbonate/acetate as carbon source</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>&lt;-0.7</td>
<td>H⁺</td>
<td>H₂</td>
<td>2.2 L/L d 98 mmol/L d</td>
<td>50 ± 2.9</td>
<td>n/a</td>
<td>(Jeremiasse et al., 2012)</td>
</tr>
<tr>
<td>Continuous</td>
<td>Mixed culture from a previous BES</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>&lt;-0.65</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.63 L/L d 25 mmol/L d</td>
<td>25</td>
<td>Desulfovibrio sp</td>
<td>(Rozendal et al., 2008) (Croese et al., 2011)</td>
</tr>
<tr>
<td>Continuous</td>
<td>Urban wastewater treatment sludge and effluent from MFC</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>&lt;-0.9</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.1 – 11.6 L/L d 4 – 479 mmol/L d</td>
<td>&gt;100</td>
<td>Hoefela sp Aquiflexum sp</td>
<td>(Batlle-Vilanova et al., 2014) Chapter 4</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Effluent from a thermophilic MFC operated for 3 months</td>
<td>Carbon cloth</td>
<td>CEM</td>
<td>&lt;-0.65</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.14 L/L d 5.02 mmol/L d</td>
<td>70</td>
<td>Firmicutes phylum</td>
<td>(Fu et al., 2013)</td>
</tr>
<tr>
<td>Batch</td>
<td>Hydrogenophilic dechlorinating culture</td>
<td>Carbon paper Glassy carbon</td>
<td>CEM</td>
<td>&lt;-0.70</td>
<td>H⁺</td>
<td>H₂</td>
<td>10⁴ L/L d 4 10⁵ mmol/L d</td>
<td>25</td>
<td>Desulfitobacterium sp</td>
<td>(Villano et al., 2011)</td>
</tr>
<tr>
<td>Batch</td>
<td>Geobacter sulfurreducens</td>
<td>Non-porous flat surface graphite</td>
<td>CEM</td>
<td>-0.8</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.44 L/L d 17.5 mmol/L d</td>
<td>43</td>
<td>n/a</td>
<td>(Geelhoed and Stams, 2011)</td>
</tr>
<tr>
<td>Batch</td>
<td>Desulfovibrio paquesii</td>
<td>Graphite rod</td>
<td>CEM</td>
<td>-0.9</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.2 L/L d 8 mmol/L d</td>
<td>100</td>
<td>n/a</td>
<td>(Aulenta et al., 2012)</td>
</tr>
<tr>
<td>Fed-Batch</td>
<td>Stormwater pond sediments mixed with engineered anaerobic sludge</td>
<td>Graphite plate</td>
<td>CEM</td>
<td>-0.75</td>
<td>H⁺</td>
<td>H₂</td>
<td>0.1 L/L d 4.4 mmol/L d</td>
<td>n/a</td>
<td>Proteobacteria Firmicutes Bacteroidetes</td>
<td>(Jourdin et al., 2015a)</td>
</tr>
<tr>
<td>Batch</td>
<td>Enriched culture – Methanobacterium palustre</td>
<td>Carbon paper</td>
<td>CEM</td>
<td>&lt;-0.65</td>
<td>H⁺, CO₂</td>
<td>H₂, CH₄</td>
<td>2.13 mmol CH₄/L d 400 mmol/m2 d</td>
<td>80</td>
<td>Methanobacterium palustre</td>
<td>(Villano et al., 2010)</td>
</tr>
<tr>
<td>Fed-Batch</td>
<td>Enriched culture – Methanobacterium palustre</td>
<td>Graphite fiber brush</td>
<td>AEM</td>
<td>&lt;-0.5</td>
<td>CO₂</td>
<td>CH₄</td>
<td>200 mmol/m2 d 1.12 mmol/L d</td>
<td>96</td>
<td>Methanobacterium palustre</td>
<td>(Cheng et al., 2009)</td>
</tr>
<tr>
<td>Anaerobic沪指</td>
<td>Sludge</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>CO₂</td>
<td>CH₄</td>
<td>0.24 mmol/L d</td>
<td>22.2 mmol/m2 d</td>
<td>23</td>
<td>n/a</td>
<td>(Van Eerten-jansen et al., 2012)</td>
</tr>
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</tr>
<tr>
<td>Fed-batch</td>
<td>Anaerobic沪指</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>&lt;-0.6</td>
<td>CO₂</td>
<td>CH₄</td>
<td>14.8 mmol/L d</td>
<td>205 mmol/m2 d</td>
<td>99</td>
<td>Methanobacterium palustre Methanobacterium aarluense</td>
</tr>
<tr>
<td>Continuous</td>
<td>Anaerobic沪指</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>-0.93</td>
<td>CO₂</td>
<td>CH₄</td>
<td>11.5 mmol/L d</td>
<td>n/a</td>
<td>79</td>
<td>n/a</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Effluent from single-chamber MECS producing methane</td>
<td>Stainless steel with platinum and carbon black layer</td>
<td>CEM</td>
<td>&lt;0.7</td>
<td>CO₂</td>
<td>CH₄</td>
<td>2.3 mmolCH₄/L d</td>
<td>328 mmolCH₄/m2 d</td>
<td>80</td>
<td>n/a</td>
</tr>
<tr>
<td>Batch</td>
<td>Anaerobic沪指</td>
<td>Carbon black powder mixed with Platinum</td>
<td>Nafion</td>
<td>&lt;-0.55</td>
<td>H⁺, CO₂</td>
<td>H₂, CH₄</td>
<td>0.25 mmolCH₄/L d</td>
<td>&gt;100</td>
<td>n/a</td>
<td>(Siegert et al., 2014)</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Effluent of a thermophilic MFC</td>
<td>Carbon cloth</td>
<td>CEM</td>
<td>-0.35</td>
<td>CO₂</td>
<td>CH₄</td>
<td>22.1 mmolCH₄/L d</td>
<td>1103 mmolCH₄/m2 d</td>
<td>&gt;90%</td>
<td>Methanobacterium sp.</td>
</tr>
<tr>
<td>Batch</td>
<td>Methanobacterium-like archaeon strain IM1</td>
<td>Graphite rod</td>
<td>No membrane</td>
<td>Salt bridge</td>
<td>-0.4</td>
<td>CO₂</td>
<td>CH₄</td>
<td>0.013 mmolCH₄/L d</td>
<td>3.5 mmolCH₄/m2 d</td>
<td>80</td>
</tr>
<tr>
<td>Batch</td>
<td>Continuous</td>
<td>Anaerobic沪指</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>0.8</td>
<td>CO₂</td>
<td>CH₄</td>
<td>5.1 mmol/m2 d</td>
<td>6.95 mmol/m2 d</td>
<td>8: 75</td>
</tr>
<tr>
<td>Continuous</td>
<td>Previous working BES enriched in Methanobacterium sp</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>0.8</td>
<td>H⁺, CO₂</td>
<td>H₂, CH₄</td>
<td>74.1 mmol/m2 d</td>
<td>100.6 mmol/L d</td>
<td>89</td>
<td>n/a</td>
</tr>
<tr>
<td>Continuous</td>
<td>Sporomusa Ovata</td>
<td>Graphite rod</td>
<td>CEM</td>
<td>0.4</td>
<td>CO₂</td>
<td>Acetate</td>
<td>1.46 mmolC/L d</td>
<td>44.9 mmolC/m2 d</td>
<td>86⁺</td>
<td>n/a</td>
</tr>
<tr>
<td>Continuous</td>
<td>Clostridium Ljungdahlii</td>
<td>Graphite rod</td>
<td>CEM</td>
<td>0.4</td>
<td>CO₂</td>
<td>Acetate</td>
<td>0.11 mmolC/L d</td>
<td>3.4 mmolC/m2 d</td>
<td>72⁺</td>
<td>n/a</td>
</tr>
<tr>
<td>Fed-Batch</td>
<td>Brewery wastewater</td>
<td>Granular graphite</td>
<td>CEM</td>
<td>-0.59</td>
<td>H⁺, CO₂</td>
<td>H₂, CH₄, Acetate</td>
<td>Acetate: 8 mmolC/L d</td>
<td>Surface n/a</td>
<td>67⁺</td>
<td>Acetobacterium sp Sulfurospirillum Methanobacterium sp</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Effluent from previous BES from (Marshall et al., 2012)</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>-0.59</td>
<td>H⁺, CO₂</td>
<td>H₂, Acetate</td>
<td>Acetate: 34.5 mmolC/L d</td>
<td>Surface n/a</td>
<td>84⁺</td>
<td>Acetobacterium sp Sulfurospirillum sp</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Anaerobic carboxydrotrophic actinomycete mixed with wastewater sludge</td>
<td>Graphite felt and stainless steel mesh</td>
<td>CEM</td>
<td>-0.9</td>
<td>H⁺, CO₂, H₂, CH₄, Acetate</td>
<td>Acetate: 2.6 mmolC/L d 41.6 mmolC/m² d</td>
<td>50⁴</td>
<td>n/a</td>
<td>(Bajracharya et al., 2015)</td>
<td></td>
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</tr>
<tr>
<td>Fed-batch</td>
<td>Stormwater pond sediments mixed with engineered anaerobic sludge</td>
<td>Multiwalled carbon nanotubes on reticulated vitreous carbon</td>
<td>CEM</td>
<td>-0.85</td>
<td>CO₂, Acetate</td>
<td>Acetate: 10.7 mmolC/L d 6500 mmolC/m² d</td>
<td>70⁴</td>
<td>n/a</td>
<td>(Jourdin et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>Activated sludge</td>
<td>Carbon felt</td>
<td>CEM</td>
<td>&lt; -0.85</td>
<td>H⁺, CO₂, H₂, CH₄, Acetate</td>
<td>Acetate: 13.16 mmolC/L d 645 mmolC/m² d</td>
<td>82⁵</td>
<td>n/a</td>
<td>(Jiang et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Anodic effluent from MFC mixed with anaerobic sludge</td>
<td>Carbon felt</td>
<td>CEM</td>
<td>&lt; -1.26</td>
<td>H⁺, CO₂, H₂, CH₄, Acetate</td>
<td>Acetate: 2 mmolC/L d 633 mmolC/m² d</td>
<td>88⁵</td>
<td>Methanobacterium sp. Acetobacterium sp.</td>
<td>(Patil et al., 2015a)</td>
<td></td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Mesophilic wastewater anaerobic sludge</td>
<td>Graphite felt</td>
<td>CEM</td>
<td>-1.1</td>
<td>H⁺, CO₂, H₂, CH₄, Acetate</td>
<td>Acetate: 24.7 mmolC/L d 342.8 mmolC/m² d</td>
<td>65⁵</td>
<td>Acetobacterium sp Acetoanaerobium sp</td>
<td>(Xafenias and Mapelli, 2014)</td>
<td></td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Enriched brewery wastewater sludge</td>
<td>Graphite rod</td>
<td>CEM</td>
<td>-0.6</td>
<td>H⁺, CO₂, H₂, Acetate, Formate</td>
<td>Acetate: 10.34 mmolC/L d Surface n/a</td>
<td>77⁵</td>
<td>Acetobacterium sp</td>
<td>(Labelle et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>Anaerobic digestion sludge mixed with retention basin sludge</td>
<td>Graphite granules</td>
<td>CEM</td>
<td>-0.6</td>
<td>CO₂, CH₄, Acetate</td>
<td>Acetate: 0.98 mmolC/L d 0.8 mmolC/m² d</td>
<td>29⁴</td>
<td>n/a</td>
<td>(Batlle-Vilanova et al., 2015a) Chapter 7</td>
<td></td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Anaerobic carboxydrotrophic culture from syngas fermentation</td>
<td>Carbon cloth</td>
<td>CEM</td>
<td>-0.8</td>
<td>H⁺, CO₂, H₂, Acetate, Butyrate, Ethanol, Butanol</td>
<td>Acetate: 3.5 mmolC/L d 467 mmolC/m² d Butyrate: 1.82 mmolC/L d 243 mmolC/m² d</td>
<td>32⁴</td>
<td>n/a</td>
<td>(Ganigue et al., 2015) Chapter 8</td>
<td></td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Anaerobic carboxydrotrophic culture from syngas fermentation</td>
<td>Carbon cloth</td>
<td>CEM</td>
<td>-0.8</td>
<td>H⁺, CO₂, H₂, Acetate, Butyrate, Ethanol, Butanol</td>
<td>Acetate: 8.3 mmolC/L d 311 mmolC/m² d Butyrate: 2.8 mmolC/L d 105 mmolC/m² d</td>
<td>63⁵</td>
<td>Megasphaera suecensis Clostridium autoethanogenum</td>
<td>(Chapter 9)</td>
<td></td>
</tr>
</tbody>
</table>

CEM: Cation Exchange Membrane; AEM: Anion Exchange Membrane.
1 Different cathode materials were studied in this work; 2 Based on liquid products; 3 based on total coulombic efficiency
First of all, it is necessary to identify the electron transport mechanisms governing the reduction of CO\textsubscript{2}. As it has been previously discussed, it is not clear whether CO\textsubscript{2}-reducing microorganisms can directly use electrons to reduce CO\textsubscript{2} into different commodity chemicals. However, hydrogen is the main intermediate metabolite in the Wood-Ljungdahl pathway, through which CO\textsubscript{2} is reduced to Acetyl-CoA, and then to different end-products, such as acetate, butyrate, ethanol, etc. Furthermore, the production of hydrogen as intermediate compound can also be used as strategy to drive hydrogenotrophic methanogenesis.

According to the thermodynamics, hydrogen can be electrochemically produced at cathode potentials below -0.41 V vs SHE. However, in the practise lower voltages are used to overcome the overpotentials of the system (Cheng and Logan, 2007). The voltage requirements for hydrogen production have to be determined experimentally, as many parameters of the BES, such as configuration and desing, electrode materials, among others, may affect its catalysis. The production of hydrogen as intermediate may cause the drop of the energy efficiency of the process due to losses of the intermediate compound. However, it also has several advantages. It can be produced \textit{in-situ} using renewable energy, and since its production takes place on the electrode surface it may overcome mass transfer limitations. Furthermore it is considered an excellent reducing power source that can be used by many microorganisms to drive different processes, such as acetogenesis, methanogenesis, denitrification or sulphate reduction (Cord-Ruwisch et al., 1988).

In this section, the results are discussed starting from the production of hydrogen as intermediate compound in BES to drive the further reduction of CO\textsubscript{2} into different compounds, such as methane, acetate and butyrate.

\textbf{10.1.1. Hydrogen production}

A synthetic medium containing bicarbonate was used in this thesis to investigate hydrogen production by autotrophic microorganisms in a BES, which is the first step
towards the hydrogen-mediated transformation of CO$_2$. Microorganisms from a urban wastewater treatment plant were used as inoculum. Experiments were performed at different cathode potentials, from -0.4 to -1.8 V vs SHE. Biological hydrogen production started at -0.9 V vs SHE. The gas produced contained mainly hydrogen. Methane was not produced likely due to the high operational pH (>8) and the lack of organic matter, which avoided both hydrogenotrophic and acetoclastic methanogenesis. Parallely to the study presented in this thesis, Jourdin and colleagues (2015) also demonstrated autotrophic bioelectrochemical hydrogen production. In this case, the authors poised the cathode potential at -0.75 V, although it was suggested that hydrogen was already produced at lower potentials, low hydrogen production rate and hydrogen losses made hydrogen not accurately measurable. Other studies already showed that microorganisms can catalyse hydrogen production when current is provided in a BES (Jafary et al., 2015), but this thesis demonstrated that the presence of microorganisms also allowed for the production of hydrogen with less energy consumption. Granular graphite with microorganisms produced 0.116 m$^3$ H$_2$ kWh$^{-1}$, whereas an abiotic cell produced 0.064 m$^3$ H$_2$ kWh$^{-1}$. In our study, the current demand increased linearly with the cathode potential, and so hydrogen production (Chapter 4). The low solubility (1.4 mmol L$^{-1}$ at 1 atm and 298 K) (Sander, 1999), low mass transfer, and safety risks, limit the efficiency, scalability and application of bioprocesses requiring external hydrogen supply. An alternative to circumvent some of these issues is the in-situ production of hydrogen. In case that the microorganisms were not catalysing hydrogen production in the biocathode, hydrogen could still be provided by poising the cathode potential at low values, such as -1 V vs SHE (i.e. in a granular graphite electrode). This represents an advantage to conventional processes in which hydrogen needs to be externally supplied. In this sense, the use of BES represents and advantage, since hydrogen could be produced in situ, only by applying electricity in the same reactor. Although economic assessment is daring at this stage, hydrogen was estimated to be produced at 3.2 USD kg$^{-1}$, a value lower than
its market value (Cusick et al., 2010; Logan, 2004) when microorganisms were present in the biocathode.

10.1.2. Methane production

Inoculum from anaerobic digestor was used to investigate electromethanogenesis. The conditions of the system were modified to promote the growth of methanogens, by providing CO\(_2\) (buffer capacity, and low pH), and operating the BES in batch (longer HRT). Methane production cannot take place when plain carbon-like electrodes without microorganism were used in a BES (Batlle-Vilanova et al., 2014; Villano et al., 2010). Therefore, methane production was biologically catalysed, and electricity was used to drive the reduction of CO\(_2\). Open circuit voltage tests revealed that methane production did not take place without electricity, due to the lack of electron donors. At the working cathode potential (-0.8 V vs SHE) methane was mainly produced via hydrogenotrophic methanogenesis by *Methanobacterium* sp. These microorganisms used hydrogen, which at the poised potential was bioelectrochemically produced in the cathode surface. Although the direct uptake of electrons could not be completely discarded, the major part of methane was produced through a hydrogen mediated mechanism. This fact has been also reported in other studies, in which the applied cathode potential is below the theoretical potential for hydrogen production, and therefore, methane is likely to be produced using hydrogen as intermediate (Cheng et al., 2009; Fu et al., 2015; Luo et al., 2014; Siegert et al., 2014; Van Eerten-jansen et al., 2013, 2012; Villano et al., 2013, 2010). *Methanobacterium* sp., which have been reported as hydrogenotrophic methanogen (Kotelnikova et al., 1998; Maus et al., 2013), were enriched in the biocathode, and therefore likely responsible for methane production. *Methanobacterium*-like species have been commonly found in methane-producing biocathodes (Table 10.1).

The increase in methane production was observed when the biocathode was changed from batch to continuous operation (Chapter 5, Figure 1). Furthermore, the operation of the BES at shorter HRT increased the methane production rate.
Chapter 10. Discussion

The increase in the microbial activity of BES at decreased HRT was already demonstrated in biocathodes by Pous et al., (2015) with a denitrifying BES and Verdini et al., (2015) with a TCE dechlorinating BES, and in bioanodes by Brown and colleagues (2014) with a BES treating domestic wastewater. Up to date, it had not been tested in BES performing electromethanogenesis. In this sense, the maximum volumetric methane production rate obtained in this study was 100.6 mmol CH₄ L⁻¹ d⁻¹, which is the highest volumetric methane production rate reported so far using a BES (table 10.1). This high rate was achieved at the cathode potential of -0.8 V, and at the HRT of 6 h. The study demonstrated that methane production rate could be even more increased at shorter HRT. However, the quality of the biogas obtained decreased in terms of methane concentration when the HRT was decreased below 6 h, which was likely caused by a partial inhibition of methanogens due to the short HRT and the low pH. In this sense, the partial inhibition of methane production by pH was suggested. The pH decrease down to 6.4 ± 0.1 was observed as a concomitant effect of the HRT decrease due to the acidic conditions of the influent (pH around 5.4). Other authors also reported the inhibition of methanogens in other biological processes, such as anaerobic digestion (Liu et al., 2008) when the pH was slightly acidic, with complete inhibition below 5.5. The partial inhibition of pH is strengthened when looking at the concentration of hydrogen in the gas, which increased while methane concentration decreased. Other authors also reported an increase in hydrogen production when methanogenesis was partially or completely inhibited (Liu et al., 2008; Lu et al., 2011). The expression of hydrogenases by hydrogenotrophic methanogens could have been the cause of the product switch from methane to hydrogen, as it is discussed in section 10.4.

10.1.3. Volatile fatty acids production

The production of these compounds was investigated using 3 different BES configurations, according to chapter 3 (Flat plate reactor, h-shape reactor, and tubular reactor), two modes of operation (batch and continuous), and two different inoculum sources (non-adapted from anaerobic digestion sludge, and adapted from
Chapter 10. Discussion

A non-adapted microbial community from an anaerobic environment was used as inoculum in the flat plate reactor operating in continuous mode. Methanogens were the main electron scavengers at the beginning of the operation. Then, they were effectively inhibited by adding 2-bromoethanesulfonate.

It was shown the increase in the bioelectrochemical activity when bicarbonate was removed from the medium and only CO₂ was flushed in the influent. Although some studies demonstrated the bioelectrochemical production of acetate when bicarbonate was directly added to the medium (Bajracharya et al., 2015; Jourdin et al., 2014; Labelle et al., 2014; Patil et al., 2015a), the microbial community was likely favoured by the direct addition of CO₂, which caused a slight pH decrease to 6.8 ± 0.2. In the last period of the experiments, the pH control at slightly acidic conditions (i.e. 5.8) was shown as an effective strategy to increase the bioelectrochemical activity and the acetate production rate in the biocathode. The increased activity was caused by (i) direct effect of the pH, (ii) higher substrate availability (i.e. both CO₂ and H₂), or (iii) both. Parallelly, a similar study also demonstrated the effects of the pH on acetate production by MES (Labelle et al., 2014). Labelle et al., obtained considerably higher hydrogen production (>100-fold increase) when the pH was decreased to 5. The same study reported acetate production rate increase at slightly acidic pH (around 5.5), but further decrease of the pH (i.e. down to 5) was found detrimental to acetate production. Recently, another study tested different operational pHs (Jourdin et al., 2016a). Jourdin and co-workers found out that acetate production was favoured at slightly acidic pH, and obtained the maximum acetate production rate at a pH of 5.2. pH below that value were detrimental for acetate production.

When an adapted inoculum from a syngas fermentation reactor was used as inoculum, the addition of 2-bromoethanesulfonate was no longer necessary since methanogenic activity was not observed. Two different reactor configurations were tested. First, batch experiments were performed in an h-shape cell. The use of an adapted microbial community resulted in a fast bioelectrochemical activity once the
biocathode was inoculated. Stable bioelectrochemical activity was observed along the whole batch tests, and the analyses of the liquid phase revealed the concomitant production of acetate and butyrate. This study represented the first proof-of-concept about the feasibility of producing butyrate during MES from CO₂ (see Table 10.1). Two hypotheses were suggested for butyrate production, both of which require two basic conditions: (i) acidic pH, and (ii) high reducing power availability. One hypothesis is that acetate could have been produced from CO₂ through the Wood-Ljungdahl pathway coupled to Acetyl-CoA reduction (Daniell et al., 2012) (see section 10.4) by some microorganisms present in the inoculum, such as *Clostridium carboxidivorans* or *C. ragsdalei*. On the other hand, butyrate could have been also produced from acetate and ethanol through chain elongation reactions by *C. kluyveri* (Thauer et al., 1977). Afterwards, a new tubular reactor design was built to test the robustness of the process in a bigger reactor (1.3 L NCC). Within this reactor, the gas phase played a crucial role, since the reactor pressure evolved in time, and therefore the availability of gas compounds (CO₂ and H₂). Therefore, the effect of the availability of the main substrates on the end product spectrum was studied. The results obtained were similar to the h-shape reactor, with acetate and butyrate as the main products, and ethanol and butanol being detected at lower concentrations.

Previous studies suggested that the product spectrum obtained from the degradation of organic matter would be different when the reactor was operated at low or at high Pₜₐₜ (Agler et al., 2011; Angenent et al., 2004; McInerney and Bryant, 1981). Peters et al., (1999) and Demler et al., (2011) demonstrated the increased formate and acetate production yields, respectively, at higher Pₜₐₜ, whereas Yerushalmi et al., (1985) demonstrated the change in the product composition when the Pₜₐₜ was increased. The Pₜₐₜ also affects some steps in the anaerobic digestion process, such as hydrogenotrophic methane production and homoacetogenesis (Angenent et al., 2004). Therefore, CO₂ supply was limited to build up hydrogen partial pressure (Pₜₐₜ) and trigger the production of compounds with a higher degree of reduction. Limiting the CO₂ supply avoided flushing H₂ out

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of the biocathode and maintained high $P_{H_2}$, favouring the transition towards the production of butyrate, which became the primary end-metabolite of MES. However, production in the biocathode stopped when the $P_{CO_2}$ reached very low values (<0.1 atm), which may be inhibitory of acetogenic activity. The operation of the biocathode under CO$_2$ famine, combined with high $P_{H_2}$ likely triggered the enrichment of the microbial community and the selection of species that consumed H$_2$, and species that produced butyrate as end-metabolite. *Megasphaera* sp. were identified as the most abundant microorganism in the biocathode (>39 %), together with other microorganism, such as *Clostridium autoethanogenum*, and other acetogens with lower relative abundance. It was hypothesised that bioelectrochemical hydrogen production was the main electron consuming process in the biocathode, and that *Megasphaera* sp. drove butyrate production through chain elongation of acetate and ethanol.

Although the continuous production of acetate was first demonstrated in the flat plate reactor, the obtained production rate was low, and the nature of the continuous operation did not allow for the accumulation of the product to reach high concentrations. Therefore, continuous operation was probably not the best option since acetate is washed out from the reactor and cannot be accumulated. The continuous production of acetate streams is interesting from the applicability point of view, however its application could be limited if production rates are not considerably increased.

The operation of the MES in batch was shown a more feasible strategy, especially in the case of the tubular reactor, which was found to be easily operated, robust, and resilient to external changes. The production rates were increased in batch operation, and it allowed for the concentration of the products. The results obtained demonstrated the possibility of producing more valuable products, such as butyrate. In this sense, butyrate was accumulated in the reactors together with acetate at the end of the process (chapters 8 and 9). The use of a tubular reactor resulted in the increase of the volumetric production, but a decrease in terms of electrode surface area, which was likely caused by the use of a large electrode (320
cm²) and voltage gradients along the surface (Doherty et al., 1996; Ruotolo and Gubulin, 2011), which could cause less active zones due to lower voltages. In general, the higher production rates in terms of electrode surface (table 10.1) were obtained using small electrodes.

10.1.4. Alcohols production

Although the concentration of alcohols was low compared to the other products synthetised in this thesis, some insights are here discussed about the feasibility of producing these compounds during MES from CO₂.

A main hypothesis for the production of alcohols is suggested. First of all, the microorganisms present in the BES need to be able to drive solventogenesis of the VFA to its corresponding alcohols. Many species of Clostridium have been reported to drive solventogenesis (Agler et al., 2011; Ramió-Pujol et al., 2015b). This happens at low pH, high concentration of organic acid, and high availability of reducing power. The acidification of the culture medium led to the establishment of a transmembrane potential, and therefore a proton gradient between inside and outside, which is used as a proton motive force through proton-gradient driven phosphorylation to obtain energy (Mohammadi et al., 2011; Thauer et al., 1977), under this conditions cells may re-assimilate VFA and convert them to alcohols (Abubacker et al., 2012) to prevent a further pH decrease. Different studies have demonstrated that in carboxydotrophic microorganisms this is observed at a pH close to the pKa (i.e. 4.5 – 5.0) of the VFA (Abubacker et al., 2012; Grethlein et al., 1990; Ramió-Pujol et al., 2015c), which resulted in increased alcohols production.

It is known that the autotrophic production of VFA requires low P₃H₂ (Agler et al., 2011) and high P₂CO₂, whereas the production of alcohols requires high P₃H₂ (Agler et al., 2011; Angenent et al., 2004). Therefore, trying to couple both reaction in the same reactor seems contradictory at this stage. Further efforts need to be conducted to study the feasibility of producing these compounds through MES from CO₂. This would have a high impact in terms of applicability for example in the
production of biofuels. Otherwise, acidogenesis and the solventogenesis steps should be separated in different reactors.

### 10.1.5. General performance of microbial electrosynthesis

During microbial electrosynthesis, CO₂ and electricity can be converted to different compounds. Therefore, the efficiency of the process can be evaluated in terms of CO₂ conversion, and also in terms of electricity conversion into products. To assess the efficiency of the whole process both efficiencies have to be considered. The efficiency in CO₂ conversion would highlight the performance of the process in terms of carbon capture and transformation, whereas energy efficiency would be indicative of the energy recovered in products. In terms of energy, the CE indicated the ratio of electrons that are recovered in products, whereas the energy efficiency is the ratio of energy (i.e. in kWh) recovered in products. The general performance, in terms of carbon conversion, and coulombic and energy efficiency of the different studies performed in this thesis is summarised in Table 10.2.

#### Table 10.2. Summary of the electron acceptors and final products obtained in the different studies performed during this thesis, together with its respective CO₂ conversion, coulombic and energy efficiencies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Electron acceptor</th>
<th>Product obtained</th>
<th>CO₂ conversion efficiency (%)</th>
<th>Coulombic efficiency (%)</th>
<th>Energy efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 4</td>
<td>H⁺</td>
<td>H₂</td>
<td>n/a</td>
<td>113&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>CO₂</td>
<td>CH₄</td>
<td>67.0 ± 1.9</td>
<td>68.9 ± 0.8</td>
<td>39.7 ± 1.3</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>CO₂</td>
<td>CH₄, H₂</td>
<td>95.8 ± 9.4</td>
<td>89.7 ± 0.2</td>
<td>44.0 ± 0.1</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>CO₂</td>
<td>Acetate</td>
<td>3.0 ± 0.1</td>
<td>28.9 ± 6.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Chapter 8</td>
<td>CO₂</td>
<td>Acetate, Butyrate, Ethanol, Butanol, H₂</td>
<td>n/a</td>
<td>28 – 32 %</td>
<td>n/a</td>
</tr>
<tr>
<td>Chapter 9</td>
<td>CO₂</td>
<td>Acetate, Butyrate, Ethanol, Butanol, H₂</td>
<td>88.6 ± 5.1</td>
<td>57.3 ± 1.0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average value obtained for tests in presence of microorganisms.

<sup>b</sup> Calculated from the lowest energy consumption reported in the study (0.116 m³ H₂ kWh⁻¹)
Relatively high CO\textsubscript{2} conversion and coulombic efficiencies were achieved for continuous methane production. In this sense, the optimisation of the HRT seems to be a key parameter of the process. The decrease of the cathode HRT to 6 h enhanced methane production and increased the CO\textsubscript{2} conversion from 67.0 ± 1.9 to 95.8 ± 9.4 \%, and the CE from 68.9 ± 0.8 to 89.7 ± 0.2 \%, when comparing to the results obtained in the previous study at a cathode HRT of 19.8 h.

Regarding the transformation of CO\textsubscript{2} into liquid organic products, the CO\textsubscript{2} conversion efficiencies are significantly different when comparing a BES working in continuous (Chapter 7) to a BES working in batch (chapter 9). In terms of CO\textsubscript{2} conversion, a BES working in continuous exhibited an efficiency of 3.0 ± 0.1 \%, whereas a BES working in batch increased the efficiency to 88.6 ± 5.1 \%. The low conversion efficiency obtained in continuous was likely related to the low kinetics of the process, and the continuous CO\textsubscript{2} feeding in the BES. Whereas the intermittent introduction of CO\textsubscript{2} was a better strategy to favour its conversion. The coulombic efficiency obtained in the last study was almost double than the obtained in the previous study. This fact was likely caused by the better BES design, and the reduction of hydrogen losses.

The energy efficiency of the process for the obtention of liquid products could not be accurately measured. In the case of gas products, the energy efficiency for both hydrogen and methane was similar, around 40 \%. Although the value is low, it has to be considered, that this work was focused on the reactions taking place in the cathode of the BES. If the anode reaction were considered (i.e. production of oxygen) the energy efficiency of the process would increase, due to a higher product recovery. One of the key points towards the potential application of the technology is the optimisation of the reactions that take place in both BES compartments.
10.2. Microorganisms capable of microbial electrosynthesis

In this section, the microorganisms enriched during MES of different compounds are discussed. Table 10.3 presents the phylum and genus of the main microorganisms detected in biocathodes after enrichment and production of different target compounds.

In this thesis, two main genus were found in the hydrogen producing biocathode, belonging to Bacteroidetes and Proteobacteria. Other authors also found Bacteroidetes and Proteobacteria phylums to be dominant, but also firmicutes (Table 10.3). Proteobacteria and Firmicutes are the phylums that have been most commonly found in hydrogen producing biocathodes. However, different genus were found in each case (Table 10.3).

Regarding the production of methane, *Methanobacterium* was the genus detected in our studies. It clearly dominated the microbial community in all the biocathodes. It is suggested that similarly to *Geobacter* sp. in bioanodes, *Methanobacterium* sp., could play a key role as a key biocathode electroactive microorganism, especially regarding methane-producing biocathodes.

The composition of the suspended microbial community of the BES producing a mixture of VFA, with butyrate being the main product, was also analysed (chapter 9). The main finding was that *Megasphaera* sp. dominated the bacteria community in the solution, and other *Firmicutes*, such as *Clostridium* sp. were also found. The complete study of the microbial community in another BES, suggested that acetogens are the dominant community in the solution, but that hydrogen-producing bacteria dominate the biofilm (Puig et al., In preparation). In the case of MES of acetate, especially *Acetobacterium* sp. from the firmicutes phylum were detected. In some studies, other microorganism were also detected. It is highlighted the presence of *Methanobacterium* in acetate-producing biocathodes. Recent reports suggest that this microorganism could not only produce methane, but at low cathode potentials it could be also responsible for hydrogen catalysis (Beese-Vaslender et al., 2015; Patil et al., 2015a), thus increasing the availability of
reducing power and the chances of hydrogen mediated MES of organic compounds. When it comes to the production of organic compounds by MES, *Firmicutes* have been generally detected as the predominant species.

<table>
<thead>
<tr>
<th>Product</th>
<th>Phylum</th>
<th>Genus</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>Proteobacteria</td>
<td>Desulfovibrio sp.</td>
<td>(Rozendal et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Bacteroidetes</td>
<td>Aquiflexum sp.</td>
<td>(Croese et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Proteobacteria</td>
<td>Hoeflea sp.</td>
<td>(Batlle-Vilanova et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Firmicutes</td>
<td>Desulfotobacterium sp.</td>
<td>(Villano et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Firmicutes</td>
<td>n/a</td>
<td>(Jourdin et al., 2015a)</td>
</tr>
<tr>
<td>Methane</td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Villano et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Cheng et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Van Eerten-jansen et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Fu et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Beese-Vasbender et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Batlle-Vilanova et al., 2015b)</td>
</tr>
<tr>
<td></td>
<td>Proteobacteria</td>
<td>Sulfospirillum sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Euryarchaeota</td>
<td>Methanobacterium sp.</td>
<td>(Patil et al., 2015a)</td>
</tr>
<tr>
<td></td>
<td>Firmicutes</td>
<td>Acetobacterium sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firmicutes</td>
<td>Acetobacterium sp.</td>
<td>(Xafenias and Mapelli, 2014)</td>
</tr>
<tr>
<td></td>
<td>Firmicutes</td>
<td>Acetoanaerobium sp.</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>Firmicutes</td>
<td>Megasphaera sp.</td>
<td>Chapter 9</td>
</tr>
<tr>
<td>and butyrate</td>
<td>Firmicutes</td>
<td>Clostridium sp.</td>
<td></td>
</tr>
</tbody>
</table>
10.2.1. Effect of the inoculum

During this thesis, different inoculum sources were used for the production of different compounds. It was observed that the use of non-enriched inoculum took longer start-up times in the experiments performed with the flat plate reactors, whereas the use of previously enriched microorganisms allowed for a faster start-up and the production of acetate and butyrate using h-shape and tubular reactors.

Since mixed cultures were used, the addition of 2-bromoethanesulfonate was necessary to inhibit methanogenesis when non-adapted inoculums were used. However, the use of enriched inoculums allowed for the withdrawal of this compound from the medium, without negative effects in the production of VFA, and without methane detected in the gas phase. The long-term enrichment of the carboxydotrophic microorganisms in a previous syngas fermentation reactor likely caused the lack of methanogens.

The use of microbial mixed cultures favoured syntrophic relationships among the microorganisms in the biocathode, which can be beneficial for the process (Dolfing, 2014). In biocathodes syntrophy can be stablished from many points of view, such as electron transfer, by-product transformation, oxygen removal, etc. In this sense, it is highlighted the role of the \textit{Methylocystis} sp., from the Alphaproteobacteria genus (Chapter 5, Figure 2), which allowed for the removal of oxygen and therefore, methanogens could carry out its metabolic activity. Acetate and butyrate production, was also likely to be allowed by the presence of different microorganisms that stablished synthophic relationship, by transforming the acetate produced into butyrate, for instance. In all cases, the applied potential was low enough to produce hydrogen, which was used as intermediate for CO$_2$ reduction into different compounds. In this sense, syntrophy was also stablished between the hydrogen-producing microbial community present in the electrode surface and hydrogen-utilising suspended cells.
10.3. Electron transfer and production mechanisms

As it has been already stated, electron transfer mechanisms governing biocathodes are yet not well understood, especially when microbial electrosynthesis is concerned. However, since carboxydrotrophic microorganisms, more specifically homoacetogens, are harnessed to drive CO₂ reduction, hydrogen can be directly provided in the biocathode of a BES by poising the cathode potential at low values. Thus, electrochemically active microorganisms are no longer needed to drive the reduction of CO₂, but they are necessary to biocatalyse hydrogen production in the electrode surface. However, from a practical point of view it would be ideal that CO₂-fixing microorganisms were also able to directly uptake electrons from the electrode surface, without the need for hydrogen as intermediate product. Thus, energy losses would be minimised and the efficiency of the process would increased (Lovley, 2011). Nonetheless, to date it is still not clear whether those microorganisms are able to reduce CO₂, are also able to directly accept electrons to reduce CO₂.

In the studies carried out during this thesis, the applied potential was always low enough to drive the electrochemical or bioelectrochemical hydrogen reduction (Rabaey and Rozendal, 2010). Thus, CO₂ reduction was likely to happen with hydrogen as intermediate. Actually, all the studies carried out to date used a potential below the necessary for catalytic hydrogen production (i.e. -0.41 V vs SHE) (Table 10.1). Only Nevin and coworkers (Nevin et al., 2011, 2010) demonstrated MES of organic products using directly electrons derived from the electrode using pure cultures at cathode potentials higher than the necessary for hydrogen production. Regarding MES of methane only two studies reported its production at a cathode potential higher than the theoretical for hydrogen production (Beese-Vasbender et al., 2015; Fu et al., 2015). In all these studies, the production rates were very low compared to other studies (Table 10.1), due to the limited amount of available reducing power.
The metabolic mechanisms that microorganism use for the production of the different compounds obtained during this thesis are discussed below.

10.3.1. Mechanisms for hydrogen production

The production of hydrogen in biocathodes require microorganisms with hydrogenase activity (Geelhoed et al., 2010). There exists a wide range of hydrogenases, and they are very efficient enzymatic catalysts for both the consumption and production of hydrogen (Lubitz et al., 2014). Up to date, the production of hydrogen from electrons have been demonstrated for [FeFe] and [NiFe]-hydrogenases (Butt et al., 1997; Pershad et al., 1999; Woolerton and Vincent, 2009), and also by Desulfovibrio vulgaris, which contain different hydrogenases (Lojou et al., 2002). Recently, Geelhoed et al. (Geelhoed et al., 2010) and Croese et al. (Croese et al., 2011) suggested that hydrogen-producing biocathodes could sustain energy conservation and growth when they are provided with enough energy and in presence of a carbon source. Therefore, becoming self-regenerating catalysts. Figure 10.1 shows the production mechanism that have been suggested for hydrogen-producing microorganisms by different researchers (Geelhoed et al., 2010; Jourdin et al., 2015a).

![Figure 10.1](image)

**Figure 10.1.** Energy conservation mechanisms by hydrogen-producing microorganisms containing hydrogenases (A), and suggested hydrogen production mechanism in the biocathode of a BES (B).
Figure 10.1 A is focused on the energy conservation, whereas figure 10.1 B is focused on the application of this mechanisms in a biocathode of a BES. Although electron transferring components that link hydrogenases to the cathode itself to allow electron transport are still unknown (Rosenbaum et al., 2011), electron carriers, such as cytochromes, ferredoxin, quinones and flavins are suspected to be key compounds in electrode-microbe interactions (Kracke et al., 2015).

### 10.3.2. Mechanisms for methane production

Although many studies demonstrated methane production from CO$_2$ in BES, the production mechanisms and the electron uptake by microorganisms is still not clear (Lovley, 2011). What most researchers meet an agreement is that electrochemical methane production mechanism is most probably similar to hydrogenotrophic methane production mechanism. Hydrogenotrophic methanogens reduce CO$_2$ though the so-called Wolfe cycle (Thauer, 2012). Costa and Leigh recently summarised hydrogenotrophic methane production mechanisms (Figure 10.2). In the Wolfe cycle, hydrogenotrophic methanogens use electron bifurcation to couple the final heterodisulfide reduction step to the initial CO$_2$ reduction (Kaster et al., 2011), using reducing power, either in form of hydrogen, electrons or formate.
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Figure 10.2. Simplified schematic representation of the Wolfe cycle. Electron bifurcation occurs at the heterodisulfide reductase complex (Hdr). Ferredoxin (Fd) is used for CO$_2$ reduction. Hydrogen (H$_2$) or electrons can be used as reducing power source for the Hdr complex and Fd regeneration. (F$_{420}$) represent a flux of electrons to intermediate reduction steps. Adapted from Costa and Leigh, 2014.

Different metabolic steps are performed to obtain methane from CO$_2$, from BES point of view, the role of reducing power, either in form of electrons, hydrogen or ferredoxin is especially interesting. Dehydrogenases were found responsible for catalysing the H$^+$ or Na$^+$ motive force-driven reduction of ferredoxin with hydrogen (Kaster et al., 2011), and were also involved in a series of reduction steps (F$_{420}$) (Thauer et al., 1977; Tzeng et al., 1975). The presence of hydrogenases in hydrogenotrophic methanogens could be the reason why some genus produced hydrogen when they have been provided with a high amount of reducing power (Beese-Vasbender et al., 2015). Altogether suggested that hydrogenotrophic methanogens, and more specifically Methanobacterium sp. are a promising microorganism to produce methane from CO$_2$ in BES. This has been already suggested by many researchers (Table 10.1), and the results obtained in this thesis (Chapters 5 and 6) highlight the potential of electrochemical methane production to become a platform technology.
10.3.3. **Mechanisms for volatile fatty acids and alcohols production**

Acetogenic microorganisms (acetogens) are those anaerobic microorganisms that are able to drive the Wood-Ljungdahl pathway using CO$_2$ as a carbon source. Many acetogens have been reported so far, some well-known acetogens belong to *Acetobacterium*, *Clostridium* and *Sporomusa* genus (Drake et al., 2008; Gottschalk and Braun, 1981; Köpke et al., 2010). Although the main product of these microorganisms is acetate they are also able to produce other compounds, such as butyrate, ethanol or butanol, among others, and they can also do it from different substrates, such as organic matter (Tracy et al., 2012). Acetogens use the so-called Wood-Ljungdahl pathway to fix CO$_2$ into the intermediate metabolite Acetyl-CoA. Wood-Ljungdahl pathway is coupled to the generation of transmembrane proton gradients, which allow for ATP generation, and energy conservation through the Rnf complex (Fast and Papoutsakis, 2012; Tremblay et al., 2012).

Acetyl-CoA can lead to the production of 2-carbon (C2) compounds such as acetate and ethanol, but it can be also more reduced to Butyryl-CoA under excess of reducing power, which can lead to the production of 4-carbon (C4) compounds, such as butyrate and butanol (Daniell et al., 2012), or even 6-carbon (C6) compounds, such as hexanoate and hexanol (Ramió-Pujol et al., 2015a) (Figure 10.3 B). Figure 10.3 shows the main pathways for the production of the mentioned compounds, starting from the Wood-Ljungdahl pathway with CO$_2$ and hydrogen (or electrons) as carbon and energy sources, respectively (Figure 10.3 A), followed by the Acetyl-CoA reductive pathway (Figure 10.3 B).

The production of C2, or C4 and further compounds, relies on the ability of the microorganisms to further reduce Acetyl-CoA to Butyryl-CoA under excess of reducing power. Previous studies demonstrated that the production of acids or alcohols relies specially on the pH of the growth medium (Ganigué et al., 2015; Ramió-Pujol et al., 2015b), with increasing alcohols production at lower pH. This fact could be caused by (i) the higher activity of the Rnf complex, and therefore, the generation of more energy in form of ATP, or (ii) the presence of undissociated...
organic acids ($\text{pH} \approx \text{pKa}$), which could present toxicity to the microorganisms, forced the solventogenesis of the VFA to their respective alcohols to avoid further pH decrease (Jones and Woods, 1986).

Production of compounds different than acetate and ethanol, could also be produced by chain elongation reactions (Agler et al., 2011; Thauer et al., 1977). In the case of butyrate, it could be produced by the combination of a one molecule of acetate and another molecule of ethanol through the reverse $\beta$-oxidation process (Angenent et al., 2016).

**Figure 10.3.** Wood Ljungdahl pathway coupled to Acetyl-CoA reductive pathway used by acetogens to drive the production of their end products. Abreviations: acs: acetyl-CoA synthase; CODH: CO dehydrogenase; fdh: formate dehydrogenase; fts: formyl-THF synthase; hyd: hydrogenase; MTC: methyl-THF cyclohydrolase; mtd: methylene-THF dehydrogenase; mtr: methyl transferase; mtrs: methylene-THF reductase; adh: alcohol dehydrogenase; adhE: aldehyde/alcohol dehydrogenase; ak: acetate kinase; bcd: butyryl-CoA dehydrogenase; bdh: butanol dehydrogenase; buk: butyrate kinase;
Chapter 10. Discussion

crt: crotonase; hbd: 3-hydroxybutyryl-CoA dehydrogenase; pta: phosphotransacetylase; ptb: phosphotransbutyrylase; thl: thiolase. Adapted from Ramió-Pujol et al., 2015a.

10.4. Productivity and product selection

Different strategies to select the final product include the modification of the operational parameters, or the addition of inhibitory substances to cut off non-interesting pathways. The most typical example of inhibitory substances is 2-bromoethanesulfonate, which has been widely used in BES and other anaerobic technologies to inhibit methanogenesis. In this thesis, 2-bromoethanesulfonate was used to effectively inhibit methanogens when non-adapted microbial communities were aimed to produce organic liquid products, whereas its use was not necessary when highly enriched microbial communities were used as inoculum of the BES.

The cathode potential was the main operational parameter that affected the production rates of the different products. In this thesis, it has been demonstrated that lowering the cathode potential, increases the production of both gas (hydrogen and methane) and liquid (acetate, butyrate, and others) compounds.

Methane production was specially affected by the cathode HRT, and it was hypothesised that other operational parameters, such as the pH, temperature and substrate concentration also affected the production of this compound. The inhibitory effect of pH was suggested. The pH decreased as a concomitant effect of the HRT decrease, and thus the pH decrease was observed because of the slightly acidic conditions of the influent. Although the methane production rate increased at lower HRT, the concomitant hydrogen production became more important, and thus the quality of the product (methane) was negatively affected. It was suggested that hydrogen was produced in the electrode surface faster than methanogens could uptake it, which resulted in the detection of hydrogen in the gas phase. Hydrogenotrophic methanogens contain hydrogenases, which allow them to obtain energy (Kaster et al., 2011; Thauer et al., 1977). Another hypothesis for hydrogen production, was that hydrogenases could be stimulated under certain conditions,
such as low HRT (chapter 6) or high cathode potential (Beese-Vasbender et al., 2015), thus resulting in the product switch.

The production of VFA was favoured by slightly acidic pH (Labelle et al., 2014). Although an external pH control had to be applied to the BES operating in continuous, the results obtained in batch experiments showed a pH decrease at the beginning of the operation, and the production of acids, with concomitant production of alcohols at very low pH and high availability of reducing power. The availability of CO₂ and hydrogen was also found to be essential for the further reduction of acetate into longer VFA. Butyrate can be favoured by limiting the amount of CO₂ that is provided to the biocathode, thus acetate-producing microorganisms lack enough substrate to drive its metabolism at high rates, whereas butyrate-producing microorganism can use the acetate produced as intermediate to metabolise its end product.

### 10.5. Economic evaluation – some insights

Using CO₂ as carbon source for the bioproduction of different compounds has several advantages. It is available in excess (atmosphere, seawater, solid minerals, and flue gases), provides buffering capacity, avoiding the use of chemicals, it is land-independent, and its use represents a positive impact on greenhouse gas budget (Rabaey et al., 2011). However, electricity is needed for its transformation in BES, which implies operational costs. Therefore, the product value of the compounds obtained by MES plays a major role in the feasibility of the process. In this thesis, the economic feasibility has been studied in terms of electricity operational costs for the production of different compounds, and its value. Only the products obtained in the cathode chamber have been considered for the economic assessment, but not the products obtained by the anodic reactions. The results obtained for each product are shown in Table 10.4. The economic assessment is based on the production of 1 ton of product. It has to be highlighted that the economic feasibility of the process could dramatically change if the anode reactions were considered. Anode reactions could affect the feasibility of the whole process, for instance
allowing for a lower energy requirement for the cathode reaction, or the production of other compounds with an economic value. On the other hand, extensive economical assessment of BES might be too preliminary taking into account that the technology readiness level (TRL) is still 3-4 (validation in laboratory environment) for most potential applications.

Table 10.4. Economic assessment of the products obtained in the cathode of the BES

<table>
<thead>
<tr>
<th>Product</th>
<th>Value (€ ton⁻¹)</th>
<th>CO₂ fixed (ton_{CO₂} ton_{product}⁻¹)</th>
<th>Electron requirement (electron mol_{product})</th>
<th>Estimated energy requirement (kWh ton⁻¹)</th>
<th>Estimated production price in BES (€ ton⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural gas (methane)</td>
<td>1082⁺</td>
<td>2.75</td>
<td>8</td>
<td>13400</td>
<td>1608</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>339⁺</td>
<td>1.47</td>
<td>8</td>
<td>3600</td>
<td>429</td>
</tr>
<tr>
<td>Ethanol</td>
<td>955⁺</td>
<td>1.91</td>
<td>12</td>
<td>6977</td>
<td>837</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>1238⁺</td>
<td>2.02</td>
<td>20</td>
<td>6154</td>
<td>738</td>
</tr>
<tr>
<td>Butanol</td>
<td>3200⁺</td>
<td>2.37</td>
<td>24</td>
<td>8678</td>
<td>1041</td>
</tr>
</tbody>
</table>

⁺ Estimated from natural gas price in the EU. Source: European Comission, 2014
⁺⁺ Source: www.molbase.com (consulted on the 12/01/2015).
⁺⁺⁺ Assuming CE=100%, and cathode potential -1 V.
⁺⁺⁺⁺ Based on average electricity price for industrial consumers in 2014 in the EU (0.12 € kWh⁻¹). Source: Eurostat.

As it can be observed in the table, the operational costs for the production of methane and acetate are higher than its actual market value. In the case of the more reduced products, such as ethanol, butyrate and butanol, the value of the product itself is higher than its cost of production. This fact makes the process more attractive.

Although methane and acetate production are unfavourable at first sight, the identification of smart application niches for the technology could make the process more attractive. In this sense, the application of BES for methane production in biogas upgrading could be an alternative. Nowadays, energy is used in water scrubbing for CO₂ stripping to allow for water reuse in the absorption tower (Appels et al., 2008), which in addition cause the emissions of GHG. If this amount of energy could be used in a BES, the emissions of CO₂ would decrease, and thus an extra amount of methane could be obtained. Another example in the case of acetate
production by BES, would be the reduction of GHG emissions in wastewater treatment plants. CO₂ could be transformed in these installations and used in-situ for nutrient removal treatment. Thus avoiding the costs of organic matter, and transport that these treatment processes usually imply (van Rijn et al., 2006).

The production of liquid compounds would usually require its purification and extraction from the liquid broth. Some compounds need to be refined to obtain a certain degree of purity before they can be further utilised, which could occur to over 60 % of the total production costs (Bechthold et al., 2008). In the case of gas products, such as methane, the separation and concentration costs would be lower compared to liquid products. Since methane has a low solubility in water, it is naturally desorbed from the water, therefore minimising extraction costs. However, they would require compression, unless they are used in situ.

An extensive economic assessment should be developed in each particular case for BES applications. There are many factors that should be considered when dealing with economic feasibility. Full economic assessment of BES technology is daring at this stage. However some of the major points that should be considered in the future are:

- Product value (both cathode and anode, if applicable).
- Production rates (will directly affect payback time).
- Operational costs (estimated in Table 10.3).
- Cost of CO₂ emissions (saving costs of CO₂ emissions)
- Capital expenditures (construction of the reactor and instruments).
- Downstream costs (separation and concentration of the products).

Previous researchers already stated the importance of using renewable energy when it comes to the operation of BES. The use of renewable energy would drastically reduce the operational costs. However, it would increase capital expenditures, so other aspects should be then considered, such as payback time of the equipment.
There are other costs that may affect BES processes in the future, which are environmental policies. This is especially interesting in the case of CO₂, as it would not be strange if taxes were applied to CO₂-emitting industries in the future.

10.6. Implications and perspectives

Despite CO₂ being the ultimate product of respiration processes, its conversion to different products has been demonstrated using a BES. From the results obtained in this thesis and recent studies by other research groups, it can be stated that MES represents a promising technological approach for the production of commodities, such as hydrogen, methane, volatile fatty acids, and alcohols. However, from an economical standpoint its application could be compromised by expensive reactor materials, the low productivity, or the requirement of extraction and concentration processes that could increase the cost of the whole process. It has to be highlighted though, that it represents also a carbon capture and transformation technology, and if the technology can be developed through the use of non-expensive materials, use of renewable energy etc. it could become a promising platform for CO₂ transformation.

In this thesis, BES systems have been operated for the production of different compounds, with increasing attention in methane and butyrate. Methane production in the biocathode was shown to rely on the applied cathode potential and the HRT. The maximum volumetric methane production rate of 100 mmol CH₄ L⁻¹ day⁻¹ obtained in this thesis represents the highest reported so far. This value was achieved with a CE of 89.7 % and CO₂ conversion efficiency of 95.8 %. It was already suggested that BES could be applied as a biogas upgrading technology (Batlle-Vilanova et al., 2015b), but these results strengthen the potential of BES to become a real alternative. The results suggest the possibility of developing decentralised compact units for this purpose, which make the process even more interesting.

The results regarding liquid compounds demonstrated that other compounds can be produced by BES, rather than acetate, and opened up the potential for this
technology to become a microbial production platform. Although alcohols were only
detected at low concentrations, it was suggested that its production was favoured
at low pH values and with high reducing power availability (low cathode potential).
However, seems to be contradictory regarding its production from CO₂, as these
conditions do not favour the production of VFA, which are the intermediate
products (Agler et al., 2011). If this issue cannot be overcome by further research, it
seems quite certain that the production of VFA and alcohols will need to be
performed in different reactors. Regarding butyrate production, something similar
was observed. Whereas acetate was produced at high CO₂ availability, butyrate
production predominated when CO₂ availability was limited. However, butyrate
production relied on acetate which was used as intermediate product. The
sustainable coproduction of these two compounds was demonstrated in this thesis,
with especial emphasis on the selective production of butyrate as end-product
under CO₂ limiting conditions. The production of butyrate is especially interesting
because it is the precursor for butanol, which is a promising alcohol with a high
energetic value, and it can be used in conventional combustion engines without
modification (Dürre, 2007).
Chapter II. Conclusions
This thesis demonstrates the feasibility of bioelectrochemical systems to produce a wide range of commodity chemicals from CO₂ gas streams. Special attention was given to the key parameters and system design for the production of biofuels (methane, bioH₂) and valuable organics (acetate and butyrate). The main conclusions obtained are summarised as follows:

- Biocatalysts reduce the overpotentials associated to hydrogen production in BES. Electrochemical hydrogen production started at -0.9 V with biocatalysts in carbon-like electrode, and at -1 V with plain carbon-like electrode, and increase linearly at decreasing cathode potential.
- Although plain carbon-like electrodes can catalyse hydrogen production, the use of microorganisms as biocatalysts allows the production of hydrogen with lower energy consumption.
- Direct electron transfer cannot be discarded, but the predominant production mechanism in all the studies performed with CO₂ as final electron acceptor was likely to be hydrogen mediated.
- Bioelectrochemical methane production or electromethanogenesis was demonstrated in batch and continuous operation, being the hydrogenotrophic methanogen, *Methanobacterium* sp. the main responsible for its production.
- Continuous bioelectrochemical methane production can took place at cathode potentials <-0.6 V vs SHE, with a relatively high CE >70%.
- Low HRT enhanced methane production rate, but compromised the quality of the gas obtained, which concentration in methane was decreased from 71.5 % at 6 h to 45.1 % at 2 h HRT at the cathode potential of -0.7 V.
- The short HRT (< 6 h) and the low pH of the biocathode (< 6.5) were likely to be the operational parameter favouring hydrogen production and causing partial inhibition of the methanogens. Thus, affecting negatively the purity of the final product in terms of methane concentration.
- The maximum methane production rate was above 100 mmol CH₄ L⁻¹ d⁻¹, with a CE of 89.7 % and CO₂ conversion efficiency of 95.8 %. This value represents the highest volumetric production rate reported so far.
The use of previously enriched inoculums allow for a faster start-up of MES process for the production of commodity chemicals (VFA and alcohols).

MES of acetate was favoured by the direct addition of CO₂. A slightly acidic pH (i.e. 5.8) also favoured acetogenic activity, either by direct effect of the pH, or by the higher substrate availability (i.e. CO₂ and H₂). At lower pH, acetate could become toxic and the microorganisms drive other reactions, such as butyrate or alcohols production to avoid further pH decrease.

Butyrate production by MES from CO₂ was demonstrated for the first time in this thesis. Its production takes place based on two essential conditions: (i) acidic pH, and (ii) high reducing power availability. Butyrate production could take place by direct production through the Wood-Ljungdahl pathway coupled to Acetyl-CoA reduction, or indirectly by chain elongation reactions.

The CO₂ feeding strategy influenced the product spectrum. Limiting the CO₂ supply caused the increase of the (P₇₇) due to the bioelectrochemical activity, and resulted in a switch of the product spectrum towards butyrate.

CO₂ famine conditions combined with high P₇₇ caused the enrichment of the biocathode community towards species that produced butyrate as end-metabolite, which was mainly produced through chain elongation of acetate and ethanol (reverse β-oxidation).

Membrane liquid extraction with hollow fibre membranes was used as purification process to selectively extract and concentrate butyrate. A concentration factor of 5.4 was achieved, and a butyrate/acetate ratio of 16 was obtained.

Low concentrated broth in liquid products was obtained during continuous operation (1 mM), whereas higher concentrations were obtained in batch (up to 100 mM), which are more attractive for further separation and purification of the final product.

The performance of the tubular modified reactor represents a promising step forward regarding the development of MES for the production of different compounds.
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- Production of gas compounds could be more economically attractive compared to liquids, due to ease separation from the liquid broth. However, specifically and low energy-consuming separation techniques for liquid compounds, such as membrane liquid extraction are being developed.

- Preliminary study based on the operational costs, suggest that the more reduced compounds are more economically attractive. However, further costs, such as extraction and concentration steps, should be considered, which could drastically capsize the economic assessment.

Since MES is still in its infancy, further research need to be conducted from the point of view of fundamental and applied research. From the fundamental point of view, the main steps of the process (mentioned in Figure 1.7) need to be elucidated and efforts should focus on improving the limiting steps.

In terms of applied research further studies regarding the conditions governing the selective production of different compounds need to be performed, with special attention to the production of highly reduced and long carbon-chain compounds (>C4 compounds) and alcohols, which have a higher value and promising substitutes of conventional fuels. Regarding the production of methane it is highlighted that the production rate obtained during this thesis represents only a half of the of the estimated CO$_2$ production rate during anaerobic digestion (Van Eerten-jansen et al., 2012), which highlight the potential application of this technology in biogas upgrading. In this light, the work presented in this PhD thesis regarding methane production in BES will continue with the construction of a laboratory scale pilot plant with a new design that will allow studying the feasibility of BES as a biogas upgrading technology.
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