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MUNICIPAL WASTEWATER TREATMENT IN MICROBIAL ELECTROLYSIS CELLS

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ABSTRACT:

Wastewater treatment often demands high infrastructure and usually uses a great amount of energy. Nowadays, there is growing consensus that organic components in the wastewater are a resource instead of an increasing problem. Bioelectrochemical Systems are emerging devices that could be developed into a new generation of sustainable wastewater treatment plants since they are able to treat wastewater while recovering part of the energy investment as hydrogen or electricity.

The main objective of this thesis is to study the feasibility of different MECs (Microbial Electrolysis Cell) configurations for organic matter removal and hydrogen production with low energy consumption during domestic wastewaters treatment.

The process scale-up from milliliters to liters of working volume of a microbial electrolysis cell (MEC) was demonstrated. Initially, a 50 mL MEC was operated on synthetic wastewater at different organic loads. It was concluded that process scale-up might be best accomplished using a “reactor-in series” concept. Consequently, 855 mL and 10 L MECs were built and operated on domestic wastewater. The three compartments 855 mL MEC, achieved a COD removal of $5.7 \text{ g-COD L}_R^{-1} \text{ d}^{-1}$ and a hydrogen production of $1.0\text{-}2.6 \text{ L L}_R^{-1} \text{ d}^{-1}$ by optimizing applied voltage and hydraulic retention time. Furthermore, a two 5 L MECs in series showed a COD removal rate of $0.5 \text{ g L}_R^{-1} \text{ d}^{-1}$, a removal efficiency of 60-76% and an energy consumption of 0.9 Wh per g of COD removed.

Furthermore, the effect of the organic loading rate, hydraulic retention time, applied voltage and the configuration of a semi-pilot modular microbial electrolysis cell (MEC) on the energy consumption and COD removal during domestic (dWW) wastewater treatment was studied. The MEC reactor consisted of twin tubular units hydraulically connected in series and was

able to reduce up to 85% of the chemical oxygen demand (COD) concentration of the influent dWW at a relatively low energy consumption 0.3-1.1Wh per g of COD removed. Overall, the results identified both an organic loading rate (OLR) threshold that makes the use of MECs for dWW treatment feasible in terms of energy consumption and COD removal efficiency and an OLR threshold that justifies the operation of two MECs in series to provide the required degree of COD removal. Hydrogen production was limited by the reduced amounts of organic matter fed into the reactor, the poor performance of the cathode, and COD consumption by non electrogenic microorganisms. The presence of COD consuming microorganism that do not contribute to electrogenic metabolism severely affected the MEC performance.

Finally, the carbon footprint of a domestic wastewater treatment plant (dWWTP) with an integrated MEC and its comparison with a conventional wastewater treatment plant was estimated. The dWWTP with an MEC technology was found to generate lower emissions than a traditional dWWTP, moreover, it would avoid a large amount of emissions if the energy contained in the hydrogen could be either consumed in the plant or injected into the grid.

RESUMEN:

Los tratamientos convencionales de aguas residuales hacen uso de una costosa y sofisticada infraestructura así como de elevadas cantidades de energía. Hoy en día se empieza a visualizar el potencial de la materia orgánica del agua, viendo este como un recurso en lugar de un problema. Así los reactores bioelectroquímicos (BES) surgen como una tecnología que podría ser la nueva generación de sistemas de tratamiento de aguas sostenibles, ya que estos equipos son capaces de transformar la materia orgánica del agua en hidrógeno o electricidad, aumentando así la eficiencia energética de los tratamientos de aguas residuales.

El principal objetivo de esta tesis es evaluar la capacidad de varias configuraciones de reactores bioelectroquímicos para la eliminación de materia orgánica y la producción de hidrógeno durante el tratamiento de aguas residuales urbanas.

Mediante este trabajo se demuestra el escalado desde un reactor de electrólisis biocatalítica de 50 mL, pasando por 855 mL hasta varios litros. Inicialmente el reactor de 50 mL fue alimentado con agua residual sintética con distintas concentraciones y a distinta carga. Los resultados obtenidos mostraron que era necesario un sistema de reactores en serie para alcanzar una depuración adecuada con consumos energéticos aceptables. Por lo que posteriormente se diseñaron reactores en serie de 855 mL y 10 L que fueron operados con agua residual urbana. El reactor de 855 mL formado por tres compartimentos anódicos en serie alcanzó unas tasas de eliminación de DQO (demanda química de oxígeno) de $5.7 \text{ g-DQO L}_R^{-1} \text{ d}^{-1}$ y tasas de producción de hidrógeno de $1.0\text{-}2.6 \text{ L L}_R^{-1} \text{ d}^{-1}$ gracias a la optimización de la tensión aplicada y del tiempo de retención hidráulico. Así mismo, el reactor formado por dos unidades de 5 L cada una logró una tasa depurativa de $0.5 \text{ g L}_R^{-1} \text{ d}^{-1}$, una eficiencia de eliminación de DQO de 60-76% y un consumo energético de 0.9 Wh por g de DQO eliminado.

Posteriormente, se estudió el efecto de la carga orgánica, el tiempo de retención hidráulico, la tensión aplicada y la configuración de un reactor semi piloto tubular bimodular en el consumo energético y la capacidad depurativa durante el tratamiento de aguas residuales urbanas. El reactor estaba constituido por dos unidades tubulares idénticas conectadas en serie. Dicho reactor fue capaz de eliminar el 85% de la concentración de DQO del influente (agua residual urbana) con unos consumos energéticos de 0.3-1.1Wh g-DOQ⁻¹. Mediante estos ensayos se identificaron las cargas orgánicas límites para garantizar el uso de esta configuración en el tratamiento de agua residual urbana basándonos en el consumo energético y la eficiencia en la eliminación de DQO.

También se determinaron las cargas orgánicas mínimas que justifican la utilización de dos unidades operadas en serie para obtener un grado depurativo acorde con la legislación vigente. La producción de hidrógeno estuvo limitada por la baja concentración de materia orgánica en el influente, por el deficiente funcionamiento del cátodo y por último por la presencia de microorganismos no electrógenos que consumieron parte de la materia orgánica del agua favoreciendo la depuración del sistema pero no la generación de hidrógeno.

Finalmente, se calculó la huella de carbono de una planta de tratamiento de aguas urbanas con la tecnología EB integrada en el proceso así como una comparación de esta con una planta convencional con tratamiento de fangos activados. Las emisiones fueron menores en la planta con el electrolizador biocatalítico, a lo cual si añadimos las emisiones que se evitan en el caso de utilizar el hidrógeno generado para el propio abastecimiento de la planta o bien para su inyección en la red, los beneficios serían aún mucho mayores.

LIST OF SYMBOLS AND ABBREVIATIONS

Ac	Acetate
ARB	Anode Respiring Bacteria
ARU	Agua Residual Urbana
BE	Biocatalyzed Electrolysis
BES	Bioelectrochemical System
BOD	Biochemical Oxygen Demand [g L^{-1}]
BODr	Biochemical Oxygen Demand Efficiency [%]
CCE	Cathodic Conversion Efficiency [%]
CE	Coulombic Efficiency [%]
CEM	Cationic Exchange Membrane
COD	Chemical Oxygen Demand [g L^{-1}]
CODr	Chemical Oxygen Demand Removal Efficiency [%]
DBO	Biological Oxygen Demand [g L^{-1}]
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
DQO	Demanda Química de Oxígeno [g L^{-1}]
DS	Dry Solids [g L^{-1}]
dWW	Domestic Wastewater
e⁻	Electron
E_{an}	Anodic Potential [V]
E°_{an}	Standard Electrode Potential [V]
EB	Electrolizador Biocatalítico
E_{cat}	Cathodic Potential [V]

E°cat	Standard Electrode Potential for Hydrogen [V]
Econs	Energy/Power Consumption [Wh g ⁻¹ COD] – [Wh L ⁻¹ H ₂]
Ecou	Coulombic Efficiency [%]
EDAR	Estación Depuradora de Aguas Residuales
e_{H2}	Number of Mol of Electrons Exchanged per Mol of Hydrogen
Eprod	Energy Production [Wh g ⁻¹ COD] – [Wh L ⁻¹ H ₂]
Eq	Theoretical Equilibrium Voltage [V]
emf	Electromotive Force [V]
EU	European Union
F	Faraday constant (96500 C mol ⁻¹)
GDE	Gas Diffusion Electrode
GHG	Green House Gas
H⁺	Proton
HC	High Conductivity
HRT	Hydraulic Retention Time [h]
HS	High Strength
I	Current (A)
i	Current density (A m ⁻²)
IEM	Ion Exchange Membrane
L_a	Liter of Anodic Chamber
LC	Low Conductivity
LCA	Life Cycle Analysis
LH₂	Liter of Hydrogen
L_R	Liter of Reactor Chamber (Anodic Chamber)

LS	Low Strength
M	Molar weight [g mol^{-1}]
M_A	Module A
M_B	Module B
MEC	Microbial Electrolysis Cell
MFC	Microbial Fuel Cell
MS	Medium Strength
OLR	Organic Loading Rate [$\text{g L}_a^{-1} \text{d}^{-1}$ or $\text{g L}_R^{-1} \text{d}^{-1}$]
PCF	Product Carbon Footprint
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffer Solution
Q_{in}	Influent Flow Rate [L d^{-1}]
Q_{H₂}	Hydrogen Flow Rate [$\text{LH}_2 \text{L}_R^{-1} \text{d}^{-1}$]
Q_{CH₄}	Methane Flow Rate [$\text{LCH}_4 \text{L}_R^{-1} \text{d}^{-1}$]
R	Universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
red	Reduction
R_{int}	Internal Resistance
rRNA	Ribosomal Ribonucleic Acid
rWW	Raw Wastewater
sCOD	Soluble Chemical Oxygen Demand [g L^{-1}]
SEM	Scanning Electron Microscope
Su	Sucrose
sWW	Synthetic Wastewater
tCOD	Total Chemical Oxygen Demand [g L^{-1}]

T	Temperature [°C or K]
TKN	Total Kjeldahl Nitrogen [g L ⁻¹]
TM	Trace Metal Solution
TOC	Total Organic Carbon [g L ⁻¹]
TRH	Tiempo de Retención Hidráulica [h]
TSS	Total Suspended Solids [mg L ⁻¹]
U_{app}	Applied Voltage [V]
V_a	Anode Volume [L]
V_{app}	Applied Voltage [V]
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids [mg L ⁻¹]
WW	Wastewater
WWTP	Wastewater Treatment Plant
Y	Yield [%]

Chapter 1

Introduction

1.1. BACKGROUND

Increasing energy demands from a growing world population, the depleting reserves of fossil fuels and their environmental impacts, are leading to a search for novel energy technologies.

Most likely, a diverse portfolio of energy producing technologies will be needed to replace fossil fuels (Logan, 2008). These technologies may rely on renewable or non-renewable resources, the former being much more interesting because they do not depend on limited reserves. A portfolio of renewable energy technologies may include a variety of systems based on sunlight, wind, rain, tides, geothermal heat, and biomass.

Another challenge is the treatment of organic waste and biomass residue produced by society. Specifically, this problem becomes imperative with wastewater (WW) as fresh water reserves are limited. Wastewater treatment often demands high infrastructure and usually uses a great amount of energy and consequently produces a significantly amount of greenhouse emissions. Nowadays, there is growing consensus that organic components in the wastewater are a resource instead of an increasing problem (Sutton et al., 2011; Verstraete and Vlaeminck, 2011; McCarty et al., 2011; McCarty et al., 2011). Thus, wastewater may be an additional significant source of renewable energy.

In addition, in Spain the wastewater sector has increased 6% in the last 10 years (DBK, 2010). Wastewater treatment requires 1% ($2.672 \text{ GWh year}^{-1}$) of the national electricity consumption to treat 3.000 hm^3 of domestic wastewater per year. That means a required power of 305 MW and an average consumption of 0.67 kWh m^{-3} . Most of the wastewater treatment plants in Spain consume $50 \text{ kWh (h.e.year)}^{-1}$. Nevertheless, the energy consumed in the wastewater treatment plants could be greatly reduced by

17.5% if we optimize the systems and manage to use the energy in the wastewater (IEA, 2011).

If these trends continue the energy bill for the water sector will be vastly higher than the current 2672 GWh (IEA, 2011). Moreover, part of the infrastructure will require long term planning and capital investment which forces us to research and invest in new options for wastewater treatment.

Among the available technologies to treat wastewater, some consume energy as conventional treatments that remove the organic matter via aerobic processes which are energy expensive, typically 3% of the electrical energy usage of many developed countries (Curtis, 2010), while others technologies, such as anaerobic digestion, are able to produce renewable energy from available organic matter (Metcalf & Eddy Inc., 2003).

The latter are most promising, but often require a step of aerobic post-treatment (polishing) to satisfy wastewater treatment norms. Replacing the aerobic activated sludge process with an anaerobic process means the energy stored in the organic content of the wastewater is converted to methane (80% efficiency) which can be combusted to produce electricity (35% efficiency) (McCarty et al., 2011). Only around 30% of the total energy in the wastewater can be captured as electricity in anaerobic systems, although with heat exchange in the combustion process, or the use of non-combustion methods of conversion, this could be increased (McCarty et al., 2011). In order to reduce the energy consumption and increase the quality of organic matter removal the process needs to extract and convert energy to an useable form at an efficiency that justifies the costs, as well as attain the legal discharge standards of both chemical oxygen demand and nutrients.

However, low strength domestic wastewater treatment is problematic for anaerobic digestion technologies (Rittmann and McCarty, 2001), also most

of the time wastewater treatment plants work at ambient, often low temperatures, again problematic for anaerobic digestion (Lettinga et al., 1999). Therefore, one novel promising technology of producing energy from low-strength wastewaters is Microbial Electrolysis Cell (MEC).

MECs have the potential to fulfill the needs of the wastewater industry nevertheless improvement is required to reach feasible commercial application. Furthermore, there are just a few studies of MECs at a larger scale (120-1000 L) that revealed that even though it is a promising technology for domestic and industrial wastewater treatment, several difficulties still need to be overcome (Cusick et al., 2011; Heidrich et al., 2012). In fact, one of the major bottlenecks of MEC application is their low hydrogen production. Therefore, further investigation in hydrogen generation and collection, as well as improvement of the electron transfer process (Reguera et al., 2005; Torres et al., 2010), optimization of operational conditions (Jadhav and Ghangrekar, 2009) and configurations would be required to make this technology feasible and competitive with the conventional technology. Moreover, to avoid unintended consequences of the new technology, it is necessary to conduct analysis of potential carbon footprint of Bioelectrochemical Systems (BES).

1.2. BIOELECTROCHEMICAL SYSTEMS (BESs)

BESs are based on the discovery of electrochemically active microorganisms which are able to transfer electrons to a solid surface by mediatorless direct electron transfer (Chaudhuri and Lovley, 2003; Potter, 1911). This discovery provided the basis for multiple applications which are now defined as BES. In BESs, electrochemically active microorganisms grow on an electrode, which is subsequently called a bioanode and forms the basis of all BESs. The bioanode is coupled through an electrical circuit to a (bio) cathode where a reduction reaction takes place. Depending on the

reaction at the cathode the system gains energy in fuel cell, or energy input is required in an electrolysis cell.

Generally the anode and cathode are separated by a membrane but also membraneless designs are being developed. The advantage of BESs when using two compartments is that they not solely convert compounds but also separate oxidation and reduction products, which makes it possible to extract useful products out of wastes.

Recently, BESs with bio-anodes use electron donors derived from wastes (e.g. wastewaters) (Logan, 2005), ocean sediments (Reimers et al., 2001), processed energy crops (as cellulose) (Niessen et al., 2005; Ren et al., 2007; Rezaei et al., 2007), photosynthetic microorganisms (Chiao et al., 2006; Fu et al., 2010; Strik et al., 2008a) or in-situ photosynthesized plant rhizodeposits (De Schamphelaire and Verstraete, 2009; Strik et al., 2008b).

In the last years BES research has also focused on producing, apart from electricity in MFCs and hydrogen in MEC (Rozendal et al., 2006b), all kinds of value added products like CH_4 (Cheng et al., 2009), H_2O_2 (Fu et al., 2009), CO_2 reduction to form hydrocarbons (Gattrell et al., 2007), caustic production (Rabaey et al., 2010), nitrogen removal from waste waters (Clauwaert et al., 2007), sulphate removal (Coma et al., 2013), nickel removal (Qin et al., 2012), transformation of strongly oxidized functional groups in persistent chemicals (Mu et al., 2009), recovery of metals such as cobalt (Huang et al., 2011; Huang et al., 2013) and magnesium and desalination (Chen et al., 2013; Ping and He, 2013).

1.2.1. Principles of Microbial Fuel Cells (MFC) and Microbial Electrolysis Cells (MEC)

A standard MFC (Figure 1.1.A) consists of two electrodes separated from each other by means of a membrane forming two separate chambers: the

anodic and the cathodic chambers. In the anodic chamber microorganism degrades organic matter producing electrons that are released to the anode through a series of respiratory enzymes in the cell. At the cathode the electrons react with a terminal electron acceptor which typically is oxygen. The two electrodes are connected by a wire containing a load which allows electron transfer from the anode to the cathode. In principle, the membrane that separates anode from cathode is permeable to protons produced in the anode, so they can migrate to the cathode where they can combine with the electrons transferred via the wire and with oxygen, forming water (Logan, 2008). The maximum electromotive force attainable (emf) in a MFC is theoretically in the order of 1.1 V (Rozendal, 2007), however due to potential losses and irreversibilities, the emf usually falls below 0.6 V under operating conditions (Logan et al., 2006).

Hydrogen production by microbial electrolysis, a technique independently discovered by two research groups a few years ago (Liu et al., 2005; Rozendal et al., 2006b) provides a feasible device to produce hydrogen from biomass. This process occurs in a system known as a microbial electrolysis cell (MEC). Microbial electrochemical cells are bioreactors that have a design similar to a fuel cell, with an anode and a cathode connected through an electrical circuit (Logan et al., 2006). These reactors present an anaerobic anode chamber containing electrochemically active microbes growing on the surface of the anode, which oxidize organic matter and transfer electrons to an electrode as a part of its metabolism. Applying a voltage to the system reaction between these protons and electrons in an anaerobic cathode is forced, which leads to hydrogen formation (Rozendal et al., 2006b; Rozendal, 2007) (Fig. 1.1.B). If acetate is used as carbon source, the anode process of an MEC is the same as in a microbial fuel cell (MFC) and the cathode process is the same as that of a water electrolyzer as shown in Table 1.1. and Fig.1.1.A and B.

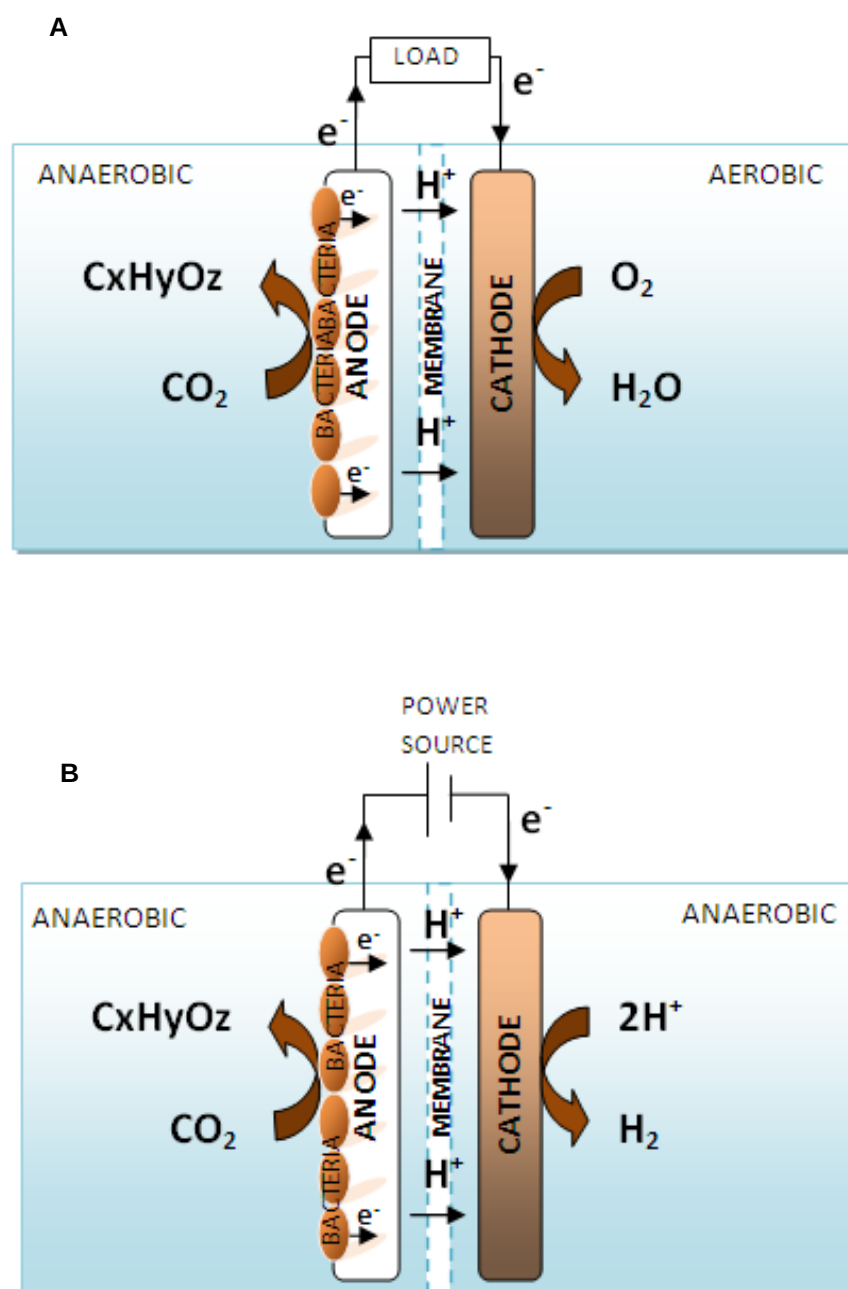


Figure 1.1. Microbial Fuel Cell (A); Microbial Electrolysis Cell (B).

Table 1.1. Electrode reactions and potentials of MEC, MFC, and water electrolysis systems under standard conditions (pH=7).

Process	Anode reaction/potential	Cathode reaction/potential	Potential difference
MEC	$\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} = 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^-$ (E=-0.279V)	$2\text{H}^+ + 2\text{e}^- = \text{H}_2$ (E=-0.414V)	-0.135 V
MFC	$\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} = 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^-$ (E=-0.279V)	$\text{O}_2 + 4\text{H}^+ + 2\text{e}^- = 2\text{H}_2\text{O}$ (E=0.806V)	1.085 V
Water electrolyzer	$2\text{H}_2\text{O} = \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ (E=0.82V)	$2\text{H}^+ + 2\text{e}^- = \text{H}_2$ (E=-0.414V)	-1.22 V

Note: Acetate is used as model electron donor for MEC and MFC.

For an MEC, the electrode potential can be written as follows for anode reaction:

$$E_{an} = E_{an}^{\circ} - \frac{RT}{8F} \ln \frac{[\text{CH}_3\text{COO}^-]}{[\text{HCO}_3^-]^2[\text{H}^+]^9}$$

where E_{an}° (0.187 V) is the standard electrode potential for acetate oxidation, R (8.314 J/K/mol) is the universal gas constant, T (K) is the absolute temperature, and F (96485 C/mol e^-) is Faraday's constant. And for the cathode reaction, the electrode potential can be written as follows:

$$E_{cat} = E_{cat}^{\circ} - \frac{RT}{2F} \ln \frac{p\text{H}_2}{[\text{H}^+]^2}$$

where E_{cat}° (0 V) is the standard electrode potential for hydrogen. For electrode reactions in MECs at pH 7, the anode potential is calculated as -0.279 V and the cathode potential is -0.414 V (Table 1.1). The equilibrium voltage of the overall reaction can be calculated as follows:

$$E_{eq} = E_{an} - E_{cat} = (-0.414) - (-0.279) = -0.14\text{V}$$

The negative equilibrium voltage indicates that hydrogen cannot be produced from acetate spontaneously and that an additional voltage (at least 0.14 V) has to be applied in order for the reaction to proceed. In practice, the applied voltage is normally larger than the theoretical equilibrium voltage (E_{eq}) due to the internal losses in the system.

Experiments have demonstrated that an applied voltage of 0.2 V or more is needed to obtain reasonable current density and thus useful hydrogen production rates (Cheng and Logan, 2007; Rozendal et al., 2007). This voltage, however, is much lower than the theoretical value of 1.2 V that required for water electrolysis. Table 1.1 compares the electrode reactions and potentials in an MEC, an MFC, and a water electrolyzer. MECs can produced hydrogen from a variety of organic materials while fermentative hydrogen production (dark fermentation) requires a fermentable (carbohydrate-rich) substrate, which limits the use of biomass containing other organic matter (e.g., proteins or organic acids) as substrate. In MECs, microorganisms are able to use a wide array of substrates, from pure compounds of sugars, carboxylic acids, alcohols, and proteins to complex mixtures such as biomass hydrolysate and domestic, animal, food-processing wastewaters.

Microorganisms which are able to transfer electrons from the substrate to the anode, generating a current in the system (Childers et al., 2002; Bond and Lovley, 2005; Pham et al., 2008a; Wrighton et al., 2008) and allowing the electricity or hydrogen production are defined as electrogens /exoelectrogens (Zuo et al., 2006; Logan et al., 2008). Many studies have been done to characterize exoelectrogens in MFCs (Zuo et al., 2006; Logan and Regan, 2006; Torres et al., 2007). While it is possible that the microbial activity and electron transfer might be affected by the applied external voltages (anode potential), and/or different solution chemistry (for example dissolved oxygen in MFCs and hydrogen in MECs), the current generation

capabilities and general electron transfer mechanisms of electrochemically active bacteria in MFCs are also applicable to MECs.

MFC studies have shown the presence of diverse bacterial communities enriched from various environments, such as domestic wastewater (Liu et al., 2004), ocean sediments (Reimers et al., 2001), and anaerobic sewage sludge (Kim et al., 2005). Isolated exoelectrogens appear to belong to diverse genetic groups, including four of the five Proteobacteria (α , β , γ and δ) (*Rhodospirillum rubrum*, *Shewanella*, *Pseudomonas*, *Aeromonas*, *Geobacter*, *Geopsychrobacter*, *Desulfuromonas*, *Desulfobulbus*) (Chaudhuri and Lovley, 2003; Holmes et al., 2004b; Holmes et al., 2004a; Rabaey et al., 2004; Kim et al., 1999; Xing et al., 2008; Zuo et al., 2008), Firmicutes (*Clostridium*) (Park et al., 2001), Acidobacteria (*Geothrix*) (Bond et al., 2002) and Actinobacteria (*Propionibacterium*) (Wang et al., 2008).

Previous research has defined three mechanisms involved in the release of electrons to the anode by electrogens:

(1) direct electron transfer using outer membrane cytochromes; *Geobacter* and *Shewanella* species, may have both the outer membrane cytochromes.

(2) biogenic soluble mediators that “shuttle” electrons from cells to anode. For example, *Pseudomonas aeruginosa* and *Geothrix fermentans* can only transfer electrons in the presence of self-produced mediators (Park et al., 2001; Bond et al., 2002; Bond and Lovley, 2005; Holmes et al., 2004a ; Holmes et al., 2004b; Rabaey et al., 2004; Pham et al., 2008a). This mechanism (Figure 1.2.) of electron transfer (i.e., aided by redox mediators) proposes the presence of a soluble electron shuttle: a compound that carries electrons from the bacteria by diffusive transport to the surface of the electrode (or metal oxide), and is able to react with it, discharging its electrons (Torres et al., 2010). Then the oxidized redox mediator diffuses back to the microorganism where it is re-reduced again being

subjected to a new oxidation-reduction cycle. The electron shuttles involved in this process can be endogenous chemical mediators (i.e., self-produced) such as quinones and phenazine (Rabaey et al., 2004; Rabaey et al., 2005) and also they can be found naturally in the environment as in the case of humic acids (Straub et al., 2001; Hernandez and Newman, 2001).

The first two mechanisms have been studied on investigations of dissimilatory metal-reducing bacteria using solid minerals during the course of their respiratory electron-transfer process (Myers and Myers, 1992; Holmes et al., 2004a; Rabaey et al., 2005).

(3) The third mechanism involves electron transfer by conductive bacterial appendages (nanowires). It requires that bacteria synthesize appendages called nanowires capable of transferring electrical current based on the measurements of electrical conductance across the wire diameter and along their length (Reguera et al., 2005; Gorby et al., 2006; El-Naggar et al., 2008).

For some electrogens, one mechanism may be dominant in the electron transfer. A previous study showed that *Shewanella oneidensis* also produces flavins that can function as an electron mediator (Von Canstein et al., 2008), indicating the possibility of the involvement of all three mechanisms in electron transfer by a single bacterial species. Mixed cultures, enriched from domestic wastewater and anaerobic sewage sludge have been used in most MEC studies (Liu et al., 2005; Rozendal and Buisman, 2005; Rozendal et al., 2006b; Rozendal, 2007; Cheng and Logan, 2007; Ditzig et al., 2007; Logan et al., 2007; Tartakovsky et al., 2009). While some isolates from mixed cultures demonstrate electrochemically active properties, most of them exhibit lower current densities when grown as pure cultures compared to the mixed cultures (Logan, 2009). The interactions between species in mixed cultures that enable these greater current

densities, however, remain unclear. While it has been demonstrated that metabolites generated by one species can be utilized by another species as a shuttle to transfer electrons (Pham et al., 2008b) further research is required to elucidate metabolic patterns of niche partitioning, interspecies communication, and food web dynamics that enable a diverse anode microbial community to optimize current output.

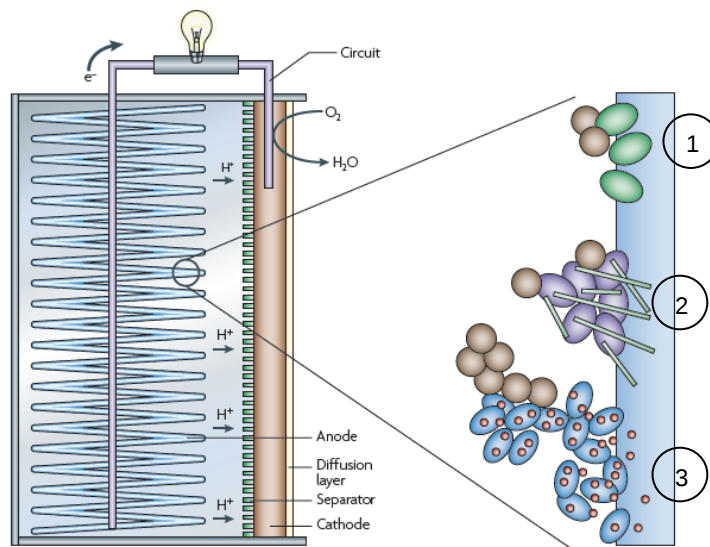


Figure.1.2. Mechanisms for extracellular electron transport in an MFC anode. 1) direct contact (top in green); (2) by nanowires (middle in purple); and (3) self-produced mediators (bottom in blue). Figure modified from the (Logan, 2009).

1.2.2. Key parameters describing the performance

The variation of MECs design and operation conditions adopted by researchers requires a necessity to uniform several parameters which will allow comparing the results among different systems. Following it is summarized the main parameters that have been used to evaluate the performance of MECs.

Treatment efficiency (COD removal efficiency, %). BESs have been proposed as technology for wastewater treatment, and as so it is important to compute its ability to remove organic matter from a waste effluent. Even though its performance can be evaluated in terms of biochemical oxygen demand (BOD) removal, total organic carbon (TOC) removal and several other parameters (Metcalf & Eddy Inc., 2003), in this work chemical oxygen demand removal (COD_r) has been selected as the preferred parameter to compute the BES treatment efficiency:

$$COD_r = \frac{(COD_{in} - COD_{out})}{COD_{in}}$$

where COD_{in} and COD_{out} are the COD concentration of MEC influent and effluent respectively.

Coulombic efficiency (CE, %) has been defined as the ratio of total electronic charges transferred to the anode from the substrate, to maximum possible charges if all substrate removal produced current (Logan et al., 2006). It is calculated as:

$$CE = \frac{\int_0^{86400} I dt}{\frac{(COD_{in} - COD_{out})}{M \cdot Q_{in} \cdot e \cdot F}} \cdot 100$$

where I is the circulating electrical current (A); M is the weight of 1 mol of COD (32 g mol^{-1}); Q_{in} is the influent flow rate (L d^{-1}); e is the number of mol of electrons exchanged per mol of COD equivalent consumed (8 mol mol^{-1}); F is the Faraday constant ($96.485 \text{ C mol}^{-1}$) and COD_{in} and COD_{out} are the COD concentration of MEC influent and effluent respectively.

Cathode conversion efficiency (CCE, %), also known as cathodic efficiency. It represents the ratio of hydrogen recovery to the maximum theoretical

production if all the electronic charges that arrive to the cathode were converted to hydrogen:

$$CCE = \frac{\frac{p \cdot Q_{H_2} \cdot V_a}{RT} \cdot e_{H_2} \cdot F}{\int_0^{86400} Idt} \cdot 100$$

where p is the pressure in the cathodic chamber (p=1 atm); Q_{H_2} is the H_2 flow rate ($L_{H_2} L_a^{-1} d^{-1}$); V_a is the anode volume (L); R is the ideal gas constant ($R=0.08205 L atm K^{-1} mol^{-1}$); T is the temperature (K); e_{H_2} is the number of mol of electrons exchanged per mol of hydrogen ($2 mol mol^{-1}$).

Hydrogen yield (Y_{H_2} , $mol mol^{-1}$), is the number of mol of hydrogen harvested from 1 mol of COD consumed:

$$Y_{H_2} = \frac{(p \cdot Q_{H_2} \cdot V_a) / (RT)}{\frac{(COD_{in} - COD_{out})}{M \cdot Q_{in}}}$$

where p is the pressure in the cathodic chamber (p=1 atm); Q_{H_2} is the hydrogen flow rate ($L_{H_2} L_a^{-1} d^{-1}$); V_a is the anode volume (L); R is the ideal gas constant ($R=0.08205 L atm K^{-1} mol^{-1}$); T is the temperature (K); Q_{in} is the influent flow rate ($L d^{-1}$); M is the weight of 1 mol of COD ($32 g mol^{-1}$) and COD_{in} and COD_{out} are the COD concentration of MEC influent and effluent respectively.

Specific energy/power consumption (E_{cons} , $Wh g^{-1}-COD$) is the electrical energy supplied to the MEC relative to the amount of organic matter consumed, and is calculated as:

$$E_{cons} = \frac{\int_0^{86400} V_{app} \cdot Idt}{\frac{(COD_{in} - COD_{out})}{M \cdot Q_{in}}}$$

where V_{app} is the applied voltage (V); I is the circulating electrical current (A); Q_{in} is the influent flow rate ($L d^{-1}$); M is the weight of 1 mol of COD ($32 g mol^{-1}$) and COD_{in} and COD_{out} are the COD concentration of MEC influent and effluent respectively.

Specific energy/power consumption (E_{cons, H_2} , $Wh L^{-1}-H_2$) is the electrical energy supplied to the MEC relative to the amount of hydrogen produced, and is calculated as:

$$E_{cons} = \frac{\int_0^{86400} V_{app} \cdot I dt}{Q_{H_2}}$$

where V_{app} is the applied voltage (V); I is the circulating electrical current (A); Q_{H_2} is the hydrogen flow rate ($L_{H_2} La^{-1} d^{-1}$).

Specific energy production (E_{prod} , $Wh g^{-1}-COD$) is the energy produced by the MEC in terms of hydrogen and methane relative to the amount of organic matter consumed, and is calculated as:

$$E_{prod} = \frac{3 \cdot 10^3 Wh Nm^3_{H_2} (Q_{H_2} + Q_{CH_4})}{\frac{(COD_{in} - COD_{out})}{M \cdot Q_{in}}}$$

where Q_{H_2} is the hydrogen flow rate ($L_{H_2} La^{-1} d^{-1}$); Q_{in} is the influent flow rate ($L d^{-1}$); Q_{CH_4} is the methane flow rate ($L_{CH_4} La^{-1} d^{-1}$); M is the weight of 1 mol of COD ($32 g mol^{-1}$) and COD_{in} and COD_{out} are the COD concentration of MEC influent and effluent respectively.

1.2.3. Factors affecting the performance

There are several factors that significantly affect the BESs performances. These factors can be classified into three catalogues: reactor configuration and materials, operational conditions and biological factors.

1.2.3.1. Reactor configuration and materials

The design of reactor and the materials used in its construction and operation play an important role in BESs performance and are especially important to large scale application. The main factors are reactor type, presence, type and size of separator, surface area of electrode, volume and electrode materials and catalysts.

Reactor design affects the performance, operation and construction cost of BESs. Numerous reactor configurations have been designed and tested over the last decade. Most studies use two-chamber systems for basic investigation, as this kind of reactor have high internal resistance due to large electrode spacing. Considering MFCs the two chambers design will add the construction cost which is not suitable for field application (Clauwaert et al., 2008). On the other hand, MEC with single-chambers has been demonstrated to have a negative effect on MECs performance (Gil-Carrera et al., 2011). In this background, single-chambers air cathode MFC seems more attractive for practical application and two chamber design for MECs.

Furthermore, BESs designs have been studied focusing on planar and tubular designs (Logan et al., 2006). There is not a clear conclusion on which design is more efficient, therefore, intense research is now focusing on the enhancement of MECs through better reactor design and configuration (Logan and Regan, 2006; Shimoyama et al., 2008; Logan, 2010).

Separator utilization, especially use of various membranes, can affect the internal resistance and construction cost of MECs, but it is helpful for minimizing the coulombic losses (Liu et al., 2004). A cationic exchange membrane (CEM) has slow proton transfer capacity and could result in a rapid accumulation of acidity in the anode, which can decrease the activity

of exoelectrogens (Harnisch et al., 2009). Moreover, the presence of a membrane has a significant effect on BESs performance due to its influence on pH (Rozendal et al., 2006a), therefore introducing anode effluent to the cathode has been proposed to alleviate the imbalance of pH (Freguia et al., 2008).

Omitting membrane from BES can also be an effective way to balance pH in the anode and cathode (Liu et al., 2004). However, the absence of a polymeric membrane between the anode and the cathode of the MEC, makes possible the conversion of the cathodic hydrogen to methane by *Methanobacteriales* in anode and cathode biofilms (Lee et al., 2009), as well as hydrogen losses in MECs due to the hydrogen recirculation, being a serious drawback affecting MEC performance since it increases artificially the energy usage, which limits significantly the overall performance of hydrogen production.

Electrode properties are one of the main factors that affect the BESs performance. The anode material can influence in the development of an electrogenic biofilm on its surface, as well as in the electron transfer in BESs. Different materials also contribute differently in internal resistance. It has been reported that use of electrode materials with high friendly microbial-accessible surface (such as carbon fiber or paper) improved current generation by 40% (Liu et al., 2004). Electrode and anode chamber design (e.g., ratio of electrode surface area and volume) can significantly affect the protons and substrate transportation (Borole et al., 2011). Using nanotechnologies to modify the surface of electrode may help to maximize the surface area and enhance the activity of electrode (Call et al., 2009; Selembo et al., 2009), the development of cathode materials (Rismani-Yazdi et al., 2008; Ter Heijne et al., 2008), or application of effective electrode catalysts that would help to decrease the energy consumption during wastewater treatment while allowing enough electron flux (electrical

current) between the anode and the cathode therefore improving organics removal in the anode and product formation in the cathode (Manuel et al., 2010; Hrapovic et al., 2010).

1.2.3.2. Operational parameters

Beside reactor design, the BESs performances are influenced by the operational parameters applied, such as pH, temperature, conductivity/ionic strength, external resistance, substrate composition, concentration and organic loading rate.

pH is one of the most important environmental factors impacting bacterial cell growth and physiology. The effect of pH on power generation has been well addressed. The pH gradients formed in MFCs by using CEM can disturb the operation of MFCs and decrease their performance and durability (Biffinger et al., 2008). A neutral pH in anode while maintaining high acidity in cathode is preferable for electricity production (He et al., 2008; Raghavulu et al., 2009; Martin et al., 2010; Borole et al., 2011). Moreover, in regular domestic wastewater, the concentration of cations other than protons (e.g., Na^+ , K^+ , NH_4^+) are typically 10^5 times higher than the concentration of protons (Rozendal et al., 2007), in this situation these species rather than protons are responsible for the transport of positive charges (Zhao et al., 2006). In bi-compartmental systems, where cation exchange membrane is interposed between both the anodic and the cathodic chamber, this transport creates a pH gradient between the anodic and cathodic chambers that can affect negatively the cell performance (Rozendal et al., 2007).

Temperature has a high impact on bacteria development. It has been observed that increasing temperature from 30 to 40 °C increased current generation by 80% (Liu et al., 2011). Min et al., (2008) found that a lag phase of 30 hours occurred at 30 °C which was half that at 22 °C. The

maximum power density was 70 mW m^{-2} at 30°C while 43 mW m^{-2} was produced at 22°C . However, this is not clear since other studies have found better performance at lower temperatures such as $8\text{--}22^\circ\text{C}$ which shown that current generation in a MFC was much higher than that at $20\text{--}35^\circ\text{C}$ (Jadhav and Ghangrekar, 2009). More recently, when studying electrochemically active biofilm activity, it was found that when temperature is in the range between 30 and 45°C , the catalytic currents increase following the Arrhenius law and that at 53°C and above negligible catalytic current was observed (Liu et al., 2011). Optimum working temperature was 45°C .

Conductivity/ionic strength. One of the main limitations in BES performance is the solution resistance (Logan, 2008; Logan et al., 2008; Rozendal et al., 2008). Min et al., (2008) found that power density was 4 times higher with phosphate buffer addition (conductivity of 11.8 mS cm^{-1}) than the value without phosphate additions (2.89 mS cm^{-1}). It becomes particularly important when BES are operated on domestic and many industrial wastewaters, which typically exhibit low conductivities (in the order of only 1 mS cm^{-1}) (Rozendal et al., 2008), leading to high ohmic losses. A practical solution could be adding salts to the electrolyte but this might not be economically nor environmentally feasible at industrial scale. Other solutions have been proposed, as more feasible and without negative effects, such as reducing electrode spacing, since conductivity is inversely proportional to the distance between electrodes (Logan et al., 2006; Ghangrekar and Shinde, 2007).

External resistance is an important electrical factor for power generation, which controls the ratio between the electric current and the working voltage. A low external resistance leads to a low working voltage and high current, which results in a high substrate conversion rate; the opposite is true in the case of a high external resistance. The maximum power could be

obtained when the external resistance is equal to the internal resistance of fuel cells. Thus, by changing the external resistance and recording the voltage and current produced, the internal resistance can be estimated (Logan et al., 2006). It has been reported that the external resistance can affect the microbial composition of anode biofilm (Lyon et al., 2010). Using an external resistance equal to or lower than the internal resistance has been proposed to promote biofilm growth and maximize the power generation of MFCs (Aelterman et al., 2008).

Applied voltage. According to previous research (Liu et al., 2005; Rozendal et al., 2006b; Rozendal, 2007; Ditzig et al., 2007) the applied voltage needed to produce hydrogen in MEC could be as low as 0.11-0.23 V. However, most studies have shown that applied voltages below 0.4 V hardly produce hydrogen and remove organic matter (Tartakovsky et al., 2009). In addition, tests performed in an acetate-fed single-chamber MEC revealed that applied voltages between 0.4-1.2 V, acetate removal and hydrogen production rates were proportional to V_{app} (Tartakovsky et al., 2009). Tests performed with other substrates showed a similar dependence between applied voltage and hydrogen production and substrate removal rates (Lu et al., 2009; Escapa et al., 2009). Applied voltages above 1.2 V did not improve significantly hydrogen production nor the substrate removal rate. It has been suggested that applied voltages above 1.2 V, electron transfer becomes limited by the metabolic activity of electrogenic microbes (Tartakovsky et al., 2008).

Substrate type and **loading rate** are important factors determining the character and amount of carbon source fed into BES. Previous research have found that power generation and microbial communities varies depending on the composition of the influent (fermentative and non-fermentative) fed to the reactors (Kim et al., 2007; Lee et al., 2008; Parameswaran et al., 2010; Kim et al., 2010; Zhang et al., 2011). In

addition, an increase in the amount of COD fed to the reactor, either through an increase in the organic loading rate or a change in the substrate composition, might lead to an increase, saturation or decrease of the power generation, hydrogen production and COD removal depending on the reactor conditions and substrate composition (Rabaey et al., 2003; Lee et al., 2008; Jadhav and Ghangrekar, 2009; Behera and Ghangrekar, 2009; Juang et al., 2011; Escapa et al., 2012b). These parameters are crucial and should be optimized to achieve an efficient BESs performance, proceed to the scale-up of these devices and obtain a feasible and competitive technology.

1.3. BIOELECTROCHEMICAL SYSTEMS FOR WASTEWATER (WW) TREATMENT

BESs can be used for wastewater treatment, since they are able to oxidize the organic matter while achieving hydrogen production in a MEC or electricity production in a MFC. This possibility was studied by Habermann and Pommer who first proposed a MFC as wastewater treatment system (Habermann and Pommer, 1991). This technology could replace the traditional high energy-demanding bioreactors, such as activated sludge systems, leading to a low energy-consuming system which can recover energy from wastewater ($3.8 \text{ kWh kg-COD}^{-1}$) without aeration cost ($1 \text{ kWh kg-COD}^{-1}$ in conventional aeration). Another advantage is the lower production of sludge in BESs, which is $0.02\text{-}0.22 \text{ g biomass-COD g-COD}^{-1}$ in a MFC compared with $0.53 \text{ g biomass-COD g-COD}^{-1}$ for conventional aerobic treatment (Clauwaert et al., 2008). Furthermore, in MECs the hydrogen produced in the cathode chamber has high purity levels which makes feasible its use.

Considering the possibility of introducing BESs in wastewater treatment plants, it is necessary to address the operation of conventional wastewater treatment plant and the type of wastewater. It is important to point the

differences in the WWTP regarding the composition of the wastewater to be treated.

In our case we will focus on a basic domestic wastewater treatment plant which includes the typical elements and steps in a water treatment process (preliminary, primary, secondary and tertiary treatment).

The preliminary treatment's purpose is to protect the operation of the wastewater treatment plant. This is achieved by removing from the wastewater any constituents which can clog or damage pumps, or interfere with subsequent treatment processes. The water stream is conducted to the primary treatment, which involves the physical separation of suspended solids from the wastewater flow using primary clarifiers. Within the primary clarifiers, suspended solids are allowed to settle. Large amounts, about 60%, of total suspended solids (TSS) are removed with the gravity separation that takes place. The biological oxygen demand (BOD) is also reduced by about 30% in this process. Then the waste stream is prepared for the secondary treatment.

The secondary treatment consists of removing or reducing contaminants or growths that are left in the wastewater from the primary treatment process. Usually biological treatment is used to treat wastewater in this step because it is the most effective type of treatment on bacteria, or contaminant, growth. This treatment can remove up to 90 percent of the organic matter in wastewater by using biological treatment processes. The two most common conventional methods, used to achieve secondary treatment, are attached growth processes and suspended growth processes.

Tertiary treatment further treats effluent to remove nitrogen, phosphorus, fine suspended particles and microbes, and to kill or disable disease-causing organisms and viruses. It is possible to treat effluent in this phase,

resulting in a non-potable reclaimed water source, which can be reused in a variety of ways.

Last step is the residual management, where solids removed by other process are collected, stabilized and subsequently disposed.

The water that usually reaches the plant might be polluted by many materials and substances, the most common contaminants found in domestic wastewater are organic materials (as measured by the biological demand for oxygen or the chemical demand for oxygen), nitrogen, which includes biological nitrogen, nitrates, nitrites and ammonium, phosphorus, suspended solids, pathogenic organisms (as estimated by coliforms) and traces of persistent organics (such as chlorinated pesticides).

Integration of BES in wastewater treatment plants has been previously investigated (Rosenbaum et al., 2010). It is well-known that the anodic chamber of a bio-electrochemical system usually contains undefined mixed cultures (including electrogenic microorganisms) that can oxidize a wide variety of organic matter with the anode as an electron acceptor (Rabaey et al., 2003; Liu et al., 2004; Liu et al., 2005; He et al., 2005; Heilmann and Logan, 2006), therefore replacing the bioreactor in the secondary treatment might be the simplest design.

However, a COD removal efficiency of 40-80% was achieved from BESs fed with domestic wastewater in most of the studies at lab-scale (Liu et al., 2004; Min and Logan, 2004; Rodrigo et al., 2007; Ditzig et al., 2007; Escapa et al., 2012a; Heidrich et al., 2012). These values of COD removal efficiency may not accomplish the legal limits establish by local regulations in terms of COD and BOD removal and BOD and COD concentration of the discharged effluent. Furthermore, nitrogen removal in a BES is usually low and it is mostly attributable to nitrogen assimilation into bacterial biomass, which accounts for only a small percent of the total removal usually needed

(Freguia et al., 2007; Logan, 2008), consequently an aerated step in the MFC (Yu et al., 2011) or an additional MFC for denitrification (Zhang et al., 2013) may be required to achieve acceptable COD and nitrogen removal efficiencies, however, this step might increase the net energy consumption. Therefore, it seems more viable that a BES may require a polishing step rather than operating alone in a wastewater treatment plant. This could be achieved by BESs operated in series or with adding another aerobic/anoxic step to reach the legal requirements.

1.4. SCALING-UP BIOELECTROCHEMICAL SYSTEMS FOR (WW) WASTEWATER TREATMENT

To further demonstrate the technical viability of BES technology, it is necessary to examine the long-term performance and stability of larger size MFCs and MECs with real wastewater.

There have been several studies reporting the long-term operation of MFCs and MECs. An upflow tubular MFC with an anode working volume of 750 mL was used to treat animal carcass wastewater and continuously produced electricity during more than a 280 days operation (Li et al., 2013). A 10-L MFC system consisting of 40 individual tubular MFCs was operated for more than 180 days on brewery wastewater with a maximum power density of 4.1 W m^{-3} . An increase in COD removal efficiency and a decrease in electricity production within time (Zhuang et al., 2012) was observed. Moreover, a 16-L MFC was operated in a municipal wastewater treatment plant and achieved good COD removal efficiency but low electricity production (Jiang et al., 2011). Two 4-L tubular MFCs were installed in a municipal wastewater treatment facility and operated for more than 400 days on primary effluents. The performance of the MFCs was largely affected by organic input and temperature and achieved a COD removal efficiency of 65-70% at a hydraulic retention time (HRT) of 11 h. The results demonstrated the technical viability of MFC technology outside the

laboratory and its potential advantages in low energy consumption, low sludge production and energy recovery from wastes (Zhang et al., 2013).

Meanwhile, there have only been a few studies reporting pilot-scale BESs operation. The first MFC pilot system consisting of 12 MFC modules with a total liquid volume of 1000 L was tested by Queensland University (Logan, 2010). The MFC was fed with brewery wastewater; however, little information is available about its performance. It is only known that low COD removal, the power density output was 8 W m^{-3} , and biofouling of the cathodes, far below the desired level for practical applications (Zhou et al., 2012).

MECs have been advanced to a pilot scale in a few separated studies treating low-strength domestic or high strength winery wastewaters. Cusick et al., (2011) showed the results of their 1000 L pilot-scale continuous flow-MEC fed with winery wastewater at the Napa Wine Company located in Oakville, California, USA. Their reactor was operated with 144 electrode pairs in 24 modules with an externally applied voltage of 0.9 V. Acetate enrichment was needed as well as an elevated wastewater temperature of $31 \pm 1^\circ\text{C}$ to achieve soluble COD removal rate of $62 \pm 20\%$. The maximum current generation was measured at 7.4 A m^{-3} after 100 days when the test ended. The total gas production rate of $0.19 \pm 0.04 \text{ L L}^{-1} \text{ d}^{-1}$ was quite low compared with laboratory studies. It was found that the majority of the gas produced ($86 \pm 6\%$) was methane.

Recently, Heidrich (Heidrich et al., 2012) discussed the results of a wastewater fed microbial electrolysis cell. A 120-L MEC was operated on a site in Northern England, using raw domestic wastewater to produce hydrogen for a period of over 3 months. The volumetric loading rate was $0.14 \text{ kg-COD m}^{-3} \text{ d}^{-1}$, just below the typical loading rates for activated sludge of $0.2\text{-}2 \text{ kg-COD m}^{-3} \text{ d}^{-1}$, at an energetic cost of $2.3 \text{ kJ g-COD}^{-1}$ which is lower than the energy required in activated sludge $0.7\text{-}2 \text{ kWh kg-COD}^{-1}$

(Pant et al., 2011). However, hydrogen production was $0.015 \text{ L}_{\text{H}_2} \text{ L}^{-1} \text{ d}^{-1}$ and recovered around 70% of the electrical energy input with a coulombic efficiency of 55-60%. These results confirm that MEC technology is capable of achieving a good COD removal efficiency and energy capture using low strength domestic wastewaters even though further research is need to deal with the hydrogen production.

1.5. CARBON FOOTPRINT OF WASTEWATER TREATMENT PLANT WITH A MEC INTEGRATED

Wastewater treatment contributes to greenhouse gases through production of CH_4 and/or CO_2 from treatment processes and from CO_2 produced from the energy required for the system. CH_4 produced from sewage treatment was found to constitute about 5% of the global methane sources (El-Fadel and Massoud., 2001). Another process that contributes to the increase of the green gas emissions (GHG) is the treatment of organic waste and biomass residue produced by society. A high amount of this waste is in the water and to eliminate it, the WW treatment requires high amount of energy as well as produces greenhouse emissions.

Bioelectrochemical systems have been found to be a novel promising technology of producing energy from low-strength wastewaters and a great potential to contribute to greenhouse gas emissions reduction in wastewater treatment processes as it has previously been explained.

Carbon footprint is used to evaluate the environmental performance of goods, processes and services in terms of its contribution to the greenhouse emissions.

With every new technology is important that its carbon footprint is very clearly estimated before its application on a large scale. It is significantly important for bioenergy systems since these technologies use renewable

biomass resources to produce a large range of products such as electricity, chemicals, heat, fuels...etc. (Clarens et al.,2010) analyzed the environmental impacts of algal farms and concluded that they require six times as much energy as growing land plants and emit more GHGs while it was previously believed that algae could be important source of biofuels. This example shows the importance of conducting an accurate estimation of the carbon footprint of the new technologies before commercializing. Carbon footprint is thus necessary to avoid unintended consequences of a new technology or mitigation strategy.

To further analyze the environmental benefits of MEC for wastewater treatment, it is necessary to conduct a complete analyses of carbon footprint of MEC technology integrated in a WWTP, which should include an examination of the construction and operation of a MEC integrated in a WWTP and a comparison with a conventional WWTP.

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Chapter 2

Scope of the thesis

2.1. OBJECTIVES

The main objective of this Thesis is to study the ability of Microbial Electrolysis Cells (MEC) to perform as a wastewater treatment for organic matter removal and hydrogen production at low energy consumption. To achieve this main objective several research works will be conducted:

- i) investigating MEC scale-up from a 50 mL to a 10 L cell when treating synthetic wastewater and domestic wastewater at several organic loads.
- ii) studying different configurations from single MEC up to three MECs in series and designs from flat MEC to tubular MEC.
- iii) evaluating the efficiency of MEC operated in order to improve the COD removal efficiency and energy efficiency.
- iv) studying the feasibility of a tubular modular semi-pilot microbial electrolysis cell for domestic wastewater treatment and hydrogen production.
- v) evaluating the influence of the applied voltage and the hydraulic retention time on the wastewater treatment and energy consumption in a tubular microbial electrolysis cell.
- vi) estimating carbon footprint from domestic wastewater treatment in a WWTP with an MEC and a conventional WWTP with activated sludge treatment.

2.2. THESIS OUTLINE

Wastewater treatment requires roughly 305 MW (0.67 kWh m^{-3}) which represents nearly 1% of the yearly electrical energy consumption in Spain, which poses a problem for our country in the future.

This energy consumption in domestic wastewater treatment plants can be reduced up to 17.5 % through online and dynamic control of aeration systems. However, this reduction could be larger if the energy content of the organic matter dissolved in the wastewater was exploited. Microbial electrolysis cells represent a new alternative to conventional wastewater treatments, since it can convert directly the energy content of the organic matter into valuable energy products such as hydrogen. However, MEC is a new technology which still needs further investigation to become viable and technical and economical competitive with traditional wastewater treatments.

The scope of this PhD thesis is to investigate the potential and further scale-up of the MEC (Microbial Electrolysis Cell) for organic matter removal, hydrogen production and low energy consumption while treating domestic wastewater.

In **Chapter 1**, a general overview is presented regarding bioelectrochemical systems and their role in the field of wastewater treatment.

In **Chapter 2**, the objectives of the thesis and the thesis outline are presented.

Methodology and materials are presented in **Chapter 3**.

In **Chapter 4**, the feasibility of a MEC scale-up is studied. An evaluation of a 50 mL MEC on several strength and composition synthetic wastewaters is presented along with a process scale-up of MECs in series in order to

achieve a good COD removal efficiency, hydrogen production and low energy consumption.

Before practical implementation of bioelectrochemical systems, it would be useful to test another configuration of the MEC to analyze which design is more competitive and in which conditions is more feasible. This issue is studied in **Chapter 5**, which evaluates a semi-pilot tubular MEC used for domestic wastewater treatment and hydrogen production with low energy consumption. The reactor consists of two tubular MEC in series. The influence of several OLRs is evaluated in tubular MECs and performance is evaluated in terms of COD removal efficiency, gas production and energy consumption. Overall, it identifies the OLR threshold that makes the use of MECs feasible for domestic wastewater treatment.

Chapter 6 presents the effect of the applied voltage and hydraulic retention time on a tubular MEC performance in terms of hydrogen production, COD removal efficiency and energy consumption in order to reduce the energy consumption and increase the hydrogen production. Moreover, the microorganisms present in the anode are identified in this chapter.

Chapter 7 presents the results of a carbon footprint estimation of a wastewater treatment plant with a MEC integrated and a traditional wastewater treatment plant and its comparison.

General conclusions are presented in **Chapter 8** and a summary in **Chapter 9**.

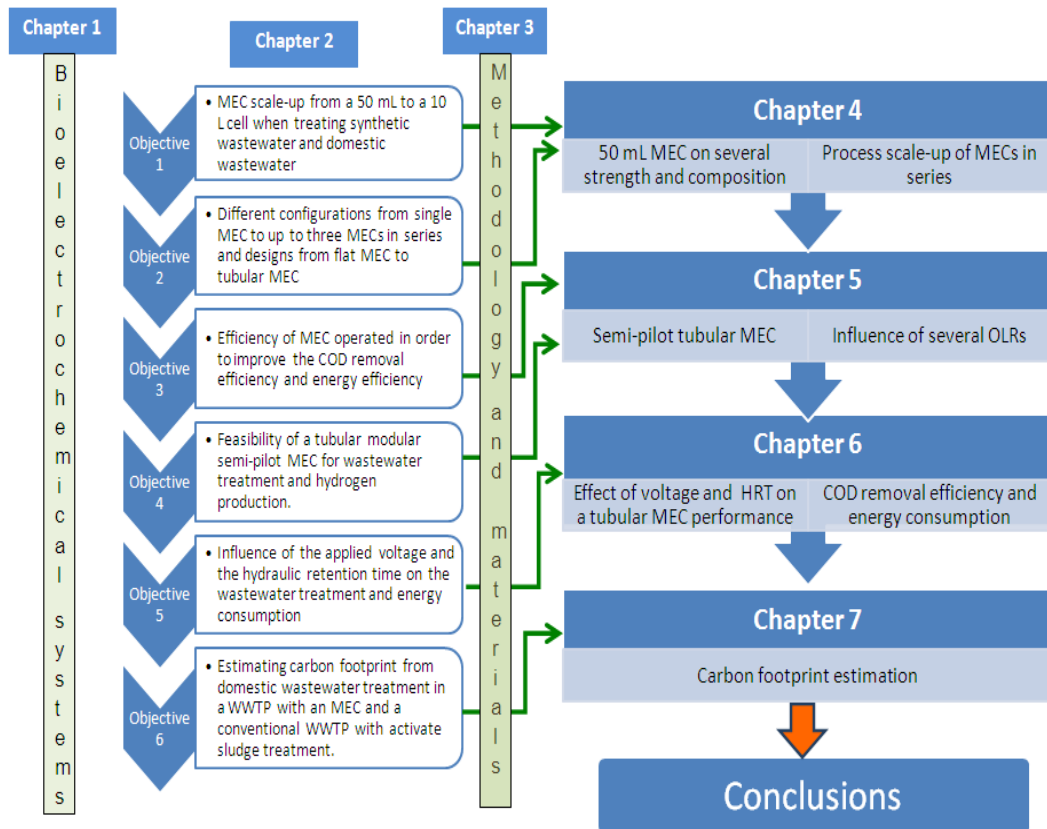


Figure 2.1. Organization of this PhD thesis.

Chapter 3

Materials and methodology

This section will present the design of MECs used in this thesis. Furthermore, details of the operating conditions, analytical methods, and inoculation and medium composition for each MEC experiment are shown.

3.1. ANALYSIS

3.1.1. Gas production and composition

Gas production in the MEC anodic and cathodic chambers were measured on-line using glass U tube bubble counters interfaced with a data acquisition system and also using a MGC-1 milli-gas counter (Ritter Co, Bochum, Germany). The gas composition from MECs 50 mL, 855 mL and 10 L was measured using a gas chromatograph (6890 Series, Agilent Technologies, Wilmington, DE) equipped with a 11 m × 3.2 mm 60/80 mesh Chromosorb 102 column (Supelco, Bellefonte, PA, USA) and a thermo conductivity detector. The carrier gas was argon. Cathodic and anodic off-gas composition from tubular MECs were analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A four-meter-long column packed with HayeSep Q 80/100 followed by a one-meter-long molecular sieve column were used to separate methane (CH₄), carbon dioxide (CO₂), nitrogen (N₂), hydrogen (H₂) and oxygen (O₂). The carrier gas was argon, and the columns were operated at 331kPa and 50°C.

3.1.2. VFA analysis

Acetate, propionate, and butyrate were analysed on an Agilent 6890 gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector and a 1m × 2mm 60/80 mesh Carbopack C column (Supelco, Bellefonte, PA, USA) coated with 0.3% Carbowax 20M and 0.1% H₃PO₄. The carrier gas was helium, which had a flow rate of 20 mL min⁻¹. The injector and the detector were maintained at 200 °C. The 0.5 µL samples were fortified at a ratio of 1:1 (v/v) using an internal standard of *iso*-butyric acid dissolved in 6% formic acid. Glucose was analysed on an HPLC

(Waters Chromatography, Milford, MA, USA) equipped with PDA detector model 2996. The total concentration of volatile fatty acids (VFAs) was calculated with respect to the COD equivalent of each component.

3.1.3. pH, conductivity and dissolved oxygen

The pH and conductivity of the effluent of MECs 50 mL, 855 mL and 10 L were measured using PHCN-37 pH meter (Omega Canada, Laval, QC, Canada) and XL-30 conductivity meter (Fisher Scientific, Ottawa, On, Canada), respectively. The pH and conductivity of the influent and effluent of the tubular MEC were measured using a pH meter (GLP 21, Crison Instruments, S.A. Spain) and a conductivity meter (LF 330 / SET WTW – Tetracon 325), respectively. Conductivity and pH measurements were obtained in replicate. Dissolved oxygen concentration was measured using a fiber optic oxygen sensor system (Ocean Optics Inc, Dunedin, FL, USA).

3.1.4. Ammonium

Ammonium was determined by an ion-selective electrode (781 pH/Ion Meter de Methrom).

3.1.5. Biological oxygen demand and chemical oxygen demand

The biological oxygen demand of the influent and effluent measurements was based on pressure measurement in a closed system during 5 days. It was measured using OxiTop kit with a software-controlled functions and infrared interface to communicate with the powerful OC 100 controller.

The chemical oxygen demand of the influent and effluent samples of MEC 50 mL, 855 mL and 10 L were measured using an spectrophotometer DR/3000 after centrifugation at 10000 RPM and digestion in the presence of dichromate and sulfuric acid at 150 °C for 2 h using a Hach reactor.

Then COD of the influent and effluent of the tubular MEC was calculated from a titrimetric determination (862 Compact Titrosampler, Metrohm) of the oxidant still remaining in the sample after digesting in the presence of dichromate at 150 °C for 2 h using a Hanna C9800 reactor.

All assays of COD and BOD₅ were performed in duplicate and mean values were presented.

3.1.6. Total suspended solids and volatile suspended solids

Total suspended solids and volatile suspended solids in the influent were determined by drying and incineration. Total suspended solids well-mixed samples were filtered through a weighed standard glass-fiber filter. The residue left on the filter is dried to a constant weight at 105 °C. The increase in weight of the filter represents the total suspended solids of the sample. After the total suspended solids value was determined volatile suspended solids test (VSS) was performed in order to determine the concentration of volatile suspended solids. Volatile suspended solids data is critical in determining the operational behavior and biological concentration throughout the system. The filter used for total suspended solids (TSS) testing was ignited at 550 °C for 30 minutes. The weight lost on ignition of the solids represents the volatile solids in the sample.

3.1.7. Protein quantification

Protein quantification was performed to determine the relative amount of microorganisms in the anodic biofilm. Carbon felt samples, 1 cm x 1 cm x 0.5 cm were taken from the top, middle and bottom of each anode, cut with scissors into pieces, and were put into sterile 2 mL tubes with 500 mg of 0.1 and 0.5 mm sterile glass beads (zirconia/silica beads, Biospec Products, Inc., Bartlesville, OK, USA). Tubes were then filled with sterile distilled water, vortexed to mix the beads and anode pieces and bead-beaten for 15

seconds twice using FastPrep® system (Bio 101 Savant, Bio/Can Scientific, Mississauga, ON, Canada) at a speed setting of 5.5. Subsequently, the samples were centrifuged and the supernatant collected and concentrated in a DNA concentrator (Savant DNA120 SpeedVac® concentrator, Thermo Fisher Scientific, Asheville, NC, USA). The samples were then analyzed following the Bio-Rad protein assay protocol (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada).

3.1.8. Microbial community analysis

Total chromosomal DNA was extracted from the anodic biofilms using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratory Inc., USA). For DNA extraction and purification, the 16S rDNA fragments were amplified by PCR (polymerase chain reaction). The region corresponding to positions 357 and 518 in the 16S rDNA of *Escherichia coli* was PCR-amplified using the forward primer EUB357f (5_-CCTACGGGAGGCAGCAG-3_) with a GC clamp (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCGCCCCGCCCC-3'). The 5'- end of forward primer is rich in GC sequences, which prevents the PCR products from completely melting during separation. PCR amplification was conducted in an automated thermal cycler (GeneAmp PCR System 9700, Applied Biosystem, USA) following the protocol of the manufacturer. PCR mixtures had final volumes of 20 µl. PCR products were electrophoresed on 1% (wt/vol) agarose gel in 1 × TAE for 90 min at 60V and then checked with GelRed™ Nucleic Acid Gel Stain (Biotium Inc, USA) to confirm the amplification.

Gene cloning and 16S rRNA gene sequencing for community analysis were conducted as previously described (Call et al., 2009). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and then ligated to vector PJET1.2 (Invitrogen) and transform the recombinant vector into competent cells *E. coli* following the manufacturer's protocol. Appropriate colonies were placed in a 96-well format on LB plates (Ampr 50 µg/ml).

Plasmid extractions were carried out on these colonies using the E-Z 96 Fast filter_ Plasmid Kit_ (www.omegabiotek.com). Once extracted, plasmids were sequenced with the BALF (5'-AGAGTTTGATCCTGGCTCAG-3') and BALR (5'-GGTTACCTTGTTACGACTT-3'). Primers using an ABI PRISM® 3100 (Applied Biosystems), 16S rRNA gene Sequences were analyzed using the BLASTN search tools.

DGGE (denaturing gradient gel electrophoresis) was carried out using the Dcode™ Universal Mutation Detection System (BioRad, California, USA). PCR products were electrophoresed in 1 × TAE buffer for 480 min at 70 V and 60°C on polyacrylamide gel (7.5%) containing a linear gradient ranging from 40% to 60% denaturant. After electrophoresis, the polyacrylamide gel was stained with GelRed™ Nucleic Acid Gel Stain for 30 min and then visualized on a UV transilluminator. Most of the bands were excised from the DGGE polyacrylamide gels for 16S rDNA sequencing. DNA fragments from the excised bands were PCR-amplified with the forward primer EUB357f without a GC clamp and the reverse primer UNIV518r. After PCR amplification, PCR products were purified using a Macherey-Nagel Nucleic Acid and Protein Purification kit (Clontech, USA). All strands of the purified PCR products were sequenced with the EUB357f primer using an ABI PRISM Big Terminator Cycle Sequencing Kit and an Amersham MegaBace DNA sequencer (GE Healthcare, USA) according to the manufacturer's instructions. Sequence data were analyzed with Chromas Lite 2.01 software (Ibis Bioscience, Carlsbad, CA, USA) and submitted to the non-redundant nucleotide database at GenBank using the BLASTN facility.

3.1.9. Microscopy analysis

Carbon felt samples, 1 cm x 1 cm x 0.5 cm were taken from the middle and bottom of each anode's layer, rinsed with phosphate buffer solution (PBS), and then the samples were fixed overnight in PBS plus 2.5% glutaraldehyde in 4°C. The fixed specimens were rinsed three times in sterile buffer

solution, and then dehydrated by a graded ethanol series (30, 50, 70, 80, 95, and 100%; 30 min each stage with very gentle periodic agitation). Electrode pieces were mounted on aluminum specimen mounts with contact adhesive, and were sputter coated in a Sputter Coater. The scanning electron microscope (SEM) (JEOL 6100, Japan) was operated.

3.2. MEC DESIGN AND OPERATION

Several microbial electrolysis cells were operated in this study. In Chapter 4 “Microbial electrolysis cell scale-up for combined wastewater treatment and hydrogen production” three different continuous flow MEC were operated (i) a 50 mL MEC equipped with a gas-diffusion cathode with Ni electrodeposited, a polyester cloth as separator and one layer of carbon felt (SGL Group, Kitchener, On, Canada), (ii) a 855 mL MEC which consisted of three anodic compartments connected in series and a shared gas collection (cathodic) compartment and (iii) a 10 L MEC consisted of two 5 L MECs in series which contained seven layers of the 5 mm thick carbon felt used as an anode, a carbon paper with electrodeposited Ni as cathode and a polyester cloth was used to separate the electrodes and the cathode was sandwiched between the plates forming the anodic and the gas collection compartments. More details are provided in Chapter 4.

Research shown in Chapter 5 and 6 was conducted in duplicate and performed in a continuous-flow single-chamber MEC, which consisted of two tubular modules (M_A and M_B , 2L each), connected in series. Each module consisted of a gas collection chamber with gas diffusion electrode (Sigracet GDL 25 BC carbon paper) with Ni electrodeposited and a anodic chamber with two layers of carbon felt (SIGRATHERM GFD 5, SGL Group) served as the anode and a piece of porous cellulosic non-woven fabric (J-cloth®) with a thickness of 0.7 mm served as electrical insulation between the anode and the cathode. Titanium wires coiled around the electrodes served as the anodic and cathodic current collectors. Every unit was

immersed in a 24.5 cm x 15 cm x 15 cm enclosure. The empty space between the anode and the inner walls of the receptacle served as the anodic chamber, retaining 2000 mL of liquid with a headspace of 200 mL. A detailed description is provided in Chapter 5 and 6.

3.3. CARBON FOOTPRINT METHODOLOGY

The product carbon footprint (PCF) was calculated for a conventional WWTP and a WWTP with an integrated bioelectrochemical system, based on the O2C™ tool.

The O2C™ carbon calculator allows to develop an evaluation of the GHG emissions of water treatment plants such as desalination plants, drinking water production plants or wastewater treatment and recycling plants.

This tool was developed by Degrémont in collaboration with Pricewater House. An exhaustive study of the sources of the emissions listed below was conducted before developing the calculator. The study considered:

- Energy consumption (fuel, electricity, natural gas, etc.)
- Procedures specific to water and waste management activities (biological treatment, etc.)
- Production of inputs (reagents, consumables, construction materials, equipment, etc.)
- Movement of persons
- Transport of goods, sludge, waste, materials (incoming freight, internal freight, outgoing freight)
- Waste treatment and sludge processing

The O2C™ tool is based on Life Cycle Analysis (LCA) and the greenhouse gas metrics (ISO 14040) defined by international guidelines. O2C™ therefore integrates the methodological rules of the Bilan Carbone® audit

defined by ADEME in France and is based on the guide published by ASTEE (Scientific and Technical Association for Water and the Environment). The inputs required to conduct a carbon assessment of a plant are known as emission factors. These emission factors are obtained from public sources (Bilan Carbone® by ADEME, ASTEE, ECOINVENT, etc.) but also – in order to adapt to the water treatment industry – investigations conducted by the CIRSEE, the research center of SUEZ ENVIRONNEMENT. This is the case for emissions related to the decomposition of organic materials in anaerobic conditions (CH_4) or the treatment of nitrogenous life forms (urea, ammonium, proteins) present in water (N_2O generated during the nitrification and denitrification phases). Research into these matters has appeared in recent publications.

The emission factors database is the foundation of O2C™, and is the result of a collaborative process. It is shared transparently with the entire water industry on the website www.lifecarbontool.com. The Bonnard & Gardel1 group carried out a critical and independent appraisal of the emission factors database.

3.3.1. Study wastewater treatment plant

This study includes the GHG emissions CO_2 (1x), CH_4 (25x) and N_2O (298x) and results in CO_2 equivalents. Methane (CH_4) and nitrous oxide (N_2O) emissions were independently calculated since they are specific emissions connected to with wastewater treatment procedures, since methane (CH_4), is produced by the decomposition of organic matter under anaerobic conditions and nitrous oxide (N_2O) is connected to the treatment of nitrogenous shapes present in water (urea, ammonium, proteins). N_2O is generated during the nitrification and denitrification phases of nitrogen.

The carbon footprint was estimated for the infrastructure, including construction materials, evacuated materials, equipment, energy needs and

transportation, and operation of both WWTPs, which includes consumables, energy, process emissions, by-products and transportation.

3.3.1.1. Scenario 1: WWTP

The wastewater plant studied was located in Andalucía, southern Spain. The plant was designed for a population of 73600 PE equivalent inhabitants (EI), a processing capacity of $12696 \text{ m}^3 \text{ d}^{-1}$, $1595 \text{ t BOD}_5 \text{ eliminated year}^{-1}$ and $215 \text{ t N eliminated year}^{-1}$. Wastewater stream is conducted to a screen and grit chamber, then waste stream is sent to an aerobic biological treatment and finally is conducted to the secondary settling tank, which involves the physical separation of suspended solids from the wastewater flow. Some solids are recirculated to the aerobic treatment and the rest conducted to a gravity thickener and finally to the centrifuge that allows the sludge to dry, recovering the water and sending it back to the entrance flow. The biological reactor has a total volume of 16000 m^3 with a hydraulic retention time of 31 hours. Chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus concentration at the exit of the grit chamber are 695 g-COD m^{-3} , $48.15 \text{ g-TKN m}^{-3}$ and 13.04 g m^{-3} , respectively. Removal efficiencies are 89%, 73% and 88% for COD, TKN and phosphorus, respectively. More details are provided in Escapa et al. (2012).

3.3.1.2. Scenario 2: WWTP+MEC

The wastewater treatment plant with an integrated MEC setup consisted of a MEC integrated as part of the biological treatment, where the effluent from the grit chamber is fed directly into the MEC reactor. This is followed by the polishing step (aerobic reactor), which removes the remaining COD, obtaining an effluent with TKN and COD concentrations below 15 and 125 g m^{-3} , respectively. This plant also includes a gas compressor and a gas

storage tank. The selected organic loading rate (OLR) of the MEC reactor is $3100 \text{ g-COD m}_a^{-3} \text{ d}^{-1}$, which corresponds to an HRT of 5.2 h. The size of the aerobic reactor was calculated so that nitrification, denitrification and COD removal can be accomplished effectively following the calculation methods described by (Metcalf & Eddy Inc., 2003; Aarne Vesilind, 2003). COD and TNK concentrations at the exit of the MEC treatment corresponded to COD and TNK concentrations at the inlet of the aerobic treatment. The HRT and the sludge retention time were selected to be 17 h and 16.5 days, respectively, when the aerobic reactor was preceded by an MEC reactor. These parameters were based on previous research (Escapa et al., 2012c). Escapa et al. (2012) evaluated a dWWTP with a MEC in three different scenarios. In this study a moderate scenario was selected. In moderate scenario, the COD removal in the MEC reactor remains at 44%, current densities on the order of 2.5 A m^{-2} , a hydrogen production up to $0.60 \text{ m}^3 \text{ ma}^{-3} \text{ d}^{-1}$, an energy consumption to $1 \text{ kWh kg-COD}^{-1}$ and CEs and CCEs of 50% and 75%, respectively.

3.4. REFERENCES

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Chapter 4

Microbial electrolysis cell scale-up for combined wastewater treatment and hydrogen production

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4.1. INTRODUCTION

Microbial Electrolysis Cells (MECs) are bioelectrochemical devices that produce hydrogen by combining the hydrogen evolution reaction at the cathode with the ability of anodophilic bacteria to oxidize organic matter and transfer electrons to the anode. Although this process requires electricity to be supplied, the specific energy consumption is much lower than that consumed by water electrolysis for hydrogen production (Logan, 2004; Rozendal and Buisman, 2005; Rozendal et al., 2006; Rozendal et al., 2008). Furthermore, MECs can operate on a variety of carbon sources, including wastewaters, thus combining the chemical oxygen demand (COD) removal with the production of a valuable energy carrier (Cusick et al., 2011; Ditzig, Liu and Logan, 2007; Wagner et al., 2009). Several laboratory studies evaluated the degradation of complex organic materials in a MEC. It was demonstrated that the mixed microbial consortium of the anodic compartment hydrolyzes and ferments the organic feed to volatile fatty acids and acetate, while the anodophilic bacteria predominantly utilize acetate as a source of carbon (Wagner et al., 2009; Escapa et al., 2012; Wang et al., 2011). Nevertheless, pure anodophilic strains were demonstrated to grow on other carbon sources (Chaudhuri and Lovley, 2003).

Owing to the process novelty, MEC experiments are typically conducted in laboratory setups with an anodic compartment volume of several mL. Very few attempts at process scale-up have been reported so far. A pilot-scale 1000 L MEC operated on winery wastewater (Cusick et al., 2011) highlighted several difficulties of MEC scale-up, including low volumetric rates of H₂ production, H₂ losses to hydrogenotrophic methanogenesis, and a relatively low efficiency of chemical oxygen demand (COD) removal. A study of electricity production from brewery wastewater in a pilot-scale 1000 L microbial fuel cell (MFC) led to similar conclusions, as this test showed

limited current generation and a low biochemical oxygen demand removal (Keller and Rabaey, 2008).

The study presented below was aimed at identifying main bottlenecks in MEC scale-up and demonstrating approaches for resolving these issues. Initially, process performance was evaluated in a laboratory-scale MEC with an anodic compartment volume of 50 mL. Process scale-up was demonstrated by increasing the anodic compartment first to 855 mL and then to 10 L, with the latter setup operated on raw municipal WW.

4.2. METHODS

4.2.1. Analytical methods and media composition

Acetate was analyzed in an Agilent 6890 gas chromatograph (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector. The gas composition was measured using a gas chromatograph (6890 Series, Agilent Technologies, Wilmington, DE) equipped with a 11 m × 3.2 mm 60/80 mesh Chromosorb 102 column (Supelco, Bellefonte, PA, USA) and a thermo conductivity detector. The carrier gas was argon. The pH and conductivity of the effluent were measured using PHCN-37 pH meter (Omega Canada, Laval, QC, Canada) and XL-30 conductivity meter (Fisher Scientific, Ottawa, On, Canada) , respectively. The concentration of COD was determined according to the Standard Methods (APHA 1995). Additional details are provided in (Tartakovsky et al., 2009).

The protein content of carbon felt anodes was measured following the Bio-Rad protein assay protocol (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). For the analysis, carbon felt samples were taken from the middle of each anode at the end of MEC operation. More details of the analytical procedure are provided in (Gil-Carrera et al., 2011).

Several synthetic carbon source solutions, including acetate-based, sucrose-based, and synthetic wastewater (sWW) were used. Details are provided in Table 4.1. Also, the MECs were fed with raw municipal wastewater (rWW), which had a total COD content of 250 – 300 mg L⁻¹ (first batch) and 100 –180 mg L⁻¹ (second and third batches).

Table 4.1. Composition of synthetic wastewater used in MEC tests. Notations: HS – high strength, LS – low strength. HC - high conductivity, LC – low conductivity, Ac – acetate, Su – sucrose, MS – medium strength, TM – trace metal solution.

Feeding Solution	Sodium Acetate	Sucrose	Peptidase	Beef Extract	Yeast Extract	NH ₄ HCO ₃	K ₂ HPO ₄	K ₂ HPO ₄	NaCl	TM
	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹
HS/HC	-	-	2.5	2.5	1.5	0.85	0.087	0.075	2.81	1
LS/HC	-	-	0.179	0.179	0.107	0.85	0.087	0.075	2.81	1
HS/LC	-	-	2.5	2.5	1.5	0.0607	0.00625	0.00537	0.2	1
Ac/HC*	8.37	-	-	-	-	-	0.49	0.42	-	-
Su/HC*	-	6.8	-	-	-	-	0.49	0.42	-	-
MS/HC**	-	0.22	0.046	-	-	0.52	1.38	0.69	0.83	1

* influent stream concentration after mixing with dilution water

** also contained (in g/L) : MgSO₄·7H₂O 0.26 and MnSO₄·7H₂O 0.26.

4.2.2. MEC design and operation

A continuous flow MEC-1x was equipped with a gas-diffusion cathode and had a 50 mL anodic compartment and a gas collection compartment of the same volume. The anodic compartment contained one layer of 5 mm thick carbon felt measuring 10 × 5 cm (SGL Group, Kitchener, On, Canada). A 50 cm² gas diffusion cathode (Sigracet GDL 25 BC carbon paper, SGL Group) containing electrodeposited Ni particles at a load of 0.25 mg-Ni cm⁻² and 0.5-0.7 mm thick pieces of polyester cloth were sandwiched between the anode and hydrogen collection compartment plates. The electrodeposition

procedure and a detailed diagram of this MEC can be found elsewhere (Hrapovic et al., 2010). In all MEC- 1x tests a HRT of 20 h was maintained.

A 855 mL MEC-17x also was equipped with a gas diffusion cathode. MEC-17x consisted of three anodic compartments connected in series (Fig.4.1A) and a shared gas collection (cathodic) compartment. Each anodic compartment had a volume of 285 mL and contained four layers of the carbon felt. Similar to the 50 mL setup, the cathode was made of Sigracet GDL 25 BC carbon paper with electrodeposited Ni ($0.28 \text{ mg-Ni cm}^{-2}$). The cathode had a total surface area of 315 cm^2 . The cathode and the non-conductive separator cloth were sandwiched between the plates. Each anodic compartment had an external recirculation loop equipped with a Masterflex (Cole-Parmer Canada, Montreal, Canada). A shared cathode condensate collector was used to collect the condensate and the anodic liquid percolated through the cathode. This liquid was returned to the influent stream as shown in Fig.4.1A.

MEC-17x was continuously fed using a 4L bottle containing the feed solution. The flow rate was controlled to obtain the desired HRT. The feed bottle was maintained at a room temperature. The WW volume in the bottle was allowed to fluctuate between 1-4 L and the bottle was replenished every 2-3 days with a fresh solution to maintain an average HRT of 4 days. This resulted in partial hydrolysis of the WW in the feed bottle. VFA and total COD content, pH, and conductivity of the feed solution was measured twice a week.

A 10 L MEC-200x consisted of two 5 L MECs in series (Fig.4.1B). Each 5 L MEC contained seven layers of the 5 mm thick carbon felt used as an anode. The gas diffusion cathode of the MEC-200x was made of the carbon paper with electrodeposited Ni ($0.25 - 0.30 \text{ mg-Ni cm}^{-2}$). The cathode had a total surface area of 3024 cm^2 . Each gas collection compartment had a volume of 1.5 L. As in the MECs described above, a polyester cloth was

used to separate the electrodes and the cathode was sandwiched between the plates forming the anodic and the gas collection compartments. Each anodic compartment of MEC-200x had an external recirculation loop. Similar to MEC-17x, there was a cathode condensate collector and the collected liquid was returned to the influent stream (Fig.4. 1B).

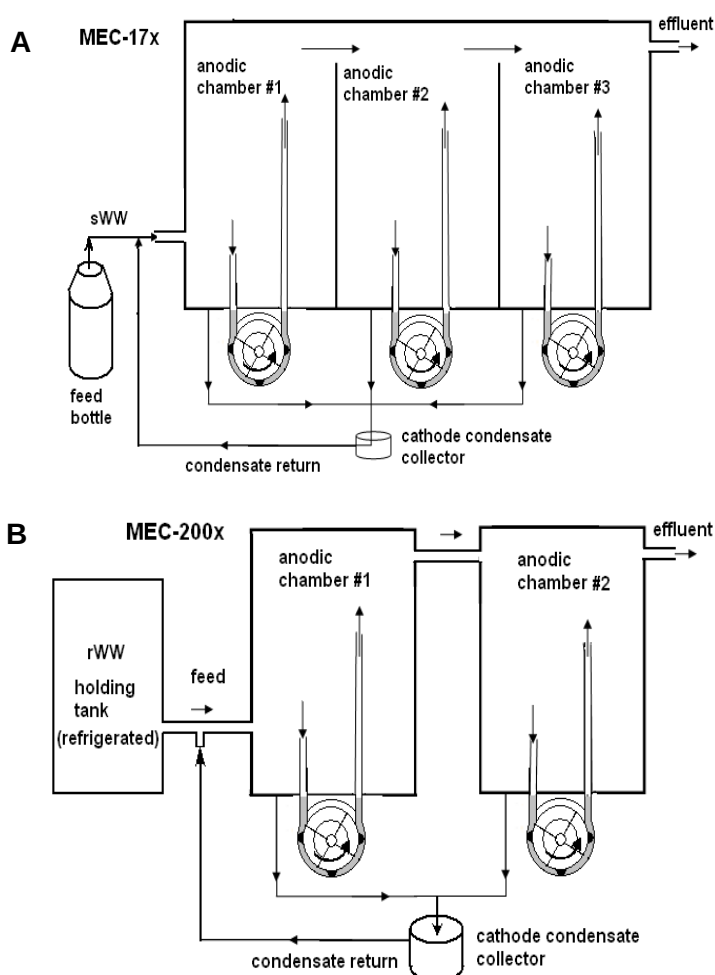


Figure 4.1. Diagrams of MEC-17x (A) and MEC-200x (B) experimental setups showing external recirculation lines and flow patterns in the anodic compartments. In all MECs cathodes and polyester cloth separators were sandwiched between the anode and H_2 collection compartment plates. The condensate collectors were installed in the cathodic (gas collection) compartments and the condensate was returned to the influent lines.

MEC-1x and MEC-17x were inoculated with a homogenized anaerobic mesophilic sludge (A. Lassonde Inc., Rougemont, QC, Canada). MEC-200x was inoculated with the effluent of MEC-17x. With the exception of acetate and sucrose stock solutions, all MECs were fed using Masterflex peristaltic pumps (Cole-Parmer Canada, Montreal, Canada). In MEC-1x tests concentrated stock solutions of acetate and sucrose were fed with an infusion pump (model PHD 2000, Harvard Apparatus, Holliston, MA, USA) at a rate of 5.0 mL d^{-1} . To maintain the desired hydraulic retention time, dilution water containing trace metals (1 mL per L) and 17 g L^{-1} of NH_4HCO_3 was simultaneously fed a peristaltic pump. The carbon source solutions used in the tests are described in 4.1.

All MECs were equipped with temperature and pH control loops. Anodic compartment mixing was provided by external recirculation loops (0.57 L h^{-1} , 1.7 L h^{-1} , and 4 L h^{-1} , for MEC-1x, MEC-17x, and MEC-200x, respectively). MEC-1x was operated at 30°C , while MEC-17x and MEC-200x were operated at $23\text{-}25^\circ\text{C}$. The pH set point was 7. Gas production in MEC-1x and MEC-17x was measured by bubble counters connected to glass U-tubes and interfaced with a data acquisition system (Tartakovsky et al., 2009). Gas production in MEC-200x was measured by MGC-1 milli-gas counter (Ritter Co, Bochum, Germany). For all tested configurations, a minimum period of 72 h was allowed after each change in operating conditions. Once stable current was observed, gas and effluent composition were measured daily to average 3-5 measurements. Steady – state conditions were considered if standard deviation did not exceed 20%. This approach ensured steady state conditions in terms of COD removal and H_2 production, but did not provide steady state distribution of microbial populations, which would require much longer operating times. In total, MEC-1x, MEC-17x, and MEC-200x were operated for 160, 99, and 45 days, respectively.

In all MECs, the applied voltage was controlled using a four-channel regulated power supply PW18-1.8 AQ (Kenwood, Japan) interfaced with a PC. The power supply was computer-controlled. Unless specified otherwise, the applied voltage (U_{app}) of each anodic compartment was optimized in order to reduce the overall energy consumption using the on-line Perturbation and Observation algorithm (Tartakovsky et al., 2011). In brief, the U_{app} was changed (increased or decreased) with an interval of 1 min by a step (ΔU) equal to 0.05 V. The direction of voltage change depended on the current measured at the end of the time interval, e.g. if a voltage increase led to a higher current as compared to its previous value, then the voltage was further increased. Otherwise, the voltage was decreased. The Perturbation and Observation algorithm was implemented in Visual Basic 6 (Microsoft Corp, Redmond, WA, USA). A data exchange interval of 10 s was used to control the applied voltage, while voltage values were recorded with an interval of 20 min.

MEC performance was characterized in terms of the volumetric hydrogen production rate expressed per liter of the reactor volume. Coulombic efficiency was calculated as the ratio of electrons relative to the total electrons available from acetate consumption. Cathodic efficiency was calculated as the ratio of electrons recovered as hydrogen gas to the total number of electrons that reach the cathode. The H_2 yield was calculated in mol of H_2 produced per mol of COD consumed (degraded) in the reactor. Details can be found elsewhere (Escapa et al., 2009). A molecular weight of 32 g/mol and an electron yield of 4 mol/mol (mol of electrons produced per mol of substrate consumed) were assumed for synthetic and municipal wastewaters (Liu and Logan, 2004).

4.3. RESULTS AND DISCUSSION

MEC scale-up was studied by progressively increasing anodic compartment volumes from 50 mL to 10 L (a scale-up factor of 200). All tested MECs had

similar design and consisted of an anodic compartment with carbon felt anode, gas diffusion cathodes, a H_2 - collection compartment, and a cathode condensate collector. All MECs were continuously fed with either synthetic or municipal wastewater.

4.3.1. MEC-1x tests

Tests in a 50 mL MEC-1x were aimed at identifying key factors affecting COD removal and H_2 production. For the tests, the influent solution was sequentially varied in terms of strength, composition, and conductivity. Importantly, in all tests described below the optimal applied voltage was estimated and maintained by the Perturbation/Observation algorithm (Tartakovsky et al., 2011), as described above. This improved the comparison, since it was always performed under optimized conditions in terms of the applied voltage.

In test #1-1, MEC-1x was fed with a high-strength and high-conductivity (HS/HC) sWW solution with an influent COD concentration of 6 g L^{-1} and a conductivity of 7 mS cm^{-1} (Table 4.1). After the startup, H_2 production stabilized at $1.5 \text{ L L}_R^{-1} \text{ d}^{-1}$ with a COD removal efficiency of 71%. The real-time optimization of applied voltage by the Perturbation/Observation algorithm resulted in an average voltage of 0.91 V. The corresponding energy consumption values were 3.7 Wh L^{-1} and 1.5 Wh g^{-1} for H_2 production and COD removal, respectively. Other performance parameters are given in Table 4. 2.

In the following test #1-2, the influent COD concentration was reduced to about 600 mg L^{-1} by diluting the feed solution while maintaining the conductivity at 7 mS cm^{-1} (LS/HC solution). The current and H_2 production started to decrease immediately after the influent change with a new steady state established within four days. At steady state, H_2 production was very low (Fig.4.2A), the COD removal efficiency decreased from 71% to 57%

(Fig.4.2B), and the hydrogen yield was only 0.02 mol/mol. Also, the optimized applied voltage fell to 0.58 V. This was consistent with carbon-source limited anodophilic activity.

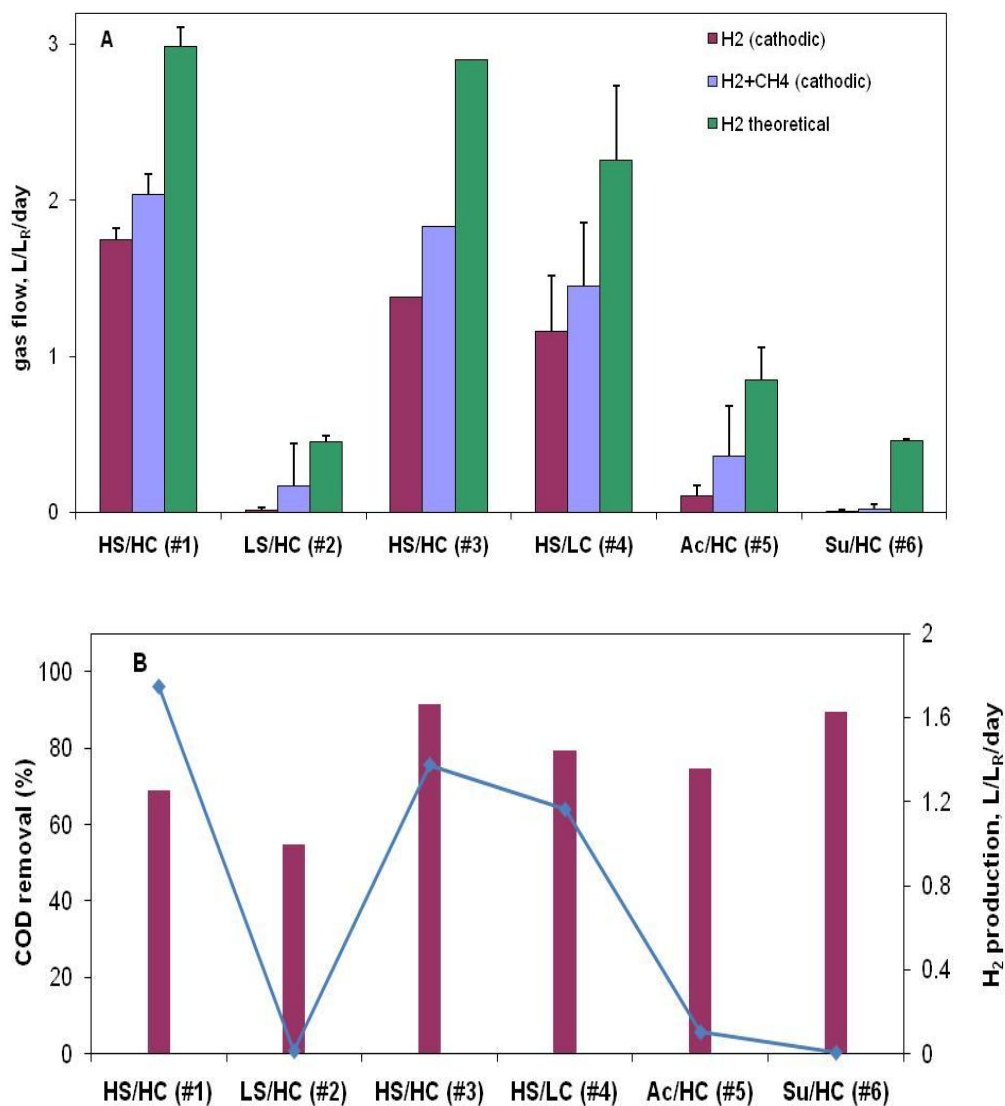


Figure 4.2. (A) Production of H₂ and CH₄ in the cathodic (gas collection) compartment of MEC-1x and (B) COD removal efficiency. A stoichiometric ratio of 4 mol/mol was used to express CH₄ production in H₂ equivalent.

Table 4.2. MEC -1x performance as a function of feeding solutions used in the test.

test	WW	OLR g L _R ⁻¹ d ⁻¹	COD _{in} g L ⁻¹	V v	I mA	COD _r g L _R ⁻¹ d ⁻¹	E _{coul} %	H ₂ yield mol / mol	Econs Wh/L H ₂	Econs kWh/ kg COD
1-1	HS/HC	6.0	4.9	0.91	15.2	4.3	50	0.60	3.7	1.5
1-2	LS/HC	0.7	0.6	0.58	1.6	0.4	43	0.02	55.8	0.2
1-3	HS/HC	6.4	5.3	0.94	13.1	5.7	25	0.32	2.5	0.8
1-4	HS/LC	6.0	4.9	0.82	11.3	4.8	38	0.40	3.8	1.0
1-5	Ac/HC	0.5	0.4	0.61	4.2	0.4	75	0.34	6.4	3.3
1-6	Su/HC	0.5	0.4	0.51	2.3	0.5	61	0.04	39.5	1.0

In test #1-3 the influent composition was restored to HS/HC. Once the strength of the influent solution was increased, H₂ production resumed and the current rebounded to the values observed in test #1-1, thus reproducing MEC-1x performance at a high organic load (Fig. 4.2). However, the decrease in the influent COD concentration during the previous test appeared to have a long-lasting effect on the H₂ / CH₄ ratio. A comparison of H₂ yields and Coulombic and COD removal efficiencies in tests #1-1 and #1-3 revealed time – related evolution of microbial populations. In test #1-3 the COD removal efficiency was improved, while Coulombic efficiency and H₂ yield were lower than in #1-1. Apparently, hydrogenotrophic methanogens proliferated during test #1-2 and continued to affect H₂ yield in test #1-3, as can be seen from the comparison of H₂ and combined H₂ / CH₄ flow rates shown in Fig.4.2A. The latter flow rate includes the stoichiometric equivalent of CH₄ recovered in the cathode off-gas. The combined flow rate observed in test #1-3 was similar to that measured during test #1-1, albeit with increasing CH₄ percentage. Consequently, H₂ yield in test #1-3 was lower than in test #1-1 (Table 4. 2).

An additional confirmation of the strong impact of wastewater strength on H₂ production was obtained in test #1-4, where MEC-1x was fed with a high

strength and low conductivity (HS/LC) solution. Starting from the first day of this test, a stable current of 11 mA was obtained. Although H_2 production somewhat decreased, it promptly stabilized at 59 mL d^{-1} . Thus, poor performance in test #1-2 was linked to the anodic liquid COD concentration rather than solution conductivity. Other performance parameters during test #1-4 are given in Table 4.2.

Overall, tests #1-1 through #1-4 demonstrated that H_2 production is mostly limited by the influent COD concentration. The next two tests were aimed at studying the effect of organic matter hydrolysis and fermentation on H_2 production. In test #1-5 acetate was fed at an influent concentration of 500 mg L^{-1} while maintaining high solution conductivity (Ac/HC solution). In this test the hydrolysis and fermentation steps in the biodegradation sequence were eliminated. After the feed was changed to Ac/HS, about 10 days were required to establish a new steady state. A COD removal of 75% was obtained. This performance was only slightly better than in test #1-2, which was carried out with a sWW solution (LS/HC) that had a comparable organic load and conductivity. In spite of MEC operation on acetate, which can be directly used by the anodophilic bacteria, the H_2 yield was lower than that on the high strength HS/HC sWW in test #1-1.

The rate of H_2 formation at the cathode depends on the activity of the anodophilic bacteria at the anode. Apparently, low acetate concentration in the anodic liquid due to either low organic load or low influent acetate concentration limited the anodophilic activity, as evidenced by the low current (Table 4.2).

At the same time, similar to test #1-2, the off-gas analysis showed an increased level of CH_4 in the cathodic off-gas, implying that the hydrogenotrophic methanogens converted H_2 produced at the cathode to CH_4 , which further decreased the apparent efficiency of H_2 production. The observed increase in CH_4 concentration at low organic loads and low COD

concentrations in the anodic liquid was in line with previously reported results (Cusick et al., 2011; Manuel et al., 2010; Tartakovsky et al., 2008).

In test #1-6 a sucrose solution was fed (Su/HC solution in Table 4.2) to evaluate the MEC performance on an easily fermentable source or carbon, which did not require hydrolysis. During the sucrose test the steady state current value was 2.3 mA, which is less than the current observed on acetate. Also, H₂ production further declined (Figure 4.2 and Table 4.2). However, the performance in terms of COD removal efficiency was good, reaching 90%. Accordingly, energy consumption for COD removal decreased to only 1.0 Wh g⁻¹ of COD. The anodophilic microorganisms can grow on glucose or sucrose (Chaudhuri and Lovley, 2003), however in a mixed anaerobic culture sucrose is most likely to be fermented to acetate (Kim et al., 2010).

From tests #1-5 and #1-6 it was concluded that the fermentation step did not limit the degradation process, while the anodophilic activity was limited by low levels of acetate in the anodic liquid. As well, sucrose feed once again led to an increased CH₄ concentration in the cathodic off-gas, which might be attributed to hydrogenotrophic activity (Manuel et al., 2010; Tartakovsky et al., 2008). At the same time, the increased methanogenic activity in the anodic chamber improved the overall COD removal efficiency (Chae et al., 2010).

Overall, the tests with different sWW strengths, compositions, and conductivities indicated that low wastewater conductivity only moderately reduces H₂ production, thus enabling H₂ production from real wastewater with relatively low conductivity. Hydrolysis is often the rate-limiting step in anaerobic degradation of complex organic matter (Johansen JE, 2006; Zhu et al., 2009). Accordingly, H₂ production might be limited by a low level of acetate, which is a readily available carbon source for anodophilic microorganisms, in the anodic liquid. At the same time, CH₄ production from

cathodic H_2 by the hydrogenotrophic methanogens appeared to be independent of the organic load, which might lead to a higher CH_4 percentage at low organic loads, where the H_2 production is the lowest.

4.3.2. MEC-17x tests

A comparison of MEC-1x performances at high and low organic loads and influent concentrations demonstrated the inefficiency of scaling-up by a simple increase of the anodic compartment volume. Indeed, while wastewater may have a relatively high COD content, to satisfy wastewater treatment norms the effluent COD concentration should be in a range of 80-120 mg L⁻¹. For a MEC with intensive mixing of the anodic liquid this requirement might lead to low volumetric H_2 production and proliferation of hydrogenotrophic methanogens. Furthermore, an increased anodic compartment volume demands a greater anode thickness, which in turn might increase proton transport resistance. These limitations were resolved by using reactor-in-series approach. This process design, often referred to as reactor staging, is used in aerobic wastewater treatment to increase the organic load in the first reactor in series, while satisfying the wastewater treatment standards by using the last reactor in series for effluent “polishing”. The staging approach agrees with the results obtained in MEC-1x tests described above, where significant H_2 production was only observed at high COD concentrations (high organic loads). Consequently, process scale-up in MEC-17x was attempted by using three anodic compartments in series.

At the startup, MEC-17x was fed with the HS/HC solution and a HRT of 41 h was maintained (test #2-1). Under these operating conditions H_2 production stabilized at 0.98 L L_R⁻¹ d⁻¹ corresponding to a H_2 yield of 1.01 mol/mol. CH_4 content in the off-gas remained at 10.8% (Fig.4.3A). Other performance parameters are given in Table 4.3.

Table 4.3. MEC -17x performance as a function of HRT used in the test.

test	HRT h	WW	OLR $\text{g L}_R^{-1} \text{d}^{-1}$	COD _{in} g L^{-1}	I mA	COD _r $\text{g L}_R^{-1} \text{d}^{-1}$	E _{coul} %	H ₂ yield mol / mol	Econs Wh/L H ₂	Econs kWh/ kg COD
2-1	41	HS/HC	3.4	5.7	176.1	1.4	104	1.01	5.3	3.7
2-2	27	HS/HC	8.3	9.8	256.3	5.7	37	0.61	3.2	1.4
2-3	27	MS/HC	1.2	1.3	64.1	0.9	64	0.13	23.4	2.2
2-4	18	MS/HC	1.2	0.9	36.5	0.8	26	na/a*	n/a	1.4

* H₂ flow not measured due to a gas leak

At a steady state, the applied voltages optimized by the perturbation/observation algorithm were 1.08, 1.07, and 0.67 V for the first, second, and third anodic compartments, respectively. The resulting currents are shown in Fig.4.3B. Notably, the current in the third compartment was the lowest. COD measurements showed an overall COD removal efficiency of 41%. A more detailed look at COD values in the anodic compartments suggested that the removal occurred in the first and second compartments (Fig.4.4). The COD concentration in the third anodic compartment was even slightly higher than in the second, apparently due to the inoculum biomass hydrolysis in this compartment. Indeed, the third compartment was subjected to a very low organic load. Under these conditions the inoculum sludge might be “self-digested” in this compartment, especially during first weeks of operation. By the end of MEC-17x test, COD concentration in the third compartment was equal to or below the second compartment level.

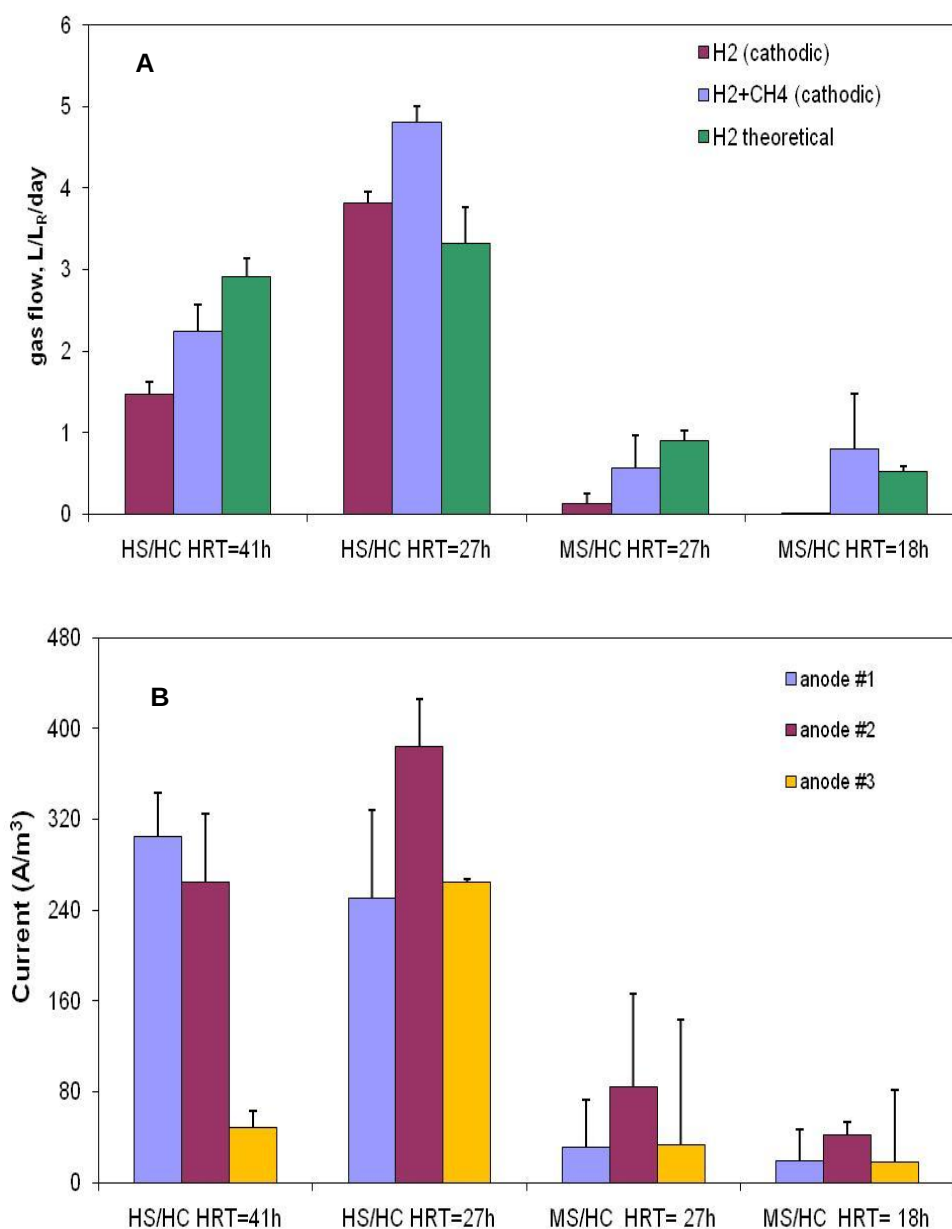


Figure 4.3. (A) H₂ and CH₄ production in the cathodic (gas collection compartment) of MEC-17x and (B) The current in each anodic compartment. The applied voltage in each anodic compartment were optimized by the P/O algorithm.

In test #2-2 the HRT was decreased to 27 h thus changing the organic load from 3.4 to 8.3 g L_R⁻¹ d⁻¹ (Table 4.3). The change improved H₂ production

increasing it to $2.5 \text{ L L}_R^{-1} \text{ d}^{-1}$ (Fig.4.3A). Energy consumption for H_2 production remained unchanged. COD removal efficiency also improved to 69%. The current in all anodic compartments increased, with the highest current observed in the second compartment (Fig.4.3B). The total current, calculated as a sum of the current measured in each compartment, increased from 176 mA to 256 mA. Although in this test the current in the third compartment was comparable to the first compartment, the COD measurements did not show COD removal in it (Fig.4.4). The poor COD removal in the third compartment in spite of measurable current might be attributed to a lack of easily available carbon source (acetate) for the anodophilic bacteria combined with a suspected short-circuit developed between the anode and cathode during test#2.

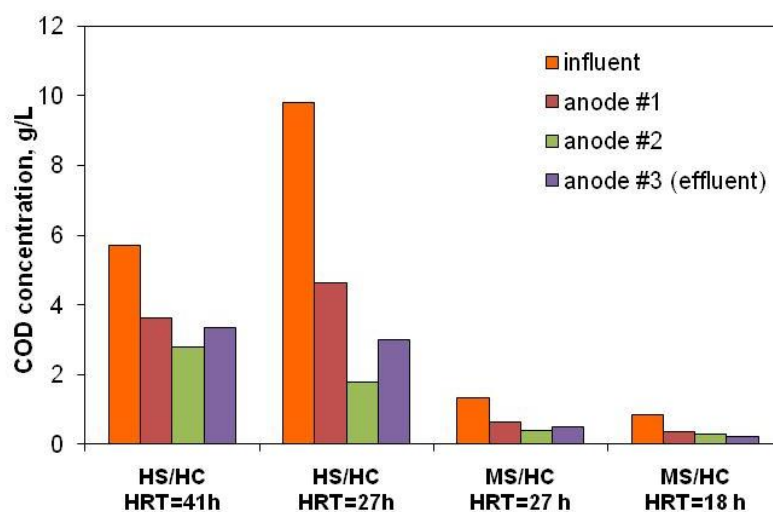


Figure 4.4. COD measurements in each anodic compartment of MEC-17x and in the influent wastewater stream.

The next two tests were carried out to study MEC performance on medium strength/high conductivity sWW (MS/HC) resulting in a lower organic load (Table 4.3). In test #2-3, MEC-17x was operated at the same HRT as in the previous test. Both the current and H_2 production declined in response, as can be seen from Fig.4.3. This decrease was attributed to the decreased

organic load, which was even lower than anticipated due to the partial degradation of the MS/HC solution in the feed bottle.

To counteract wastewater degradation in the feed bottle, in test #2-4 the HRT was decreased to 18 h. This stabilized the organic load, which remained at $1.2 \text{ g L}_R^{-1} \text{ d}^{-1}$ throughout this test, however the influent COD concentration was relatively low at 0.9 g L^{-1} . Figure 4.3A shows that although in test #2-4 HRT was decreased to 18 h, H_2 production further decreased. This drop in H_2 production was consistent with the increased percentage of CH_4 in the cathodic (gas collection) compartment due to hydrogenotrophic activity. Overall, experimental results obtained in MEC-1x tests #1-2 and #1-3 were reproduced. As mentioned above, H_2 losses to hydrogenotrophic methanogenesis are often observed at low influent COD concentrations corresponding to low acetate concentration in the anodic liquid (Cusick et al., 2011; Gil-Carrera et al., 2011; Rozendal et al., 2007). Also, H_2 losses due to its recycling at the anode could contribute to the observed performance decrease (Ditzig, Liu and Logan, 2007; Lee and Rittmann, 2010). To evaluate the possibility of accumulation of organic solids in the carbon felt used for the anodes, felt samples were taken from each anodic compartment at the end of MEC-17x operation for protein analysis. The following values were obtained: 0.56, 0.15, and 0.04 mg of protein per mL of anode for the first, second, and third anodic compartments, respectively. These values were in good agreement with the COD removal rates. Indeed, no COD removal was observed in the third anodic compartment and protein analysis of this compartment showed no biomass attachment to the carbon felt. The first anodic compartment with the highest protein density had the highest rate of COD removal and, overall, the highest current. Also, a protein concentration of 0.56 mg mL^{-1} agreed well with the previously obtained value in a MEC operated on acetate (Gil-Carrera et al., 2011). The correlation between the average current and the protein density in the corresponding compartment confirms

that the proteins were related to active biomass rather than to the accumulation of non-degraded organic materials.

The observed dependence of H_2 production on the organic load agreed with the results of MEC-1x tests, both at low and high organic loads, where the organic underload also led to a low H_2 production and a high CH_4 percentage in the off-gas. CH_4 production in the anodic compartment of MEC-17x was negligible with the headspace only containing CH_4 and CO_2 . At the same time, COD removal was acceptable even at low organic loads and energy consumption was low, e.g. 1.4 kWh/kg COD in tests #2-2 and #2-4. At low organic loads in tests #2-3 and #2-4 COD removal mostly occurred in the first anodic compartment. It can be hypothesized, that H_2 was mostly produced in the first anodic compartment, while the second compartment predominantly produced CH_4 . At least at low organic loads, a two-reactor setup was deemed sufficient for achieving an acceptable level of COD removal, while avoiding excessive energy consumption.

4.3.3. Municipal wastewater treatment in MEC-200x

Based on MEC-17x performance, the 10 L MEC-200x was designed with two 5 L compartments connected in series. From the startup, MEC-200x was fed with raw municipal wastewater at a HRT of 32 h. Then the HRT was progressively decreased to 24, 16, and 10 h to increase organic load (Table 4.4).

Effluent and off-gas monitoring during the startup procedure (test #3-1) showed the relatively fast development of the exoelectricigenic activity in both anodic compartments. Within two weeks, the total current increased to 100.9 mA and the total COD (tCOD) concentration in the effluent of the second anodic compartment decreased to 110 mg L⁻¹. The corresponding soluble COD (sCOD) concentration was at 80 mg L⁻¹. The municipal wastewater used during the startup had a tCOD concentration of 150 mg L⁻¹

- 180 mg L⁻¹. At this wastewater strength the MEC was underloaded, as can be seen from the sCOD measurements in the first and second anodic compartments showing similar values (70 mg L⁻¹ and 80 mg L⁻¹, respectively). Consequently, in test #3-2 the organic load was increased by decreasing the HRT to 24 h. However, the wastewater strength was decreased, apparently due to partial wastewater degradation during the prolonged storage at +4°C. Accordingly, the organic load slightly decreased (Table 4.4).

Table 4.4. MEC-200x performance as a function of HRT used in the test.

test	HRT h	OLR g L _R ⁻¹ d ⁻¹	COD _{in} g L ⁻¹	I mA	COD _r g L _R ⁻¹ d ⁻¹	E _{coul} %	H ₂ yield mol / mol	Econs Wh/L H ₂	Econs kWh/ kg COD
3-1	32	0.13	0.17	108.9	0.06	129	0.36	23.2	7.4
3-2	24	0.10	0.99	90.7	0.07	103	0.12	79.3	5.2
3-3	16	0.23	0.16	62.7	0.1	42	0.13	22.8	1.7
3-4	10	0.66	0.27	186.3	0.5	23	0.19	8.7	0.9

The total current remained at around 90 mA, but the COD removal efficiency improved to 66%, the effluent tCOD decreased to 40 mg L⁻¹ and the H₂ production somewhat decreased. Likely, the methanogenic population of MEC-200x continued to evolve leading to increased COD removal as well as CH₄ production from H₂. Nevertheless, by the end of test #3-2 the energy consumption for COD removal decreased to 3.6 Wh g-COD⁻¹, although it remained somewhat higher than in MEC-17x tests. Most of the COD removal observed in MEC-200x was achieved in the first anodic compartment, as at the exit of this compartment, the tCOD concentration was also at 40 mg L⁻¹.

In the following tests, the HRT was decreased to 16 hours (0.23 g COD L_R⁻¹ d⁻¹, test #3-3) and then to 10 hours (0.65 g COD L_R⁻¹ d⁻¹, test #3-4). The onset of test #3-3 coincided with a fresh batch of wastewater with an even

lower tCOD content of around 100 mg L^{-1} , so that the OLR increase was less than initially anticipated. The increased organic load led to H_2 production of 119 mL d^{-1} . Considering that the COD was mostly removed in the first 5 L compartment, a volumetric rate of H_2 production of $0.05 \text{ L L}_R^{-1} \text{ d}^{-1}$ can be projected, which agrees with the results obtained in both the MEC1x and MEC-17x at comparably low organic loads. Energy consumption for H_2 production remained high and the off gas contained only 50 – 52% of H_2 . However, the COD removal efficiency notably improved reaching 76% at an HRT of 10 h. Effluent tCOD remained at 80 mg L^{-1} (60 mg L^{-1} sCOD) (Figure 4.5).

In all tests described above, $2 - 5 \text{ L d}^{-1}$ of liquid was collected in the cathode condensate collector and returned to the influent stream, as shown in Fig.4.1. Liquid return was considerably higher than in MEC-1x, where only $5 - 20 \text{ mL d}^{-1}$ was returned. The collected liquid consisted both of cathodic condensate and anodic liquid percolating through the cathode surface. Apparently, increased liquid pressure in MEC-200x led to the higher percolation rate, however cathode flooding was avoided by liquid return.

Calculations of energy consumption for COD removal showed that only $0.9 \text{ Wh g-COD}^{-1}$ was required in test #4-4. This value was consistent with the energy consumption observed in MEC-1x and MEC-17x and is lower than an average energy consumption of $1.5 \text{ Wh g-COD}^{-1}$ for aerobic wastewater treatment (Tchobanoglous et al., 2003). Similar performance was obtained in a smaller MEC operated on domestic wastewater (Escapa et al., 2012). Energy consumption for H_2 production was quite high (Table 4.4), but consistent with the results obtained in MEC-1x and MEC-17x on low-strength sWW. This implies that the volumetric performance in terms of H_2 production can be only improved by further reducing the HRT to achieve OLRs above $1 - 2 \text{ g L}_R^{-1} \text{ d}^{-1}$. In consequence, the COD removal efficiency might be reduced. It can be suggested that in a MEC in-series H_2 production

might be accomplished in the first unit, while the following units might be used for COD removal with low energy consumption.

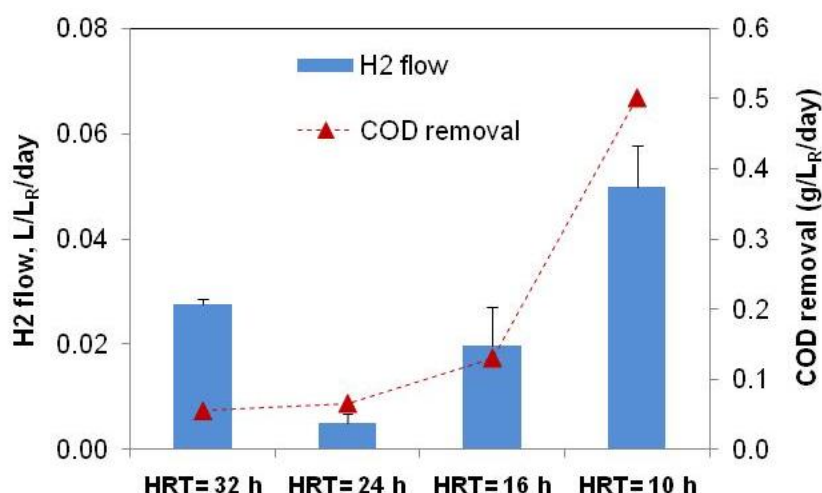


Figure 4.5. COD removal and hydrogen production of MEC-200x operated with raw wastewater at different hydraulic retention times.

4.4. CONCLUSIONS

This work demonstrates a successful scale-up of the combined wastewater treatment and H₂ production process from a 50 mL laboratory MEC fed with synthetic wastewater to a 10 L MEC treating raw municipal wastewater. A MEC-in series design was implemented for scale-up to achieve an acceptable rate of H₂ production while satisfying wastewater treatment norms. A comparison of key performance parameters of the three tested MECs confirmed process scalability. MEC operation at organic loads above 1-2 g L_R⁻¹ d⁻¹ might be suggested in order to maximize H₂ production. Also, to minimize energy consumption the applied voltage might be individually (per cell) controlled and preferably optimized in real time as a function of the actual organic load.

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Chapter 5

Reduced energy consumption during low strength domestic wastewater treatment in a semi-pilot tubular microbial electrolysis cell

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5.1. INTRODUCTION

Microbial electrolysis cells (MECs) are electrochemical devices that produce hydrogen by combining the ability of electrogenic bacteria to oxidize organic matter (using the anode as an electron acceptor) with the hydrogen evolution reaction at the cathode. Although this process requires the input of a small amount of electrical energy, the specific energy consumption is lower than that typically associated with hydrogen production through conventional water electrolysis (Logan, 2004; Rozendal and Buisman, 2005; Rozendal et al., 2006). In addition, the utilization of MECs in the treatment of wastewater in general, and domestic wastewater in particular, may provide further environmental benefits through reduction of the CO₂ footprint (Foley et al., 2010) and reduction of the energy use typically associated with the chemical oxygen demand (COD) removal by aerobic treatment (Greenberg et al., 1992).

Since hydrogen production by MECs was first reported (Liu et al., 2005; Rozendal et al., 2006), the reactor configuration has been constantly improving, resulting in a substantial enhancement in performance (Cheng and Logan, 2011; Logan et al., 2008; Rozendal et al., 2008; Tartakovsky et al., 2009). Among the several configurations investigated, the tubular design allows close to optimal distribution of the anode and the cathode in bioelectrochemical systems (BES) (Kim et al., 2010). First, cylindrical-shaped acetate-fed BES designs were operated in electricity production mode (microbial fuel cell (MFC)) and achieved power outputs in the range of 5-90 W m⁻³ with coulombic efficiencies of ~70% (Kim et al., 2009; Rabaey et al., 2005). Tubular MFCs have also proven to be feasible in the treatment of real wastewaters, achieving moderate power outputs and removing up to 80% of the COD of the wastewater (Liu et al., 2004). Moreover, a configuration of two tubular MFC reactors linked hydraulically has been

proposed to act as a polishing stage to treat anaerobic digester effluent (Kim et al., 2010).

Cylindrical designs have also proven to be feasible for hydrogen production, yielding production rates and energy efficiencies of $2.3 \text{ L L}_a^{-1} \text{ d}^{-1}$ and 240%, respectively, fed with glucose at medium-low applied voltages (Ishikawa et al., 2006).

The aim of this study was to assess the ability of an MEC semi-pilot reactor to treat real wastewater. The energy consumption, the hydrogen production and the chemical oxygen demand removal (CODr) efficiency while treating low organic loads at several hydraulic retention times were studied. The MEC reactor consisted of two tubular modules hydraulically connected in series and fed with domestic wastewater (dWW).

Importantly, the results helped to identify the organic loading rate (OLR) limits that make the use of MECs feasible for dWW treatment, as well as identifying the OLR limits that justify the operation of two MECs in series.

5.2. MATERIALS AND METHODS

5.2.1. Influent

The MEC was fed with effluent from the pretreatment systems of a domestic wastewater treatment plant (Navalmorales, Spain). The wastewater (its physico-chemical characterization is provided in Table 5.1) was fed directly from a 20 L tank stored at 2 °C.

Wastewater was continuously fed to the anodic compartment of the first module of the MEC with a peristaltic pump (Dosiper C1R; León, Spain), and the effluent flooded into the anodic compartment of the second module (Figure 5.1). Hydraulic retention time was controlled by adjusting the flow rate of the pump.

Table 5.1. Characteristics of the five sets of domestic wastewater used in this study (duplicate or triplicate measurements). ND (not determined).

HRT (h)	COD in mg-COD L ⁻¹	Conductivity uS cm ⁻¹	pH	NH ₄ ⁺ ppm	TSS mg L ⁻¹	VSS mg L ⁻¹
25	54.0	396	6.8	24.1	ND	ND
23	84.0	394	6.7	11.6	42.1	35.2
15	118.2	442	6.8	14.8	54.6	40.7
11	83.2	549	6.9	20.3	71.2	64.5
7	97.0	587	7.0	25.7	47.3	42.4
4	112.2	605	7.3	28.7	39.2	36.5

5.2.2. MEC design and operation

All tests were conducted in duplicate and performed in a continuous-flow single-chamber MEC, which consisted of two tubular modules (M_A and M_B , 2L each), connected in series (Figure 5.1). From the inside to the outside, each module consisted of the following: (i) A polypropylene tube 23 cm long, 12 cm diameter, with equally spaced 2 cm diameter holes at 1 cm intervals served as a gas collection chamber (ii) A 23 cm x 36.5 cm gas diffusion electrode (Sigracet GDL 25 BC carbon paper) was wrapped around the polypropylene tube. The electrode (cathode) was electrodeposited with nickel (nickel load of 0.4 mg cm⁻²) prior to use, as described by Hrapovic et al. (2010). A 2 m long and 0.125 mm thick titanium wire coiled around the electrode was used as a current collector. (iii) A 23 cm x 37 cm piece of porous cellulosic non-woven fabric (J-cloth®) with a thickness of 0.7 mm served as electrical insulation between the anode and the cathode. (iv) Two layers of 24 cm x 40 cm carbon felt (SIGRATHERM GFD 5, SGL Group) served as the anode. The optimum thickness of the anode (1 cm) was selected based on the results from a previous study (Gil-Carrera et al.,

2011). Again, a 2.2 m long and 0.125 mm thick titanium wire coiled around the electrode served as the anodic current collector. Every unit was immersed in a 24.5 cm x 15 cm x 15 cm enclosure. The empty space between the anode and the inner walls of the receptacle served as the anodic chamber, retaining 2000 mL of liquid with a headspace of 200 mL.

Mixing in the anodic chamber of every module was provided by an external recirculation loop at a rate of 500 mL h⁻¹ using a peristaltic pump (Dosiper C1R; León, Spain).

Each module was inoculated with the effluent from other dWW-fed MECs operated in the same lab for more than 2 years. MEC was operated for more than 2 months before performing the test in order to allow microbial community to develop and mature in the anode's biofilm.

MEC electrical outputs in each module were monitored separately, and the anodic compartments were individually controlled. Two adjustable DC power supplies made in-house were used to maintain the voltage at the predetermined set point. The power supplies were computer controlled (data recording at 30 min intervals) using an analog output board (PCI-6713; National Instruments, Austin, TX).

MEC temperature was maintained at 20°C by means of a thermocouple placed in the anodic chamber of the first module, a temperature controller (PCI-6221; National Instruments, Austin, TX) and a heating plate located on the anodic chamber side of the MEC.

Gas production in each module was measured by an MGC-1 milli-gas counter (Ritter Co, Bochum, Germany).

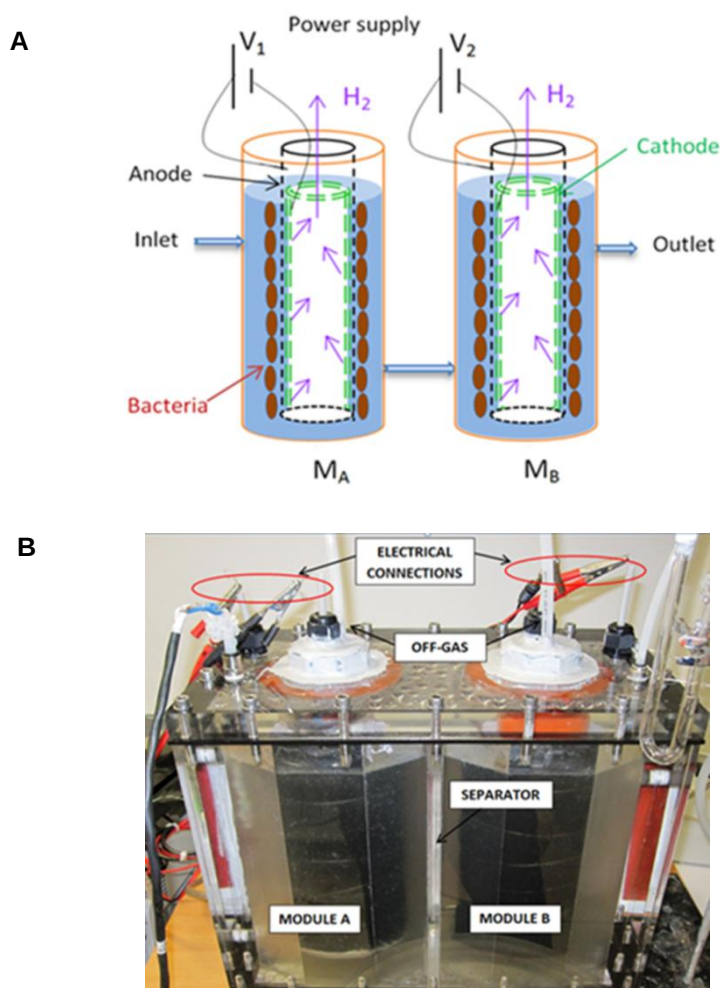


Figure 5.1. View of the two-module tubular MEC used in the experiments. (A) Scheme (M_A , module 1; M_B , module 2; V_1 , V_2 , applied voltage in each module); (B) Overall picture.

5.2.3. Experimental design and MEC characterization

The performance of the MEC was studied at several HRTs ranging from 25 hours to 4 hours (Table 5.1). Although it was initially planned to study the effect of hydraulic retention time (HRT), the variability in the strength of the real dWW fed into the reactor (Table 5.1) prompted us to select OLR rather than HRT as the independent variable.

The hydraulic retention reported in the tables and the text corresponds to the hydraulic retention time (HRT) in the whole reactor (i.e., $M_A + M_B$), while the OLR was computed for each module independently (and was denoted as OLR_{MA} and OLR_{MB} respectively) as it is shown in the tables and the figures.

Each set of operating conditions was maintained for at least six retention times to assure stable conditions. The MEC was considered to be in stable conditions when the hydrogen production rate and the current generation did not change ostensibly (i.e., no trends were observable within a period of 24 h to 48 depending on the HRT), with variations not exceeding 20% of the average values.

MEC voltage scans were performed by changing the applied voltage between +0.2 V and +1.4 V in 0.2 V increments and 10 min intervals. The current was measured 10 min after the voltage setting was changed to allow for current stabilization. Internal resistance (R_{int}) was calculated as the slope of the linear section of the voltage scan plot. More details are provided in Manuel et al. (2010).

The MEC performance was characterized in terms of (i) volumetric hydrogen production rate, expressed in liters of hydrogen per liter of the reactor volume ($L_{H_2} L_a^{-1} d^{-1}$), (ii) coulombic efficiency (CE, %), calculated as the ratio of electrons evacuated from the anode relative to the total electrons available from COD consumption, (iii) cathodic conversion efficiency (CCE, %), calculated as the ratio of the electrons recovered as hydrogen gas to the total number of electrons that reach the cathode, (iv) power consumption, expressed as kilowatt-hour of electrical energy supplied by the power source per kg of COD removed ($kWh\ kg-COD^{-1}$), (v) energy production, calculated as kilowatt-hour of energy stored in the hydrogen and methane produced in the cathode per kg of COD removed ($kWh\ kg-COD^{-1}$) and (vi) net energy consumption, calculated as the difference between power

consumption and energy production. A detailed explanation of the calculation methods for these performance parameters can be found elsewhere (Escapa et al., 2009; Lee et al., 2009; Logan et al., 2006).

5.2.4. Analytical methods

Ammonium was determined by an ion-selective electrode (781 pH/Ion Meter de Methrom). The biological oxygen demand of the influent and effluent measurements was based on the pressure measurement in a closed system over 5 days. COD concentrations of the influent and effluent samples were measured using an automatic potentiometric titrator. The pH and conductivity of the influent and effluent were measured using a pH meter and a conductivity meter, respectively. More details can be found elsewhere (Escapa et al., 2012a). Total suspended solids and volatile suspended solids in the influent were determined (Eaton et al., 2005).

Cathodic off-gas composition was analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A four-meter-long column packed with HayeSep Q 80/100 followed by a one-meter-long molecular sieve column were used to separate methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen (H_2) and oxygen (O_2). The carrier gas was argon, and the columns were operated at 331kPa and 50°C.

5.3. RESULTS

5.3.1. COD removal and energy consumption

As table 5.2 shows, at the four lower OLRs in M_A (OLR_{MA}) (0.10 to 0.35 g-COD $L_a^{-1} d^{-1}$ in M_A), the COD removal in M_A increased steadily as OLR_{MA} increased, while low or negligible amounts of COD were removed in the second module (M_B) (Figure 5.2A). Only when the OLR_{MA} was set at or above 0.67 g-COD $L_a^{-1} d^{-1}$, a significant amount of the COD entering the

reactor was removed in M_B . Total COD removal efficiency in both modules ranged from 60 to 85% (Figure 5.2B) and it was maximized (together with the biological oxygen demand (BOD) removal efficiency) at an OLR_{MA} of $0.35 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$.

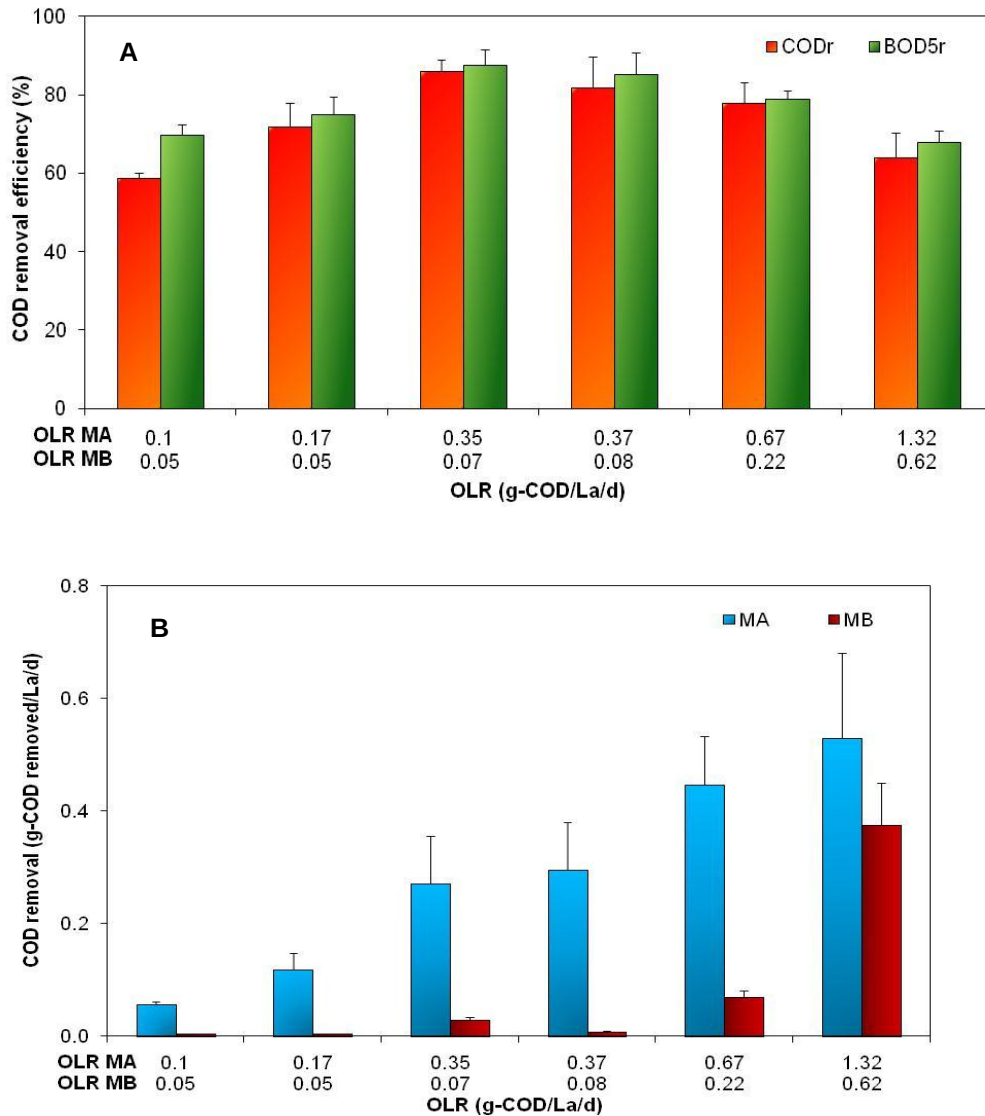


Figure 5.2. (A) COD removed in each module (M_A , module 1; M_B , module 2) under different OLRs; (B) COD and BOD_5 removal efficiency under different OLRs.

The relatively large differences registered in COD removal between the two modules at all OLR_{MA} tested (Figure 5.2A) did not correspond with the current densities observed (Table 5.2), resulting in relatively large energy consumptions (Figure 5.3) and columbic efficiencies in M_B (Table 5.2).

Table 5.2. MEC performance as a function of OLR in M_A and M_B .

	HRT	OLR	COD out ^a	Current	CH ₄ flow	CE	CCE
	(h)	(g L _a ⁻¹ d ⁻¹)	(mg-COD L ⁻¹)	(Am ⁻² Anode)	(mL L _a ⁻¹ d ⁻¹)	(%)	(%)
MODULE A	25	0.10	24.7	0.15±0.01	0.00	94.3	15.9
	23	0.17	25.6	0.14±0.01	0.00	39.6	17.6
	15	0.37	23.5	0.16±0.01	0.05	19.2	13.3
	11	0.35	19.0	0.19±0.00	0.43	24.6	8.0
	7	0.67	32.3	0.20±0.01	2.40	15.3	17.6
	4	1.32	70.1	0.22±0.01	2.91	10.6	24.3
MODULE B	25	0.05	22.2	0.10±0.01	0.00	719.6	4.4
	23	0.05	23.3	0.11±0.01	0.00	806.9	2.5
	15	0.07	20.7	0.15±0.02	0.01	568.5	3.3
	11	0.08	12.0	0.20±0.01	0.42	229.7	7.9
	7	0.22	22.3	0.22±0.01	0.30	111.8	3.3
	4	0.62	40.3	0.17±0.00	6.13	44.8	54.7

^aNote that CODout in M_A corresponds to CODin in M_B .

The effluent of M_A consistently maintained a COD concentration in the range of 19-32 mg-COD L⁻¹ at OLR_{MA} below 1.32 g-COD L_a⁻¹ d⁻¹ (HRTs above 4h), increasing sharply to 70 mg-COD L⁻¹ when the OLR_{MA} was set at 1.32 g-COD L_a⁻¹ d⁻¹ (Table 5.2). Similarly, the COD concentration of the effluent of M_B averaged 20 mg-COD L⁻¹ when OLR_{MA} was set below 1.32 g-COD L_a⁻¹ d⁻¹.

d^{-1} (HRT above 4h), doubling to 40 mg-COD L^{-1} at an OLR_{MA} of $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$. Only when the OLR_{MA} was above $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRT at 4 and 7 hours) the energy consumption was lower than $1 \text{ kWh kg-COD}^{-1}$ (Figure 5.3). Notably, with an OLR_{MA} of $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ the net energy consumption fell below $0.5 \text{ kWh kg-COD}^{-1}$ (Figure 5.3).

NH_4^+ analysis in the influent and effluent showed that there was minimal ammonium reduction (data not shown).

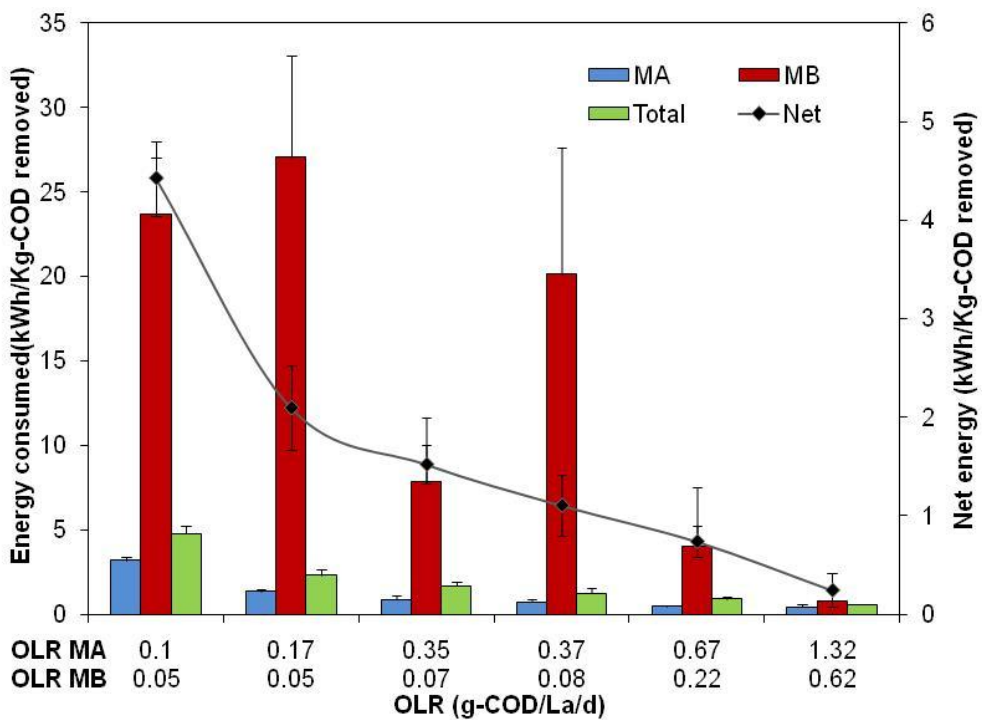


Figure 5.3. Energy consumption distribution in each module, total and net, under different OLRs.

5.3.2. Hydrogen production

The hydrogen production rate in the whole reactor ($M_A + M_B$) increased steadily with the OLR_{MA} , undergoing a rapid growth when the OLR_{MA} was above $0.37 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRT <11h) (Figure 5.4A). Notably, when the

OLR_{MA} and the OLR_{MB} were at $0.6 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ and above, the hydrogen production increased sharply. The gas production in M_A was consistently higher than in M_B , except when the OLR_{MA} was $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRT = 4h) (Figure 5.4A).

Cathodic conversion efficiency followed a similar trend to hydrogen production, ranging from 8%-18% and 3-8% in M_A and M_B , respectively, at OLR_{MA} below $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$, and experiencing a significant boost when OLR_{MA} was $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRT= 4h) (Table 5.2).

The proportion of methane in the cathodic off-gas was below 1% in both modules at OLR_{MA} below $0.37 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRTs of 11-25 h) and raised to 11% and 8.6% in M_A and M_B , respectively, with an OLR_{MA} of $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (Table 5.2).

The energy produced (as hydrogen and methane) was always lower than the energy consumed (Figure 5.3), which means that there is no net energy production during the process. Moreover, as the OLR_{MA} decreases, the difference between the energy consumed and the energy produced widens.

The hydrogen yield decreased with the OLR, achieving 0.2 and $0.9 \text{ mol H}_2 \text{ mol COD}^{-1}$ in M_A and M_B , respectively when OLR_{MA} was $0.10 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ (HRT=25h). These yields represent only 10% and 45% of the theoretical maximum H_2 yield ($2 \text{ mol H}_2 \text{ mol COD}^{-1}$). Interestingly, the hydrogen yield in M_B was consistently higher than that in M_A at all the OLRs tested (Figure 5.4B).

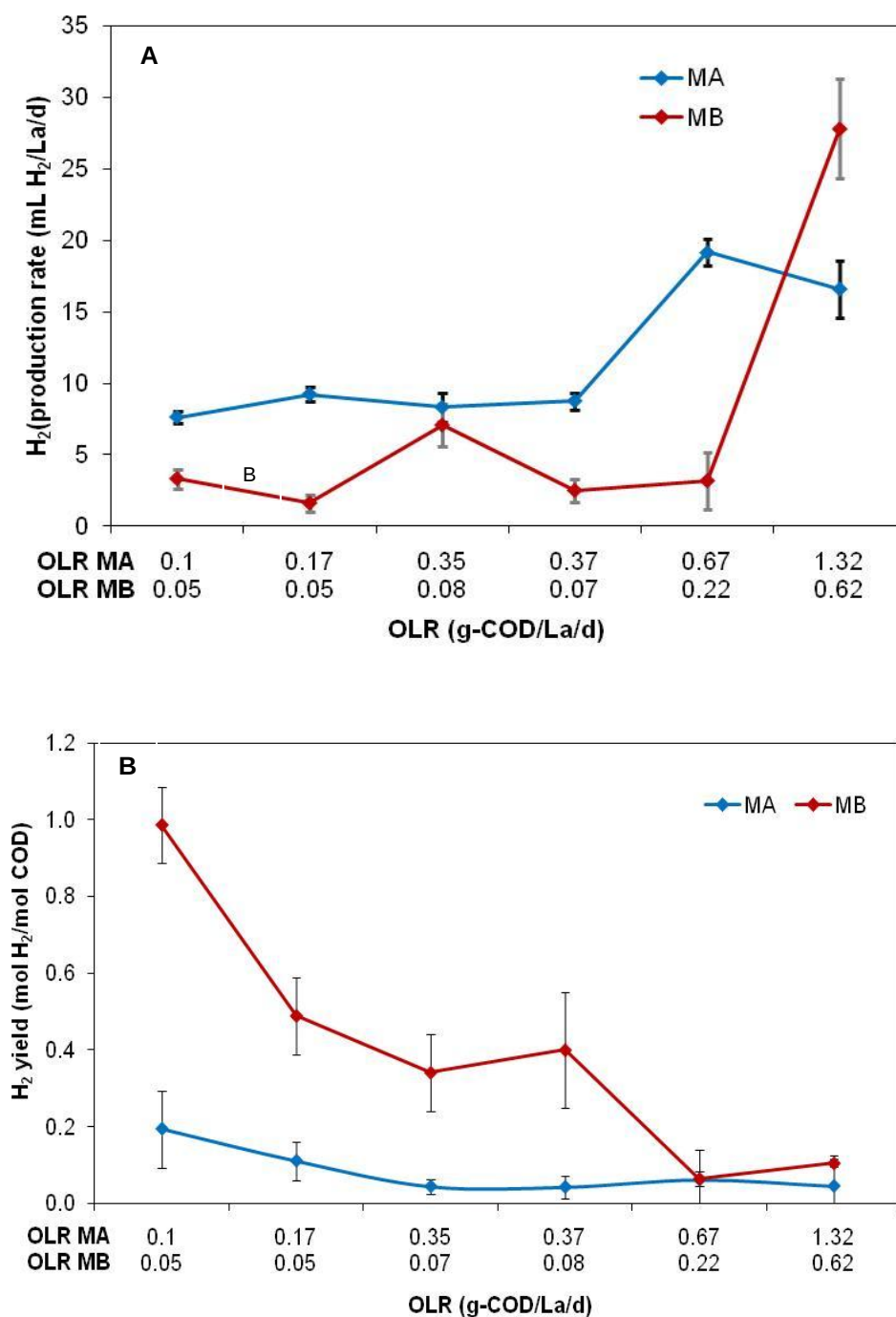


Figure 5.4. (A) Hydrogen production; (B) Hydrogen yield under different OLRs.

5.4. DISCUSSION

5.4.1. Influence of OLR on the COD elimination and hydrogen production rates

At medium-low $OLRs_{MA}$ (HRTs of 7-25 h) the COD concentration in the effluents of M_A and M_B (Table 5.2) was similar, which suggests that M_A (and therefore M_B) received a limited supply of COD. Apparently, the low COD concentrations inside the anodic chamber hampered the ARB activity, thus limiting the COD removal and hydrogen production. Still, the MEC was able to decrease the COD concentration of the influent by up to 86%, obtaining an effluent with a relatively low COD concentration. Although more than 90% of the total COD removal was attributable to M_A (Figure 5.2A), the electrical current produced in this module represented only 50-60% of the total current in the MEC (Table 5.2), which also resulted in large differences between the CEs computed in both modules. These discrepancies suggest that at least in the second module, a significant amount of current is related to non-COD-removal activity. Lee et al. (2009) and Parameswaran et al. (2009) have claimed that in membrane-less MECs, a significant amount of the hydrogen produced in the cathode can diffuse back to the anode where it is re-oxidized and converted into electricity, increasing artificially the current, although the existence of electrical short-circuits cannot be ruled out. Another possible explanation for the large CEs registered in M_B compared to those in M_A is that the main organics reaching M_B have already been fermented in M_A , therefore being more available for electrogenic bacteria. In fact, other authors have reported that MECs fed with non-fermentable substrates usually outperform those MECs fed with complex wastewaters in terms of CE (Ditzig et al., 2007). Conversely, the relatively low CE values computed in M_A suggested that part of the COD was removed by other mechanisms rather than electrogenic activity, which might be related to the complex nature of real wastewater and the limited

substrate accessibility of electrogenic bacteria in M_A . Lefebvre et al. (2011) found a similar result when a MFC was operated on real WW.

When the OLR_{MA} was set above $0.37 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ ($HRT < 11 \text{ h}$), the hydrogen production increased substantially in M_A , from 8.3 to $19.2 \text{ mL-H}_2 \text{ L}_a^{-1} \text{ d}^{-1}$ (Figure 5.4A). Interestingly, by further increasing the ORL to $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$, the hydrogen production did not improve, which suggests that it may be reaching saturation conditions (at least in the first module) at OLR_{MA} above $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$. This boost in hydrogen production at high OLR_{MA} was accompanied by an important increase of methane in the cathode off-gas, which might be related to the substantial increase in the input of organic matter to the reactor, creating a niche for the growth of methanogens under these favorable conditions. Moreover, the presence of fermentable substrates in the anodic chamber of a bioelectrochemical reactor has been associated with methanogenic activity (Parameswaran et al., 2009; Wang et al., 2009).

When the OLR_{MA} was set at $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ ($HRT=4\text{h}$), COD removal in both modules became more balanced (58% and 42% in M_A and M_B , respectively), and the hydrogen production in the second module was boosted to $27.8 \text{ mL L}_a^{-1} \text{ d}^{-1}$, outperforming the first module by almost 70% . This boost may be explained by the combined effect of the increased supply of organic matter at higher $OLRs$ and the presence of hydrolyzed and easily degradable components in the second chamber (Hallenbeck and Benemann, 2002).

Although the hydrogen production rates here reported are relatively low compared to those obtained in other MEC studies where easily degradable substrates such as acetic acid were fed to the reactor at a higher OLR (Escapa et al., 2012b), they are similar to or even higher than those rates reported for MECs fed with real WW (Table 5.3).

Table 5.3. Hydrogen production rates of MECs reported in various studies.

Study	Operation mode	Total reactor volume (L)	OLR (g-COD L _a ⁻¹ d ⁻¹)	H ₂ production (L-H ₂ L _a ⁻¹ d ⁻¹)
(Ditzig et al., 2007)	Batch	0.58	--- ^a	0.01
(Cusick et al., 2011)	Continuous	1000	0.7-2	0.07
(Escapa et al., 2012a)	Continuous	0.10	2	0.32
(Heidrich et al., 2012)	Continuous	120	0.14	0.0015
This study	Continuous	4	M _A =1.32; M _B =0.62	0.045 ^b

^a COD wastewater in= 368 mg L⁻¹ at a cycle of 30 ±5 hours.

^bNote that H₂ production corresponds to H₂ production in M_A+M_B.

Nonetheless, in a previous study carried out in this laboratory in a 100 mL membrane-less MEC fed with dWW, we achieved a hydrogen production of 0.32 L_{H2} L_a⁻¹ d⁻¹, which could be set as a target rate for future improvements in the cylindrical design (Escapa et al., 2012a).

The relatively low hydrogen yields achieved in both modules (Figure 5.4B) may be explained by low fermentation and hydrolysis rates. In fact, longer retention times are known to increase the hydrolysis rates, which would explain the improvement in hydrogen yield with increased retention times. In addition, the significantly higher yields achieved in M_B compared to those in M_A (especially at high HRTs) suggest that easily degradable fermentation products arriving at the second module are readily used by electrogenic microorganisms. This result supports the idea that a pre-acidification step would help to improve the hydrogen recovery (Gómez et al., 2011). Moreover, higher HRTs leads to low substrate concentration in the anodic chamber, which limits the growth of methanogens leading to a higher proportion of organics available for ARB (Esteve-Núñez et al., 2005; Lawrence and McCarty, 1969), and therefore increasing the hydrogen yield.

Total cathodic conversion efficiency (CCE) was relatively low (9-24%) compared to other studies where domestic wastewater was used as substrate (Ditzig et al., 2007). Low CCEs (Table 5.2) indicated either significant hydrogen loss or substantial percentage of the electrons which reached the cathode could have been involved in other parallel reactions apart from hydrogen evolution (Parameswaran et al., 2009) leading to an increase of current without increasing the net yield of hydrogen (Lee and Rittmann, 2010).

NH_4^+ analysis showed that there was minimal ammonium reduction. This low reduction implies that an additional treatment might be necessary for nitrogen removal, although previous research has confirmed ammonium reduction in BES (Aboutalebi et al., 2011).

5.4.2. Tubular modular configuration in series. Energy consumption and the selection of the optimal configuration

This study helped to estimate the OLR limits that make use of MEC technology in dWW treatment feasible. In fact, the limited COD removal in the M_B , together with its relatively large energy consumption and low H_2 production at OLR_{M_A} below $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$, clearly does not justify the use of this second module as a polishing step. Only when the OLR in M_A was set at $1.32 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ did M_B achieve a significant rate of COD removal with acceptable energy consumption.

Moreover, above $0.17 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ the energy consumption in the first module fell below the energy consumption threshold traditionally associated with aerobic dWW treatments ($1.5 \text{ kWh kg-COD}^{-1}$) (Metcalf & Eddy Inc., 2003). This low energy consumption combined with the relatively high quality of the effluent (in terms of COD concentration) at the exit of the first module when the OLR_{M_A} was set below $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$, suggests that,

at least in terms of energy consumption and treatment efficiency, a single MEC module may be competitive with aerobic methods for dWW treatment at OLRs in the range of $0.17\text{--}0.67\text{ g-COD L}_a^{-1}\text{ d}^{-1}$. A recent study has proved that MECs can be regarded as a competitive technology for dWW treatment (at least in terms of energy consumption and compared to conventional aerobic treatments) at OLRs as low as $0.14\text{ kg-COD m}^{-3}\text{ d}^{-1}$ (Heidrich et al., 2012). In this work the authors reported an energy consumption of $0.64\text{ kWh kg-COD}^{-1}$ when treating dWW in a 120L MEC.

At OLR_{MA} of $0.10\text{ g-COD L}_a^{-1}\text{ d}^{-1}$, even with the low COD concentration in the effluent of the first module, the relatively large energy consumption prevents MECs from being competitive with aerobic methods for dWW treatment.

There must be kept in mind that throughout the tests performed in the present study, the applied voltage in both modules was set at 1 V; therefore, different applied voltages may substantially modify the OLR limits from those previously discussed, and therefore further investigation on the effect of the applied voltage on MEC performance will help to increase its energy efficiency. For instance, using an optimization tool such as that described by Tartakovsky et al. (2011) could significantly reduce the global energy consumption.

5.5. CONCLUSIONS

The MEC reactor was able to reduce up to 85% of the chemical oxygen demand of a domestic wastewater, with a net energy consumption lower than that typically associated with aerobic treatments of domestic wastewater.

The OLR of $0.10\text{ g-COD L}_a^{-1}\text{ d}^{-1}$ can be established as the threshold above which the tubular MEC starts to compete with aerobic treatments in terms of

energy consumption and COD concentration in the effluent. Likewise, an OLR of $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ was selected as the threshold above which the addition of a polishing module operating in series with the first module is recommended. This study also proves the feasibility of scaling up our design by adding modules in series for use at OLRs high enough to warrant these additional modules. However, new developments are required for nitrogen removal and management of the hydrogen produced to make this technology fully competitive with aerobic treatments and to achieve practical implementation

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Chapter 6

Performance of a semi-pilot tubular microbial electrolysis cell (MEC) under several hydraulic retention times and applied voltages

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6.1. INTRODUCTION

Microbial electrolysis cells (MECs) are electrochemical devices that employ electrogenic bacteria to produce hydrogen by oxidizing organic matter (using the anode as an electron acceptor) through the hydrogen evolution reaction at the cathode of the electrochemical reactor. This process requires a small amount of electrical energy compared to hydrogen production through water electrolysis and also has the additional advantage of simultaneously treating biowastes or wastewaters (Logan et al., 2006; Gómez et al., 2011), offering a renewable and potentially autonomous energy technology for wastewater treatment (Logan et al., 2006; Logan, 2004; Rozendal and Buisman, 2005; Rozendal et al., 2006).

MECs are a relatively new technology, and therefore few scale-up experiences have been reported so far. In a previous work it was demonstrated a successful scale-up of the combined wastewater treatment and H₂ production process from a 50 mL laboratory MEC to a 10 L MEC (Gil-Carrera et al., 2013a). Yet, other studies conducted at a larger scale (120-1000 L), revealed that even though it is a promising technology for urban and industrial wastewater treatment, several difficulties still need to be overcome (Cusick et al., 2011; Heidrich et al., 2012).

The scaling-up of tubular MECs (all the above referred scaling-up experiences were carried-out on flat designs) has also proven to be feasible, achieving 85% COD removal using domestic wastewater as substrate in a semi-pilot 4-L reactor with relatively low energy consumption (Gil-Carrera et al., 2013b). Furthermore, it has been suggested that a tubular design provides a nearly optimal distribution of the anode and cathode in bioelectrochemical systems (BES) (Kim et al., 2010), yielding production rates and energy efficiencies of 2.3 L L_a⁻¹ d⁻¹ and 240%, respectively, at medium-low applied voltages (Hu et al., 2009). Tubular

MFCs have also achieved moderate power outputs and removed up to 80% of the COD of the wastewater (Liu et al., 2004).

In an effort to further assess the ability of the tubular design for urban wastewater treatment, the present study tries to identify the influence of different hydraulic retention times (HRTs) and applied voltages (Vapps) on the performance of the reactor in terms of energy consumption, hydrogen production and chemical oxygen (COD) demand removal. The MEC reactor consisted of two tubular units hydraulically connected in series. A similar configuration has been previously used to assess the ability of an MEC semi-pilot reactor to treat real wastewater (Gil-Carrera et al., 2013b).

6.2. MATERIAL AND METHODS

6.2.1. Analytical methods and MEC characterization

The biological oxygen demands of the influent and effluent measurements were based on pressure measurements in a closed system over 5 days. COD concentrations of the influent and effluent samples were measured using an automatic potentiometric titrator. The pHs and conductivities of the influent and effluent were measured using a pH meter and a conductivity meter, respectively. More details can be found elsewhere (Escapa et al., 2012a). Total suspended solids and volatile suspended solids in the influent were determined (Eaton et al., 2005). Cathodic off-gas composition was analyzed using a gas chromatograph (Varian CP 3800 GC) equipped with a thermal conductivity detector. A four-meterlong column packed with HayeSep Q 80/100 and a subsequent a one-meter long molecular sieve column was used to separate methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen (H_2) and oxygen (O_2). The carrier gas was argon, and the columns were operated at 331k Pa and 50°C.

MEC voltage scans were performed by changing the applied voltage between +0.2 V and +1.4 V in steps of 0.2 V at 10 min intervals at each voltage setting to allow for current stabilization.

The performance of the MEC was characterized in the following terms: (i) volumetric hydrogen production rate expressed in liters of hydrogen per liter of the reactor volume ($L_{H_2} L_a^{-1} d^{-1}$), (ii) coulombic efficiency (CE, %) calculated as the ratio of electrons evacuated from the anode relative to the total electrons available from COD consumption, (iii) cathodic conversion efficiency (CCE, %) calculated as the ratio of the electrons recovered as hydrogen gas to the total number of electrons that reached the cathode, (iv) energy consumption expressed as kilowatt-hours of electrical energy supplied by the power source per kg of COD removed ($kWh\ kg-COD^{-1}$), (v) energy production calculated as kilowatt-hours of energy stored in the hydrogen and methane produced in the cathode per kg of COD removed ($kWh\ kg-COD^{-1}$), and (vi) net energy consumption calculated as the difference between energy consumption and energy production. Detailed explanations of the calculation methods for these performance parameters can be found elsewhere (Logan et al., 2006; Escapa et al., 2009).

6.2.2. Microbial community analysis

Total chromosomal DNA was extracted from the anodic biofilms using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratory Inc., USA). For DNA extraction and purification, the 16S rDNA fragments were amplified by PCR (polymerase chain reaction). The region corresponding to positions 357 and 518 in the 16S rDNA of *Escherichia coli* was PCR-amplified using the forward primer EUB357f (5'-CCTACGGGAGGCAGCAG-3') with a GC clamp (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCGCCCC-3'). The 5'-end of forward primer is rich in GC sequences, which prevents the PCR products from completely melting during separation. PCR amplification was conducted in an automated thermal cycler (GeneAmp

PCR System 9700, Applied Biosystem, USA) following the protocol of the manufacturer. PCR mixtures had final volumes of 20 μ l. PCR products were electrophoresed on 1% (wt/vol) agarose gel in 1 $\times$ TAE for 90 min at 60V and then checked with GelRedTM Nucleic Acid Gel Stain (Biotium Inc, USA) to confirm the amplification.

Gene cloning and 16S rRNA gene sequencing for community analysis were conducted a previously described (Call et al., 2009). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and then ligated to vector PJET1.2 (Invitrogen) and transform the recombinant vector into competent cells *E. coli* following the manufacturer's protocol. Appropriate colonies were placed in a 96-well format on LB plates (Ampr 50 ml/ml). Plasmid extractions were carried out on these colonies using the E-Z 96 Fast filter_ Plasmid Kit_ (www.omegabiotek.com/). Once extracted, plasmids were sequenced with the BALF (5'-AGAGTTTGATCCTGGCTCAG-3') and BALR (5'-GGTTACCTTGTTACGACTT-3'). Primers using an ABI PRISM® 3100 (Applied Biosystems), 16S rRNA gene Sequences were analyzed using the BLASTN search tools.

DGGE (denaturing gradient gel electrophoresis) was carried out using the DcodeTM Universal Mutation Detection System (BioRad, California, USA). PCR products were electrophoresed in 1 $\times$ TAE buffer for 480 min at 70 V and 60°C on polyacrylamide gel (7.5%) containing a linear gradient ranging from 40% to 60% denaturant. After electrophoresis, the polyacrylamide gel was stained with GelRedTM Nucleic Acid Gel Stain for 30 min and then visualized on a UV transilluminator. Most of the bands were excised from the DGGE polyacrylamide gels for 16S rDNA sequencing. DNA fragments from the excised bands were PCR-amplified with the forward primer EUB357f without a GC clamp and the reverse primer UNIV518r. After PCR amplification, PCR products were purified using a Macherey-Nagel Nucleic

Acid and Protein Purification kit (Clontech, USA). All strands of the purified PCR products were sequenced with the EUB357f primer using an ABI PRISM Big Terminator Cycle Sequencing Kit and an Amersham MegaBace DNA sequencer (GE Healthcare, USA) according to the manufacturer's instructions. Sequence data were analyzed with Chromas Lite 2.01 software (Ibis Bioscience, Carlsbad, CA, USA) and submitted to the non-redundant nucleotide database at GenBank using the BLASTN facility.

6.2.3. Microscopy analysis

Carbon felt samples, 1 cm x 1 cm x 0.5 cm were taken from the middle and bottom of each anode's layer, rinsed with phosphate buffer solution (PBS), and then the samples were fixed overnight in PBS plus 2.5% glutaraldehyde in 4°C. The fixed specimens were rinsed three times in sterile buffer solution, and then dehydrated by a graded ethanol series (30, 50, 70, 80, 95, and 100%; 30 min each stage with very gentle periodic agitation). Electrode pieces were mounted on aluminum specimen mounts with contact adhesive, and were sputter coated in a Sputter Coater. The scanning electron microscope (SEM) (JEOL 6100, Japan) was operated.

6.2.4. MEC configuration and operation

The MEC examined in this study was a continuous-flow single-chamber MEC that consisted of two tubular units (M_A and M_B , 2 L each) connected in series. Each module consisted of an anodic compartment and a gas collection (cathodic) compartment (Figure 6.1). Each anodic compartment retained 2000 mL of liquid with a headspace of 200 mL and contained two layers of carbon felt. The cathode was a 23 cm x 36.5 cm gas diffusion electrode (Sigracet GDL 25 BC carbon paper) with electrodeposited nickel (nickel load of 0.4 mg cm^{-2}). Further details can be found elsewhere (Gil-Carrera et al., 2013b).

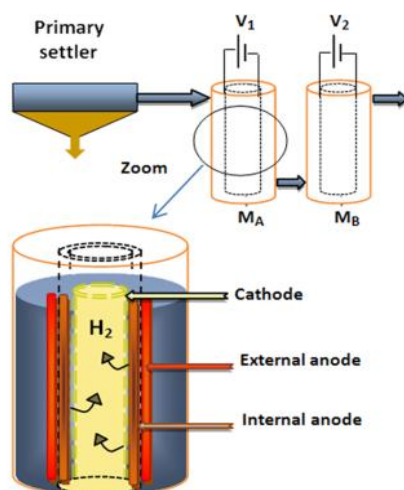


Figure 6.1. Scheme of the two-modular tubular MEC.

6.2.5. MEC operation and experimental design

The MEC units were inoculated with the effluent from other dWW-fed MECs operated in the same lab for more than 2 years. After inoculation, the MEC was operated in continuous-flow mode with domestic wastewater. The domestic wastewater used in this study was collected from a primary settler of a wastewater plant in León (Spain). A different batch of wastewater was consumed in each HRTs tested. The wastewater was characterized in terms of the general physico-chemical parameters, which are shown in Table 6.1.

Table 6.1. Characteristics of the five sets of municipal wastewater used in this study (duplicate or triplicate measurements) ND (not determined).

HRT	V_{M_A/M_B}	OLR in ($\text{g La}^{-1} \text{d}^{-1}$)			CODin	Conductivity	pH	TSS	VSS
(h)	(V)	Total	M_A	M_B	(mg L^{-1})	($\mu\text{S cm}^{-1}$)		(mg L^{-1})	(mg L^{-1})
10	1/1	0.29	0.58	0.16	121.0	617.0	6.9	ND	ND
	0.6/1	0.23	0.46	0.19	95.9	579.3	7.3	42.2	35.1
7	1/1	0.37	0.74	0.27	107.1	524.0	7.4	54.6	40.7
	0.6/1	0.32	0.64	0.32	93.0	576.0	7.3	71.2	64.5
4	1/1	0.66	1.32	0.79	112.2	605.0	7.3	47.0	42.4
	0.6/1	0.79	1.59	0.90	135.0	637.0	7.2	39.1	36.5

Wastewater was continuously pumped (Dosiper C1R; León, Spain) to the anodic chamber of the first module. The flow rate was controlled to obtain the desired hydraulic retention time (HRT). The MEC was operated for more than 3 months before testing, to allow the microbial community to colonize the anode.

A peristaltic pump (Dosiper C1R; León, Spain) was used to establish an external recirculation loop flowing at a rate of 500 mL h⁻¹ that was installed to mix the liquid in the anodic chambers of every module.

The temperatures of the MECs were maintained at 20°C with a thermocouple placed in the anodic chambers of the first unit, a temperature controller (PCI-6221; National Instruments, Austin, TX) and heating plates located on the anodic chamber side of each module.

The MEC electrical outputs of each module were monitored separately, and the anodic chambers were individually controlled. Two adjustable DC power supplies (made in-house) were used to maintain the voltage at the predetermined set point. The power supplies were computer controlled (data were recorded at 30 min intervals) using an analog output board (PCI-6713; National Instruments, Austin, TX).

The gas production of each unit was measured with an MGC-1 milli-gas counter (Ritter Co, Bochum, Germany).

The MECs were operated at several organic loads ranging from 0.46 to 1.59 g-COD L_a⁻¹ d⁻¹ under three hydraulic retention times (10, 7 and 4 hours). The OLRs presented in table 6.1 were computed for the entire reactor and for each module independently, and the HRT corresponds to the whole reactor. The hydraulic retention times were selected based on previous studies (Gil-Carrera et al., 2013b). At each hydraulic retention time, the first module was operated at two different applied voltages (1V and 0.6 V), while the second

module of the MEC was maintained at 1V throughout the entire study. Vapps were randomly selected to minimize the effect of microbial adaptation on MEC performance (Tartakovsky et al., 2008).

6.3. RESULTS AND DISCUSSION

6.3.1. Effect of HRT and Vapp on COD removal

COD and BOD₅ removal efficiencies were moderately affected by the HRT (Figure 6.2A), with COD removal efficiency decreasing from 79% to 64% as HRT decreased from 10 to 4 hours respectively (Vapp= 1V in both modules), and most of the COD fed to the reactor being removed in the first module (M_A) (Figure 6.2B). In an attempt to achieve a more balanced COD removal, Vapp in M_A was reduced to 0.6 V (Vapp in M_B was maintained at 1V), with the hope that more carbon source would reach M_B, thus improving the overall COD removal efficiency while reducing the energy consumption in M_A. The amount of COD removed in each module became indeed slightly more balanced at medium-high HRTs, but at the expense of a significant reduction in COD removal in M_A accompanied by only a moderate increase in M_B (Figure 6.2B). Contrary to what was expected, by changing the Vapp from 1.0 to 0.6 V at HRT of 4 h, the amount of COD removed in the whole reactor slightly increased (Figure 6.2A), suggesting that non-electrogenic microorganisms might be consuming a relatively large amount of the organic matter fed to the reactor, thereby deteriorating the general performance of the MEC.

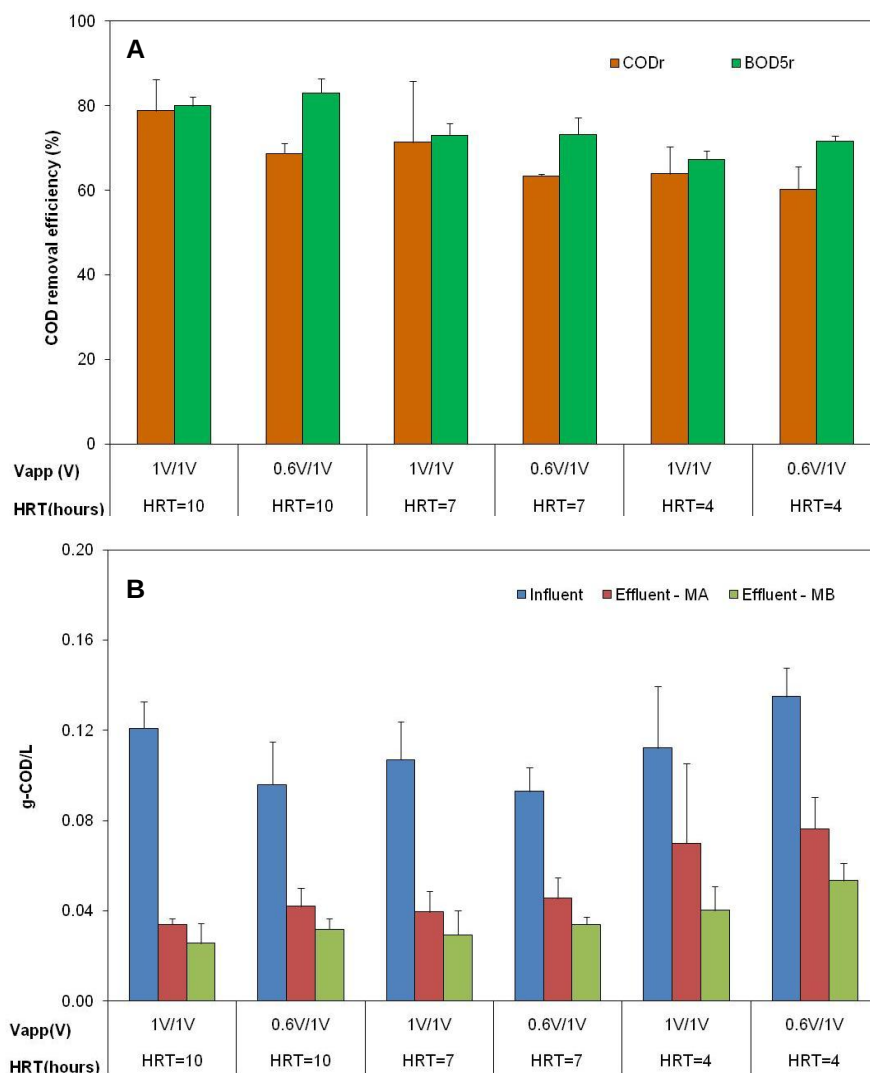


Figure 6.2. (A) COD and BOD₅ removal efficiency under different HRTs and Vapps. (B) COD concentration in the influents and the effluents of each module under different HRTs and Vapps.

In addition, the low total CEs (Table 6.2) achieved in all experiments indicates that a large percentage of electrons were not successfully transferred into current, confirming the presence of mechanisms independent of electrogenic microorganism.

Table 6.2. Electrical parameters as a function of HRT and V_{app} . Cathodic conversion efficiency was calculated based on H_2 flow.

HRT (h)	Voltage M_A/M_B (V)	Current M_A/M_B $A\ m^{-2}$ Anode	CCE (%)			CE (%)		
			Total	M_A	M_B	Total	M_A	M_B
10	1/1	0.19/0.22	8.7	20.1	4.8	30.7	15.4	189.3
	0.6/1	0.04/0.20	4.8	0.00	6.4	28.8	5.02	126.5
7	1/1	0.16/0.19	9.3	7.5	9.4	23.2	12.1	95.9
	0.6/1	0.06/0.21	21.2	0.00	30.9	21.8	6.4	90.7
4	1/1	0.22/0.19	23.9	24.3	40.6	16.8	10.6	44.8
	0.6/1	0.06/0.17	15.3	0.00	21.1	9.3	3.1	21.8

A computation of the CE independently in each module revealed that this parameter was consistently much lower in M_A than in M_B . This result might be explained by one or both of the following phenomena: (i) main organics reaching the second module have already been fermented, therefore being more available for electrogenic bacteria. Previous research has reported that CEs are higher in MEC tests with non-fermentable compounds than with fermentable sugar and complex synthetic wastewaters (Gil-Carrera et al., 2013a) and domestic wastewater (Ditzig et al., 2007). Lefebvre et al., (2011) found similar results when an MFC was operated on real wastewater, which was explained by the complex nature of real wastewater and the limited substrate accessibility of electrogenic bacteria. Interestingly, this work confirmed that the vertical configuration of tubular reactors allowed sludge accumulation at the bottom of the anodic compartment, favoring the proliferation of microorganisms that compete with electrogenic bacteria for substrate uptake. (ii) CEs above 100% in M_B strongly suggest that at least in this module a significant hydrogen recirculation was taking place. This phenomenon has been observed in planar and tubular MECs in previous studies (Gil-Carrera et al., 2013a, b). (iii) As shown in Table 6.2, CE

increases with the HRT, suggesting that at low HRTs the substrate uptake by bacterial communities other than electrogenic bacteria might be favored.

Once the tests were completed, the MEC was opened and we observed a small tendency for sludge accumulation at the bottom of the anodic compartment, where fermentation most likely took place (Lefebvre et al., 2011). Furthermore, Ren et al. (2011) found aggregates of dead and dormant cells and non electrogenic bacteria accumulated in the biofilm which do not contribute to electrogenic metabolism and may be responsible for part of the organics' degradation. This finding is consistent with the large clumps or agglomerations of biomass found in the anodic biofilm of this set-up (see the supplementary information for the lower SEM magnification (400x) of the anode's samples).

The gene cloning of the amplified 16S rRNA gene fragments from the anode community of this set-up showed the presence of non-electrogenic bacteria (Figure 6.3): *Syntrophus sp.* (reported as anaerobic bacterium that, in culture, degrades fatty acids in syntrophic association with hydrogen-utilizing micro-organisms (Hatamoto et al., 2007)), *Trichococcus sp.* (involved in acid production. These bacteria are characterized by heterotrophic fermentation and are usually found in active sludge (Bergey and Holt, 1994) and they might degrade organic matter in MECs as well), *Chlorobium limicola* (it consumes the hydrogen produced in fermentative process (Silke et al., 1999)) and *Clostridium sp.* (reported to take part in fermentation processes). Interestingly, the latter was found mainly in the external layer of the anode which is in direct contact with the raw wastewater fed to the reactor. This observation together with the prevalence of electrogenic microorganisms found in the internal layer (Figure 6.3), suggests that complex organic matter was degraded to simpler compounds in the external section of the anode, while the microorganisms found in the inner anode used these organics to produce electricity.

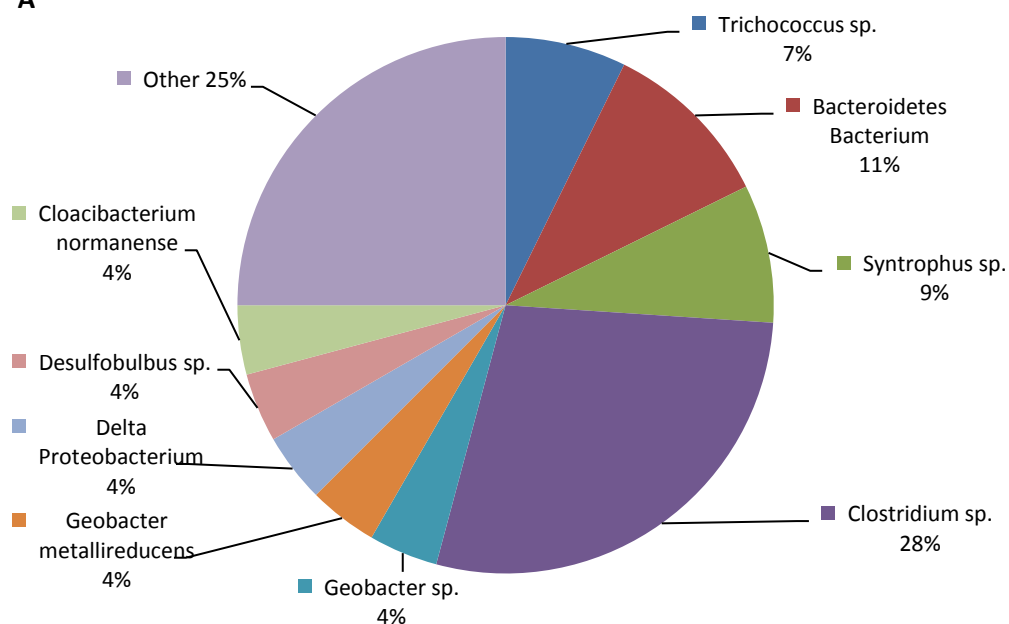
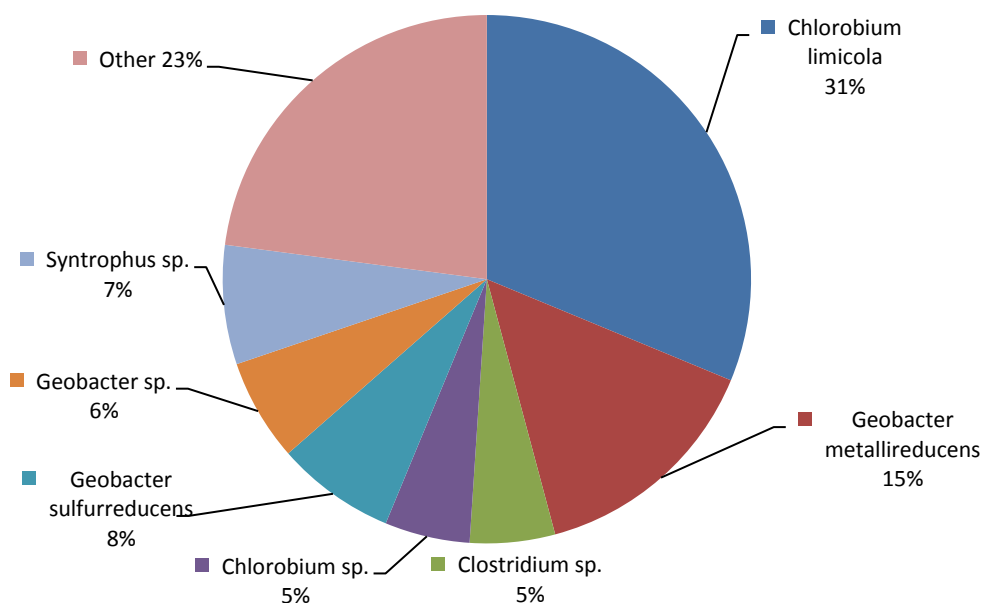
A**B**

Figure 6.3. Anodic bacterial communities based on cloned 16S rRNA gene sequences. (A) External anode, (B) Internal anode.

6.3.2. Effect of HRT and V_{app} on gas production

When the applied voltage was set at 1V in both modules, a reduction in the HRT from 10 to 4h led to a significant improvement in the rate of hydrogen production (Table 6.3) which might be explained by an increase in the amount of organic matter fed to MEC. Moreover, at HRTs of 7 and 4 h, hydrogen production in M_B was consistently higher than in M_A regardless of the V_{app} , which suggests that a higher amount of organic compounds reached M_B and a significant amount of the organic matter was already fermented in the first module, therefore being easily available for electrogenic bacteria at medium-low HRTs.

Table 6.3. Gas production as a function of HRT and V_{app} .

HRT (h)	V M_A/M_B (V)	H ₂ flow (mL d ⁻¹)			CH ₄ flow (mL d ⁻¹)		
		Total	M_A	M_B	Total	M_A	M_B
10	1/1	37.6	28.9	8.7	1.5	1.5	0.0
	0.6/1	11.5	<0.1	11.5	0.6	<0.1	0.6
7	1/1	32.7	12.7	21.8	2.0	0.8	1.2
	0.6/1	52.7	<0.1	51.9	5.0	0.0	5.0
4	1/1	89.4	33.2	55.6	19.6	6.4	13.2
	0.6/1	31.1	<0.1	30.0	5.5	<0.1	5.5

By changing the V_{app} in M_A from 1.0 to 0.6 V, the current density and notably the hydrogen production declined sharply. Hu et al. (2008) also found that a reduction in the V_{app} by 0.25 V results in a 55-70% decrease in hydrogen production rates. The relatively low H₂ production (12-89mL d⁻¹) and CCEs obtained in this study compared to other works were wastewater was used as a substrate too (Escapa et al., 2012a; Ditzig et al., 2007), suggest the existence of significant H₂ losses either (i) through the tubing used to record gas production or by proton recycling and hydrogen consumption by homo-acetogens, hydrogenotrophic methanogens or ARBs,

(ii) an internal recycling of hydrogen that may be consumed by ARBs and homo-acetogens (Lee and Rittmann, 2010; Parameswaran et al., 2011) or (iii) that a substantial percentage of the electrons that reached the cathode were involved in other parallel reactions apart from hydrogen evolution (Parameswaran et al., 2009), which would lead to an increase in current without increasing the net yield of hydrogen (Lee and Rittmann, 2010).

Methane formation inside the anodic chamber is another factor that determines the reduced energy conversion in bioelectrochemical reactors as methanogens compete with electrochemical microorganisms for substrate uptake (He et al., 2005; Kim et al., 2005; Rabaey et al., 2005). Methane production in the tubular reactor followed the same trend as hydrogen regarding HRT, although there was no clear dependency on V_{app} (Table 6.3), suggesting that organic matter rather hydrogen is the most likely source of methane. In fact it was observed an increase in methane formation with the organic loading rate, implying that at higher substrate concentrations, it will be difficult to prevent methane production in MECs (i.e. relatively large substrate concentrations in the anodic chamber might create a niche for the growth of methanogens under favorable environments).

6.3.3. Effect of HRT on energy consumption/recovery

Both HRT and applied voltage exhibited a clear influence over energy efficiency as shown in Figure 6.4. For instance a decrease from 10 to 4 hours in the HRT reduced the amount of electrical energy consumed from 1.02 to 0.56 kWh kg⁻¹-COD (kilowatt hour per kilogram of COD removed), whereas an increase from 0.6V to 1V in M_A led to increments of 0.12 and 0.28 kWh kg-COD⁻¹ at HRTs of 10 and 4 hours, respectively. Interestingly, these values are lower than the average energy consumption threshold traditionally associated with aerobic dWW treatments (0.7-2 kWh kg-COD⁻¹) (Pant et al., 2011), which reveals the great potential of MEC technology for

domestic wastewater treatment, and demonstrates a promising opportunity to scale-up.

However, the lowest value of energy consumption ($0.23 \text{ kWh kg-COD}^{-1}$) is below the threshold of energy consumption required for the hydrogen evolution reaction to occur in an MEC ($0.47 \text{ kWh kg-COD}^{-1}$, assuming the $V_{app} = 0.11 \text{ V}$). Similar results have also been found in previous studies where an energy consumption of $0.11 \text{ kWh kg-COD}^{-1}$ was achieved in an MEC fed with VFAs at a HRT of 12 h and an applied voltage of 0.6 V (Escapa et al., 2012b). This discrepancy is mainly attributable to substrate removal by microorganisms other than ARBs, which makes computation of the real value of energy consumption difficult. This is consistent with the microbial diversity analysis, which confirmed the presence of other non-electrogenic microorganisms as discussed above.

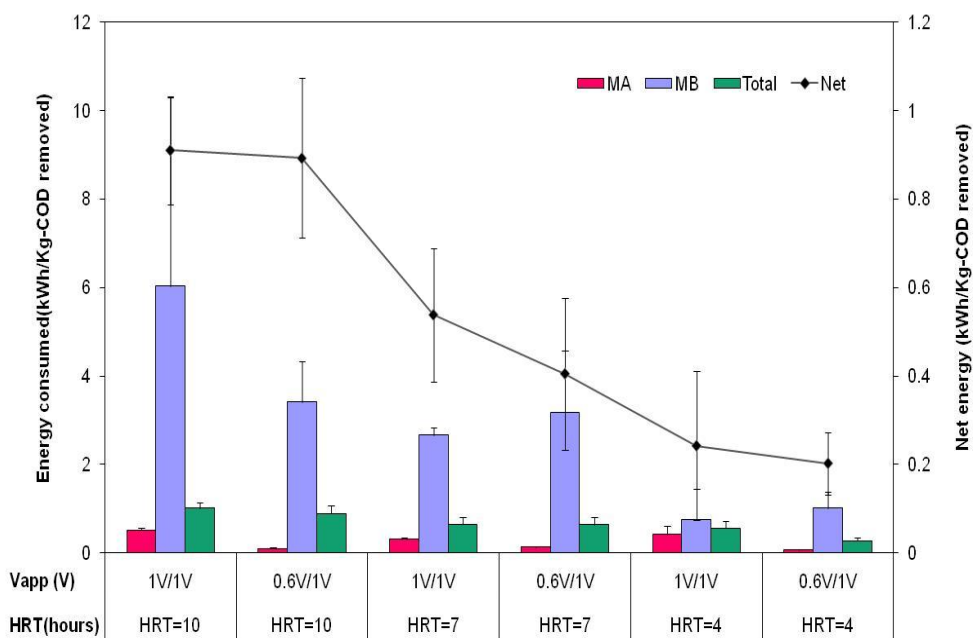


Figure 6.4. The Energy consumption distributions of each module, total and net, under different HRTs and Vapps.

6.3.4. Selection of the operational conditions (HRT and applied voltage)

Even though optimal operating conditions would depend on several factors such as effluent water quality standards and the type and composition of the influent wastewater, this study also tries to offer a preliminary estimation of the applied voltages and HRTs limits that would make feasible the use of a tubular MEC in a dWW treatment plant for COD removal and hydrogen production. Based on the results above presented, we can assume that a HRT of 4 hours could be selected as the threshold below which the addition of a polishing module operating in series with the first module is recommended. In contrast, HRTs above 4 hours would not require a second module due to the low COD removal and the high energy consumption found in M_B . Moreover, by using only one module at HRTs of 10 and 7 hours the effluent reaches a relatively high quality (in terms of COD concentration) at relatively low energy consumption.

Regarding the applied voltage, a voltage of 1 V in each module would be required to achieve an acceptable performance at relatively low energy consumption since, as it was observed, an applied voltage of 0.6V the overall COD removal efficiency as well as the total hydrogen production would be reduced.

6.4. CONCLUSIONS

The HRT and V_{app} showed significant influences on hydrogen production and energy consumption in a tubular semi-pilot MEC fed with dWW. In contrast COD elimination rate was less affected by these two parameters due to the presence of non-electrogenic microorganism. HRTs below 4 hours would require two MEC modules operating in series to achieve a relatively high quality (COD concentration) effluent while removal efficiencies above 70% were achieved with only one module at HRT=10h.

This study also confirms that the net energy consumption associated to wastewater treatment in an MEC is lower than that traditionally attributed to aerobic treatments.

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Chapter 7

***Carbon footprint analysis in a
domestic wastewater treatment
plant with an integrated
microbial electrolysis cell and
comparison with a conventional
wastewater treatment plant***

7.1. INTRODUCTION

One of the highest contributions to the climate change is the anthropogenic greenhouse gas (GHG) (Soloman et al, 2007). During the last 200 years atmospheric concentrations of greenhouse gases, CO₂, CH₄ and N₂O have significantly increased due to anthropogenic activities such as extraction, production and use of fossil fuels and other industrial activities (El-Fadel and Massoud, 2001). Wastewater treatment contributes to greenhouse gases through production of CH₄ and/or CO₂ from treatment processes and from CO₂ produced from the energy required for the system. CH₄ produced from sewage treatment was found to constitute about 5% of the global methane sources (El-Fadel and Massoud, 2001).

Another process that contributes to the increase of the GHG is the treatment of organic waste and biomass residue produced by society. A high amount of this waste is in the water and to eliminate it, the WW treatment often demands high infrastructure and usually uses a great amount of energy. Nevertheless, WW may be an additional significant source of renewable energy. Nowadays, there is growing consensus that organic components in the wastewater are a resource instead of an increasing problem (Verstraete and Vlaeminck, 2011, Sutton et al., 2011, McCarty et al., 2011).

In Spain the wastewater sector has increased 6% in the last 10 years (DBK, Depuración de aguas, 2011). Wastewater treatment requires 1% (2.672 GWh year⁻¹) of the national electricity consumption to treat 3.000 hm³ of domestic wastewater per year. That means a required power of 305 MW and an average consumption of 0.67 kWh m⁻³. Most of the wastewater treatment plants in Spain consume 50 kWh/ (h.e.year). The energy consumed in the wastewater treatment plants could be reduced by 17.5% if we optimize the systems and manage to use the energy in the wastewater (IEA, 2011). This higher efficiency would lead to a better carbon footprint.

Among the available technologies to treat wastewater, some consume energy as in the case of conventional treatments that remove the organic matter via aerobic processes which are energy expensive. Typically 3% of the electrical energy usage of many developed countries (Curtis, 2010), while others technologies, such as anaerobic digestion, are able to produce renewable energy from available organic matter (Metcalf & Eddy Inc., 2003).

Replacing the aerobic activated sludge process with an anaerobic process means the energy stored in the organic content of the wastewater is converted to methane (80% efficiency) which can be combusted to produce electricity (35% efficiency) (McCarty et al., 2011). Only around 30% of the total energy in the wastewater can be captured as electricity in anaerobic systems, although with heat exchange in the combustion process, or the use of non-combustion methods of conversion, this could be increased (McCarty et al., 2011).

One novel promising technology of producing energy from low-strength wastewaters are microbial electrochemical systems. Microbial electrolysis cells (MECs) are electrochemical devices that produce hydrogen by combining the ability of electrogenic bacteria to oxidize organic matter (using the anode as an electron acceptor) with the hydrogen evolution reaction at the cathode. Although this process requires the input of a small amount of electrical energy, the specific energy consumption is lower than that typically associated with hydrogen production through conventional water electrolysis (Logan, 2004; Rozendal and Buisman, 2005; Rozendal et al., 2006). In addition, the utilization of MECs in the treatment of wastewater in general, and domestic wastewater in particular, may provide further environmental benefits through reduction of the CO₂ footprint (Foley et al., 2010) and reduction of the energy use typically associated with the chemical oxygen demand (COD) removal by aerobic treatment (Greenberg et al., 1992).

The objectives of this paper are:

- To estimate carbon footprint from domestic wastewater treatment in a WWTP with an MEC and a conventional WWTP with activate sludge treatment.
- To compare two wastewater treatments systems mentioned above.
- To identity the critical steps and the factors with higher contribution to the carbon footprint in both systems.
- To identify the reasons behind a high carbon footprint and suggest reduction potential.

7.2. MATERIAL AND METHODS

7.2.1. Carbon footprint methodology

The product carbon footprint (PCF) was calculated for a conventional WWTP and a WWTP with an integrated bioelectrochemical system, based on the O2C™ tool. The O2C™ carbon calculator allows to develop an evaluation of the GHG emissions at water treatment plants such as desalination plants, drinking water production plants or wastewater treatment and recycling plants.

This tool was developed by Degrémont in collaboration with Pricewater House. An exhaustive study of the sources of the emissions below before developing its calculator was conducted in:

- Energy consumption (fuel, electricity, natural gas, etc.).
- Procedures specific to water and waste management activities (biological treatment, etc.).
- Production of inputs (reagents, consumables, construction materials, equipment, etc.).
- Movement of persons.

- Transport of goods, sludge, waste, materials (incoming freight, internal freight, outgoing freight).
- Waste treatment and sludge processing.

The O2C™ tool is based on Life Cycle Analysis (LCA) and the greenhouse gas metrics (ISO 14040) defined by international guidelines. O2C™ therefore integrates the methodological rules of the Bilan Carbone® audit defined by ADEME in France and is based on the guide published by ASTEE (Scientific and Technical Association for Water and the Environment). The inputs required to conduct a carbon assessment of a plant are known as emission factors. These emission factors are obtained from public sources (Bilan Carbone® by ADEME, ASTEE, ECOINVENT, etc.) but also – in order to adapt to the water treatment industry – investigations conducted by the CIRSEE, the research center of SUEZ ENVIRONNEMENT. This is the case for emissions related to the decomposition of organic materials in anaerobic conditions (CH₄) or the treatment of nitrogenous life forms (urea, ammonium, proteins) present in water (N₂O generated during the nitrification and denitrification phases). Research into these matters has appeared in recent publications.

The emission factors database is the foundation of O2C™, and is the result of a collaborative process. It is shared transparently with the entire water industry on the website www.lifecarbontool.com. The Bonnard & Gardel1 group carried out a critical and independent appraisal of the emission factors database.

7.2.2. Wastewater treatment plants

This study includes the GHG emissions CO₂ (1x), CH₄ (25x) and N₂O (298x) and results in CO₂ equivalents. Methane (CH₄) and nitrous oxide (N₂O) emissions were independently calculated since they are specific emissions connected to wastewater treatment procedures, since methane (CH₄), is

produced by the decomposition of organic matter under anaerobic conditions and nitrous oxide (N_2O) is connected to the treatment of nitrogenous materials present in water (urea, ammonium, proteins). N_2O is generated during the nitrification and denitrification phases of nitrogen.

The carbon footprint was estimated for the infrastructure, including construction materials, evacuated materials, equipment, energy needs and transportation, and operation of both WWTPs, which includes consumables, energy, process emissions, by-products and transportation.

7.2.2.1. WWTP

First scenario considers a wastewater treatment plant located in Andalucía, southern Spain. The plant was designed for a population of 73600 PE equivalent inhabitants (EI), a processing capacity of $12696 \text{ m}^3 \text{ d}^{-1}$, $1595 \text{ t BOD}_5 \text{ eliminated year}^{-1}$ and $215 \text{ t N eliminated year}^{-1}$. Wastewater stream is sent to a screen and grit chamber, then waste stream is circulated to an aerobic biological treatment and finally is conducted to the secondary settling tank, which involves the physical separation of suspended solids from the wastewater flow. Some solids are recirculated to the aerobic treatment and the rest conducted to a gravity thickener and finally a centrifuge sludge dewatering, recovering the water and sending it back to the entrance flow. The biological reactor has a total volume of 16000 m^3 with a hydraulic retention time of 31 hours. Chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus concentration at the exit of the grit chamber are 695 g-COD m^{-3} , $48.15 \text{ g-TKN m}^{-3}$ and 13.04 g m^{-3} , respectively. Removal efficiencies are 89%, 73% and 88% for COD, TKN and phosphorus, respectively. More details are provided in Escapa et al. (2012).

7.2.2.2. WWTP +MEC

The second scenario studies a WWTP+MEC setup consisted of a MEC integrated as part of the biological treatment, where the effluent from the grit chamber is fed directly into the MEC reactor. This is followed by the polishing step (aerobic reactor), which removes the remaining COD, obtaining an effluent with TKN and COD concentrations below 15 and 125 g m⁻³, respectively. This plant also includes a gas compressor and a gas storage tank. The selected organic loading rate (OLR) of the MEC reactor is 3100 g-COD m_a⁻³ d⁻¹, which corresponds to an HRT of 5.2 h. The size of the aerobic reactor was calculated so that nitrification, denitrification and COD removal can be accomplished effectively following the calculation methods described by (Metcalf & Eddy Inc., 2003; Aarne Vesilind, 2003). COD and TNK concentrations at the exit of the MEC treatment corresponded to COD and TNK concentrations at the inlet of the aerobic treatment. The HRT and the sludge retention time were selected to be 17 h and 16.5 days, respectively, when the aerobic reactor was preceded by an MEC reactor. These parameters were based on previous research (Escapa et al., 2012). Escapa et al, (2012) evaluated a dWWTP with a MEC in three different scenarios. In this study a moderate scenario was selected. In moderate scenario, the COD removal in the MEC reactor remains at 44%, current densities on the order of 2.5 A m⁻², a hydrogen production up to 0.60 m³ m_a⁻³ d⁻¹, an energy consumption to 1 kWh kg-COD⁻¹ and CEs and CCEs of 50% and 75%, respectively.

7.3. RESULTS

7.3.1. Carbon footprint of the conventional WWTP

Using the model described in section 7.2, the carbon footprint of a WWTP with an aerobic treatment (activated sludge) was calculated as 17525 t CO₂e generated by the infrastructure and 6164 t CO₂e year⁻¹ by the

operation of the WWTP. This data and the contribution of individual activities are presented in Figures 7.1 and 7.2.

7.3.1.1. Effect of construction of the WWTP on carbon footprint

According to the type of activities of infrastructure, 62% of the emissions are associated with the construction materials (11179 t CO₂e), where standard reinforced concrete generated the higher impact, 7328 of t CO₂e, followed by the reinforcing steel (1196 t CO₂e) and the pavement (942.8 t CO₂e).

The use of electricity contributes 34% to the total GHG emissions. In this case energy from a mix UCTE was calculated 20000 MWhe during the entire construction stage. This consumption was estimated by the working hours of the equipment. This category generates a total of 6120 t CO₂e.

Transportation of the equipment and the construction materials contributes to 2% and 424 t CO₂e, being 420 t CO₂e generated by the material of construction and only 4 t CO₂e generated by the transportation of the equipment. Finally, equipment was found to make a minor contribution of 1% to the product CF, or 243 t CO₂e. PVC pipeline has the highest contribution, followed by the steel pipelines, 85.5 t CO₂e and 17.3 t CO₂e respectively.

Transportation of the equipment and the construction materials contributes to 2% and 424 t CO₂e, being 420 t CO₂e generated by the material of construction and only 4 t CO₂e generated by the transportation of the equipment. Finally, equipment was found to make a minor contribution of 1% to the product CF, or 243 t CO₂e. PVC pipeline has the highest contribution, followed by the steel pipelines, 85.5 t CO₂e and 17.3 t CO₂e respectively.

This data and the contribution of individual activities are presented in Figure 7.1.

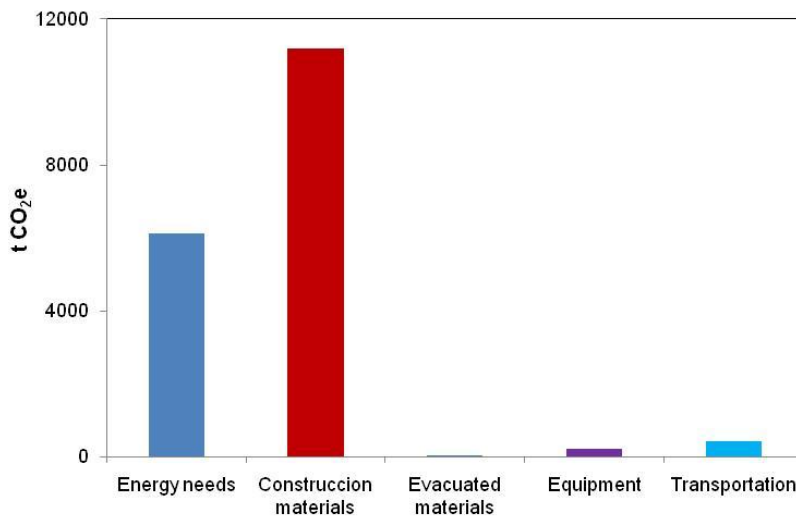


Figure 7.1. Distribution of the contribution of infrastructure to WWTP to CFP.

7.3.1.2. Effect of operation of the WWTP on carbon footprint

With regard to the operation, by-products make the highest contribution to the production of CF, $4004 \text{ t CO}_2\text{e year}^{-1}$, being the dewatered mixed sludge and its landfilling the main generator of CO_2 according to the $7040 \text{ t DS year}^{-1}$. Consumables such as chemical products, services, office suppliers, etc generate $991 \text{ t CO}_2\text{e year}^{-1}$ and it is mainly produced by the services and chemical products required in the water treatment process (Figure 7.2). Energy consumption contributes only 10% and $588 \text{ t CO}_2\text{e year}^{-1}$. Transportation has a small contribution, only 1% and it is mainly due to the by-products transport ($42 \text{ t CO}_2\text{e year}^{-1}$) rather than consumables ($28 \text{ t CO}_2\text{e year}^{-1}$). In terms of process emissions linked to treatment, it is estimated an amount of $330 \text{ t CO}_2\text{e year}^{-1}$ for 2990 t year^{-1} of COD removed and 170 t year^{-1} if N nitrified. Moreover, emissions from discharge are estimated in $180 \text{ t CO}_2\text{e}$ for 240 t year^{-1} of COD discharged and 15 t year^{-1} of N-NK.

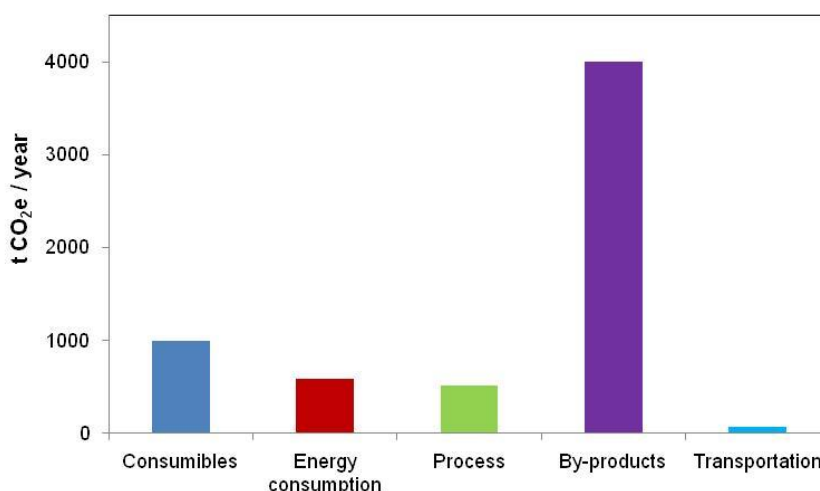


Figure 7.2. Distribution of the contribution of operation of WWTP to CFP.

7.3.2. Carbon footprint of WWTP with MEC

The carbon footprint of a WWTP with an integrated system of microbial electrolysis cells was calculated as 17765 t CO₂e generated by the infrastructure and 5504 t CO₂e year⁻¹ by the operation.

7.3.2.1. Effect of construction of the WWTP+MEC on carbon footprint

In Figure 7.3 it is shown the distribution of GHG emissions generated for the infrastructure. Construction materials have the highest impact, since they produce 11395 t CO₂e, which contributes to the 63% of total CF. Standard reinforced concrete is the main source of GHGs, 7288 of t CO₂e, followed by the reinforcing steel (1177 t CO₂e). It was also found a high contribution of the equivalent to activated carbon which is required as anode for the MECs. In terms of GHG emission produced by the infrastructure, carbon would be the highest down side of this technology.

The energy needs contribute to the total GHG emissions from infrastructure as a 34% (6120 of t CO₂e).

Equipment was found to make a minor contribution of 1% to the product CF, or 249 of t CO₂e and transportation a 2% (425 t CO₂e).

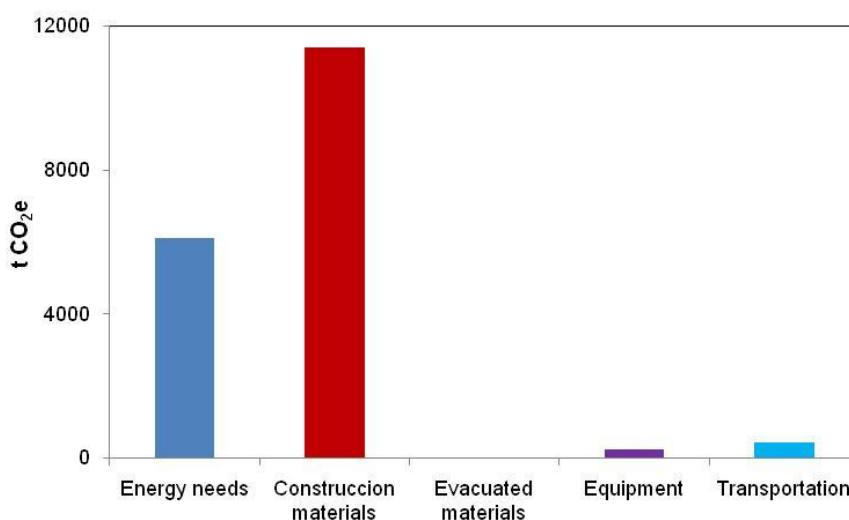


Figure 7.3. Distribution of the contribution of infrastructure of WWTP+MEC to CFP.

7.3.2.2. Effect of operation of the WWTP+MEC on carbon footprint

Operation of the WWTP with MEC technology is estimated to generate 5500 t CO₂e which is mainly produced by by-products, being the dewatered mixed sludge and its landfilling the main source of CO₂ (3353.2 t CO₂e year⁻¹) according to the 5914 t DS year⁻¹. Consumables generate 1087 t CO₂e year⁻¹ and the main contributors are services (493 t CO₂e year⁻¹) and the consumption of 40% solution of FeCl₃ (284 t CO₂e year⁻¹). Energy required for the process is estimated to produce 759 t CO₂e year⁻¹ from 1416.2 MWh year⁻¹ consumed. Transportation has a small contribution, only 1% and it is mainly due to the by-products' transportation. This data and the contribution of individual activities are presented in Figure 7.4.

According to the amount of biogas produced that could be injected into the network ($1\,373\,568\text{ Nm}^3$), energy production would avoid the emission of $2267\text{ t CO}_2\text{e year}^{-1}$.

In terms of process emissions linked to treatment, it is estimated an amount of $54.3\text{ t CO}_2\text{e year}^{-1}$ for 2990 t year^{-1} of COD removed and 170 t year^{-1} if N nitrified. Moreover, emissions due to discharge are estimated in $180\text{ t CO}_2\text{e year}^{-1}$ for 240 t year^{-1} of COD discharged and 15 t year^{-1} of N-NK.

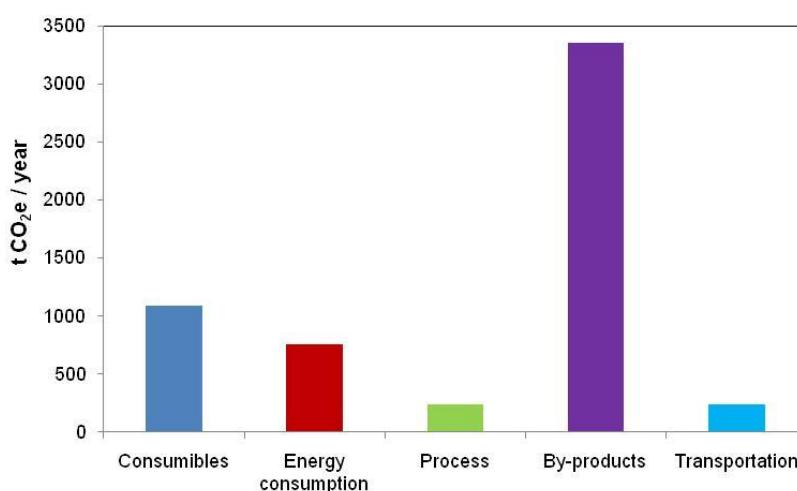


Figure 7.4. Distribution of the contribution of operation of dWWTP+MEC to CFP.

7.4. DISCUSSION

The construction of both wastewater treatment plants has a high impact on the carbon footprint. A large contribution of emissions is from the materials. The highest contribution to the carbon footprint was the standard reinforced concrete in both scenarios; however, emissions are higher in the second scenario mainly due to the “activated carbon” used as anode and cathode in the microbial electrolysis cells. The production of electrodes (activated carbon) significantly increases the amount of CO_2 released to the

atmosphere. This increase leads to a higher amount of the emissions in the WWTP+MEC than in the WWTP. In terms of GHG emission produced by the infrastructure, carbon would be the highest down side of this technology. Investigation in new materials anodes would be required to reduce its impact in the global carbon footprint of the WWTP. In this regard, there are several studies on anode's material and BEs configurations in order to improve the process efficiency therefore reducing emissions (Kipf et al., 2013; Lanas and Logan, 2013)

Operations of the both WWTPs were estimated in both scenarios (Table 7.1). It was found a significant difference of t CO₂ emissions in the second scenerio, which was calculated as 660 t CO₂e year⁻¹. This would contribute to a significant reduction in CO₂ emissions over the life time of a wastewater treatment plant. The lower generation of GHG in the second scenerio is mainly due to the reduction of by-products such as sludge (from 7040 t DS year⁻¹ to 5914 t DS year⁻¹) which is significantly lower than in the first scenario. This reduction in sludge production has been confirmed by other authors (Villano et al., 2013) and it is one of the main advantages of the microbial electrolysis cells. In terms of GHG from transportation a similar value was reported for both scenarios as well as GHG from discharge into natural environment since we are analyzing the same amounts of wastewater. Our results from the CFP study also estimated the amount of avoided emissions, which is over 2000 t CO₂e year⁻¹ in the second scenario due to the reduction in sludge production and also the reduction in energy consumption and its own energy generation from the biohydrogen produced at the cathode which could be either used in the process to became self-sustainable or could be injected into the network and sell to the gas distributor. A potential reduction in the emissions can be achieved improving the sludge management.

Table 7.1. Comparison of several indicators of infrastructure and operation of WWTP and WWTP with a MEC.

Carbon footprint report			
	WWTP	WWTP+MEC	
Infrastructure	17525	17765	t CO ₂
Operation	6164	5504	t CO ₂ year ⁻¹
Plant's annual greenhouse gas emissions report	12682	12446	t CO ₂ year ⁻¹
Sum of direct GHG emissions from process	510	234	t CO ₂ year ⁻¹
Greenhouse gas emissions from transportation	84	89	t CO ₂ year ⁻¹
Emissions from discharge into natural environment	180	180	t CO ₂ year ⁻¹
Avoided emissions report	0	2267	t CO ₂ year ⁻¹
Climate change			
Equivalent population GHG emissions	84	75	kg CO ₂ e year ⁻¹ PE ⁻¹
Emissions per m ³ of treated water	1	1	kg CO ₂ e year ⁻¹ m ⁻³
t BOD5 eliminated	3865	3449	kg Ce year ⁻¹ kg-BOD ₅ ⁻¹
Share of direct GHG emissions process	8	4	%
Share of avoided emissions	0	41	%

Using of sustainable resource			
Final waste production	116	116	t DS to landfill site
Sand production	70	70	t DS year ⁻¹
Evacuated quantity of land settling	7039	5913	t DS year ⁻¹
Drinking water consumption	4055	3446	m ³ year ⁻¹
Total energy needs	1922430	1416200	kWh year ⁻¹
Energy recovery			
Overall on-site energy autonomy	0	37	%

The calculation of net emissions for the two scenarios is calculated by assuming a 20 years life time of the facilities. Therefore, the annual emissions are multiplied and added to the emissions caused by the implementation of the infrastructure.

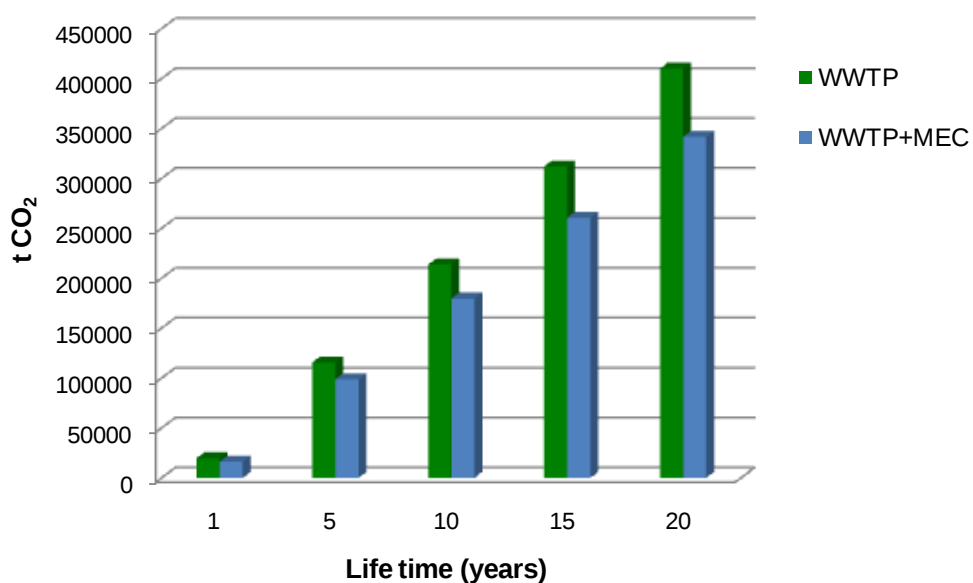


Figure 7.5. Total CFP estimated at different life time of the facilities.

The total emissions of the two scenarios are shown in the Figure 7.5. This figure shows that the WWTP+MEC facility has more advantages, the greater the period of useful life is. Current studies indicates that the durability of the microbial electrolysis cells is not clear and that could be very short which could affect the results of the carbon footprint analysis.

7.5. CONCLUSIONS

This study has tried to estimate the CFP from a domestic wastewater treatment plant with aerobic treatment (activated sludge) and a dWWTP with an integrated system of MECs. The data used was taken from a real dWWTP in the south of Spain and from test run on MECs at lab and pilot-scale. We found that the infrastructure has a strong impact on carbon footprint since 74 and 77% of greenhouse emissions came from the construction and maintenance of the infrastructure of the dWWTP and dWWTP+MEC, respectively. Contributions from transportation were negligible in both cases. The differences between those two set-ups are found in the operation, where dWWTP+MEC was estimated to generate over 600 t CO₂e year⁻¹ lower emissions than the conventional dWWTP. Furthermore, dWWTP+MEC would avoid the emission of 2267 t CO₂e year⁻¹ if the energy produced as biohydrogen could be injected into the grid network. However, more data from a larger scale MEC may be needed to acknowledge an accurate estimation.

This study proves the reduction in the CFP from a substitution of aerobic systems for microbial electrolysis systems in wastewater treatment plants.

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Chapter 8

General Conclusions

8.1. CONCLUSIONS

1. A successful scale-up of the combined wastewater treatment and H_2 production process from a 50 mL laboratory MEC fed with synthetic wastewater to a 10 L MEC treating raw municipal wastewater was demonstrated.
2. A MEC in series design was implemented for scale-up to achieve an acceptable rate of H_2 production while satisfying wastewater treatment norms. MEC operation at organic loads above $1\text{-}2\text{ g L}_a^{-1}\text{ d}^{-1}$ might be suggested in order to maximize H_2 production.
3. To minimize energy consumption the applied voltage might be individually (per cell) controlled and preferably optimized in real time as a function of the current organic load.
4. The tubular MEC reactor was able to reduce up to 85% of the chemical oxygen demand of a domestic wastewater, with a net energy consumption lower than that typically associated with aerobic treatments of domestic wastewater.
5. The OLR of $0.10\text{ g-COD L}_a^{-1}\text{ d}^{-1}$ can be established as the threshold above which the tubular MEC starts to compete with aerobic treatments in terms of energy consumption and COD concentration in the effluent. Likewise, an OLR of $0.67\text{ g-COD L}_a^{-1}\text{ d}^{-1}$ was selected as the threshold above which the addition of a polishing module operating in series with the first module is recommended.
6. This study also proves the feasibility of scaling up our design by adding modules in series for use at OLRs high enough to warrant these additional modules.

7. The HRT and Vapp showed significant influences on hydrogen production and energy consumption in a tubular semi-pilot MEC fed with dWW. In contrast, the COD elimination rate was less affected due to the presence of microorganism that consume a significant amount of the COD fed to the reactor and do not contribute to electrogenic metabolism.
8. WWTP+MEC was estimated to generate 660 t CO₂e year⁻¹ lower emissions than the conventional dWWTP. Moreover, WWTP+MEC would avoid the emission of 2267 t CO₂e if the energy produced as biohydrogen could be injected into the network.

8.2. FUTURE PERSPECTIVES

1. New improvements in the cathode material are required to make this technology fully competitive with aerobic treatments and to achieve practical implementation, since cathodic reaction seems to be the limited step leading to hydrogen recirculation and low kinetics for hydrogen production.
2. New strategies to limit the proliferation of methanogens and hydrogenotrophic microorganisms are required in order to improve MEC performance.
3. The management of cathodic hydrogen represents another critical point in the current design of tubular MEC, and deserves further investigation to achieve practical implementation in dWW treatment.
4. New developments are required for nitrogen removal.

Capítulo 9

Resumen Global

9.1. TRATAMIENTO DE AGUAS RESIDUALES URBANAS

En los últimos años, como consecuencia de la aplicación de la Directiva Europea 91/271/CEE, por la que se obligó a los estados miembros a sanear las aguas residuales de aquellas poblaciones con más de 2.000 habitantes equivalentes, se ha observado un aumento en la implantación de nuevas Estaciones Depuradoras de Aguas Residuales (EDAR).

En las EDAR, y tras un primer proceso de desarenado, desengrasado y retirada de gruesos, el agua es sometida a un proceso primario de decantación, lo que permite que las partículas suspendidas más pesadas que el agua se separen sedimentándose. Como resultado, se eliminan aproximadamente el 90% de las materias decantables y el 65% de las materias en suspensión. Se consigue también una disminución de la demanda biológica de oxígeno (DBO) de alrededor del 35%.

A continuación, una vez superadas las fases de pretratamiento y tratamiento primario, la reducción de materia orgánica presente en las aguas residuales se logra sometiendo a esta a un tratamiento secundario que suele consistir en un proceso biológico aerobio seguido por una decantación, denominada secundaria. En la mayoría de las EDAR, este proceso biológico aerobio se lleva a cabo mediante un procedimiento conocido como fangos activos. Este es un proceso continuo en el que el agua residual se estabiliza biológicamente en tanques o balsas de activación, en las que se mantienen condiciones aerobias.

El efluente de los decantadores primarios pasa a estas balsas de fangos activos que necesitan un aporte de oxígeno para la acción metabólica de determinados microorganismos (*Pseudomonas*, *Bacillus* o el hongo *Penicillium*, entre otros). Este aporte se efectúa mediante turbinas, o bien a través de difusores dispuestos en el interior de la balsa, en este último caso, el suministro de aire se realiza mediante turbocompresores. Además,

se tiene que garantizar el aporte de los nutrientes necesarios para que el sistema funcione correctamente. El aporte de aire por medio de turbinas o turbocompresores supone un importante coste para el proceso, y dado el régimen continuo del mismo, se aprecia la necesidad de diseñar un mecanismo que nos permita disminuir el consumo energético lo máximo posible.

En España el sector del tratamiento de aguas se ha incrementado en un 6% en los últimos diez años (DBK, 2010). El tratamiento de aguas residuales requiere alrededor del 1% ($2.672 \text{ GWh año}^{-1}$) del consumo nacional de electricidad para tratar 3.000 hm^3 de aguas residuales domésticas cada año. Esto supone una potencia de 305 MW y un consumo medio de 0.67 kWh m^{-3} . La mayoría de las plantas de tratamiento de aguas en España consumen $50 \text{ kWh (h.e.año)}^{-1}$.

Sin embargo, ese consumo de energía se podría ver reducido en un 17.5% si se optimizasen los sistemas y se utilizase la energía contenida en la materia orgánica del agua (IEA, 2011). Por lo tanto las aguas residuales urbanas pueden albergar una fuente de energía aún por explotar, lo que proporcionaría diversas alternativas energéticas (Angenent et al., 2004).

Además del elevado consumo de energía de los tratamientos de aguas residuales estos también contribuyen a la producción de gases de efecto invernadero a través del metano y del dióxido de carbono del propio tratamiento en sí, así como del generado a partir de los requerimientos energéticos. Estudios previos han estimado que una planta de tratamiento de agua podía incrementar en un 5% la producción global de metano (El-Fadel and Massoud, 2001).

Esto nos confirma la necesidad de encontrar nuevas tecnologías capaces de generar energía a partir de las aguas residuales reduciendo los

consumos energéticos en dicho proceso al igual que la generación de gases de efecto invernadero.

9.2. ELECTRÓLISIS BIOCATALÍTICA

La electrólisis biocatalítica surge como una tecnología que pretende ofrecer soluciones alternativas al tratamiento de aguas residuales permitiendo tanto la generación de H_2 , como de electricidad (Liu et al., 2005). Los reactores bioelectroquímicos están basados en el descubrimiento de microorganismos activos electroquímicamente los cuales son capaces de transferir los electrones a una superficie sólida sin necesidad de mediadores (Potter, 1911; Chaudhuri and Lovley, 2003). Esta novedad supuso la base para muchas aplicaciones entre las que tenemos los BESs. En estos reactores los microorganismos activos crecen sobre un electrodo que será el ánodo, el cual se conecta mediante un circuito eléctrico a un cátodo donde se llevan a cabo las reacciones de reducción.

Dependiendo de la reacción llevada a cabo en el cátodo, el sistema gana energía, como es el caso de las pilas de combustible microbianas o el sistema requiere un aporte de energía para la generación de otros productos, como es el caso de los electrolizadores biocatalíticos. La producción de hidrógeno mediante electrólisis biocatalítica se consigue en cierto modo, invirtiendo el funcionamiento normal de una pila de combustible microbiana.

Pilas de combustible microbianas

Una pila de combustible microbiana (Figura 9.1.) es un dispositivo capaz de convertir la energía química de una amplia variedad de compuestos orgánicos en energía eléctrica mediante la acción catalítica de cierto tipo de microorganismos en condiciones anaerobias. La estructura de dicho dispositivo es similar a la de una pila de combustible convencional. Dispone

de un compartimiento anódico donde crecen diversas comunidades bacterianas, que son las encargadas de oxidar un sustrato orgánico generando electrones, protones y CO_2 . Los electrones son captados por el ánodo y conducidos a través de un circuito externo hacia el cátodo. Por otro lado, los protones cruzan hacia el compartimento catódico a través de un separador que actúa como aislante entre el cátodo y el ánodo. En el cátodo es donde finalmente se combinan los electrones y los protones junto con un agente oxidante presente en el compartimento catódico que generalmente es O_2 , el cual actúa como aceptor final de electrones.

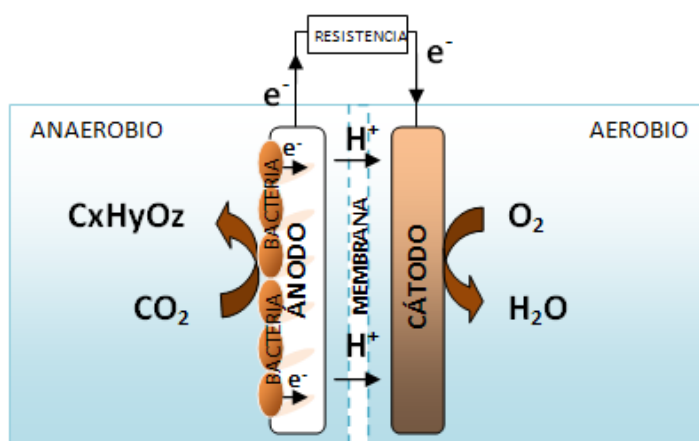


Figura 9.1. Esquema de una pila de combustible microbiana (MFC).

Electrolizadores biocatalíticos

En un electrolizador biocatalítico EB o MEC siglas en ingles (Figura 9.2.), por el contrario, los electrones no circulan libremente hacia el cátodo. Esto es así porque en el compartimento catódico no existe ninguna sustancia oxidante capaz de actuar como aceptor final de electrones, por lo cual deben ser impulsados desde el ánodo mediante una fuente de tensión. Así, los electrones y protones procedentes del ánodo se combinan en el cátodo dando lugar al hidrógeno, ya que en este caso el compartimento catódico se encuentra en condiciones anaerobias. Los procesos que ocurren en el

ánodo son semejantes a los que tienen lugar en el compartimento anódico de una pila de combustible microbiana MFC.

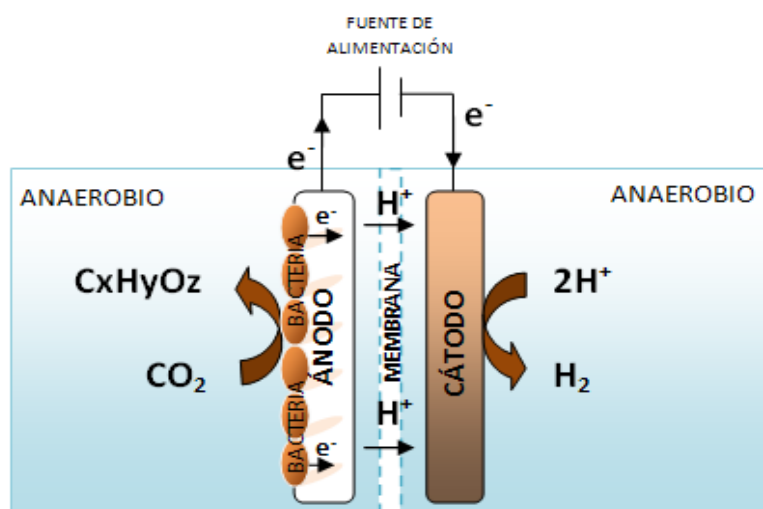


Figura 9.2. Esquema de un electrolizador biocatalítico EB (MEC).

Una revisión de la literatura ofrece un amplio rango de valores en cuanto a la tasa de producción de hidrógeno, tasa de depuración y el consumo de electricidad. Los primeros estudios realizados en MECs obtuvieron unos volúmenes de hidrógeno de $0.36 \text{ Nm}^3 \text{ H}_2 \text{ m}_R^{-3} \text{ d}^{-1}$ con un consumo de $1 \text{ kWh Nm}^3 \text{ H}_2$ empleando acetato como sustrato (tensión aplicada de 0.45 V) (Liu et al., 2005) pasando por los $10 \text{ Nm}^3 \text{ H}_2 \text{ m}_R^{-3} \text{ d}^{-1}$ con una eficiencia catódica del 90% con unos niveles de depuración entorno a $7 \text{ kg-DQO m}_R^{-3} \text{ d}^{-1}$ con un sustrato sintético (Rozendal et al., 2007) hasta lograr producciones de $17 \text{ Nm}^3 \text{ H}_2 \text{ m}_R^{-3} \text{ d}^{-1}$ con agua residual sintética (Cheng and Logan, 2011). Tras obtenerse buenos resultados con sustratos sintéticos se han evaluado sustratos reales como es el caso de aguas residuales con los que se obtuvieron valores que van desde los $0.01 \text{ Nm}^3 \text{ H}_2 \text{ m}_R^{-3} \text{ d}^{-1}$ con un consumo de $2.5 \text{ kWh Nm}^3 \text{ H}_2$ (tensión aplicada de 0.5 V) (Ditzig et al., 2007), comparable a los $0.015 \text{ L}_{H_2} \text{ L}_R^{-1} \text{ día}^{-1}$ generados en una planta piloto MEC operada con aguas residuales urbanas que logró un consumo energético

de $0.64 \text{ kWh Kg-DQO}^{-1}$ (Heidrich et al., 2012), así como los $0.3 \text{ L H}_2 \text{ L}_R^{-1} \text{ d}^{-1}$ con consumos de $1.2 \text{ kWh Kg-DQO}^{-1}$ eliminado (Escapa et al., 2012a) utilizando también aguas residuales urbanas (aplicando una tensión de 1V). Como observamos los resultados obtenidos con sustratos sintéticos aun son notablemente superiores a los obtenidos con aguas residuales reales, por lo que todavía es necesario llevar a cabo muchas mejoras en el funcionamiento de esta tecnología para alcanzar buenos rendimientos y lograr que sea competitiva con los sistemas convencionales.

A esto hay que añadir que la mayoría de las celdas bioelectrolíticas estudiadas hasta el momento se han realizado en escala de laboratorio con compartimentos anódicos con volúmenes menores a un litro y solamente se tiene conocimiento de varios intentos de escalado. Uno de ellos se llevó a cabo en una planta piloto de un reactor MFC de 1 m^3 que fue destinado al tratamiento de aguas residuales de cervecería, en este caso se encontraron varios factores que afectaron negativamente al funcionamiento de la celda, como fueron la baja densidad de corriente generada y la limitada capacidad depurativa (Keller and Rabaey, 2008). Posteriormente se estudió la eficacia de una planta piloto MEC de 1000 L donde se evaluó la capacidad de depuración de aguas residuales de producción de una bodega de vino (Cusick et al., 2011). En este caso los resultados mostraron bajas tasas de eliminación de DQO así como importantes pérdidas de hidrógeno debido a su transformación en metano a través de los microorganismos metanógenos hidrogenotróficos. Por último recientemente se ha evaluado una planta piloto MEC de 120 L operada con agua residual doméstica, en este caso se han obtenido unos consumos energéticos de $0.64 \text{ kWh kg-DQO}^{-1}$, lo cual está por debajo de la energía requerida en los tratamientos aeróbicos tradicionales $0.7\text{-}2 \text{ kWh kg-DQO}^{-1}$ (Pant et al., 2011). Sin embargo, los resultados de producción de gas no fueron tan alentadores ya que la tasa de generación de hidrógeno fue tan solo de $0.015 \text{ L H}_2 \text{ L}^{-1} \text{ d}^{-1}$, con lo que se concluyó que sería necesario una mejora en

la captura del hidrógeno así como en el diseño de la celda para poder incrementar notablemente la eficacia de la MEC para aguas residuales urbanas de baja carga a temperaturas ambientales.

BES en general y MECs en particular son una tecnología prometedora para satisfacer las necesidades de la industria del tratamiento de aguas (Foley et al., 2010). Así en este estudio nos centramos en la capacidad depurativa, la producción de hidrógeno y el consumo energético, aunque hay que destacar la flexibilidad del proceso para generar otros productos siendo esto un punto a tener en cuenta en su viabilidad económica.

Antes de que los reactores a escala de laboratorio puedan ser transformados en reactores comerciales técnica y económicamente viables, es necesario investigar el efecto que tienen ciertos parámetros de operación (tales como el tiempo de retención, la tensión aplicada, el pH, la temperatura, etc.) así como el diseño y configuración de los reactores.

Además de lo indicado anteriormente, antes del escalado y comercialización de la tecnología BE es necesario realizar una estimación de la huella de carbono de este proceso para poder así evitar consecuencias posteriores e intentar mejorar el proceso para evitar la emisión de gases de efecto invernadero y su contribución al cambio climático.

9.3. OBJETIVOS

El presente estudio se lleva a cabo con el objetivo de valorar la viabilidad de una celda bioelectrocatalítica como posible tecnología a emplear en el tratamiento de aguas residuales urbanas y la simultánea producción de H_2 para así aumentar su eficiencia energética y proceder con el escalado. El estudio se centra en cuatro aspectos que son:

- ✓ Evaluación de varios diseños y configuraciones de las celdas MEC destinadas al escalado.
- ✓ Optimización de distintos parámetros de funcionamiento (OLR, TRH, Vapp) de la MEC, tanto en celdas planas como tubulares.
- ✓ Evaluar la capacidad depurativa de aguas residuales urbanas de baja carga minimizando el consumo energético en MEC tubulares en serie.
- ✓ Estimar la huella de carbono en una instalación de tratamiento de aguas con una MEC integrada en el sistema y su posterior comparación con un sistema de tratamiento de aguas residuales convencional.

9.4. EMPLEO DE LA TECNOLOGÍA EB PARA EL TRATAMIENTO DE AGUAS RESIDUALES Y LA PRODUCCIÓN DE HIDRÓGENO - ESTUDIO DEL PROCESO DE ESCALADO

Como se ha comentado anteriormente existen pocos estudios de MECs a gran escala, por lo que mediante este trabajo se intenta llevar a cabo un escalado desde una celda biocatalítica de 50 mL, pasando por 855 mL hasta 10 L pudiendo identificar así las mayores limitaciones en el escalado de las MEC.

9.4.1. Resultados de los ensayos en una MEC de 50mL (MEC-1x)

Los ensayos comenzaron con la MEC de 50 mL (MEC-1x), esta tiene una configuración plana con una cámara catódica, una cámara anódica y un separador entre ambas para evitar el contacto entre el cátodo y el ánodo. Los ensayos realizados en esta MEC trataron de identificar los factores que afectan a la producción de hidrógeno y a la tasa de eliminación de DQO. Para lograr identificar los factores limitantes se modificó la concentración de

la fuente de carbono, así como la composición y la conductividad del influente administrado a la MEC, que en este caso fue agua residual sintética (Tabla 9.1).

Tabla 9.1. Composición del agua residual sintética utilizada en los ensayos con MEC-1x y MEC-17x (MEC-50 mL y MEC-855 mL). Anotaciones: HS – carga alta, LS – carga baja. HC –conductividad alta, LC – conductividad baja, Ac – acetato, Su – sacarosa, MS – carga media, MT – disolución de metales traza.

Influente	Acetato Sodio	Sacarosa	Peptosa	Ext. Carne	Levadura	NH ₄ HCO ₃	K ₂ HPO ₄	K ₂ HPO ₄	NaCl	MT
	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹
HS/HC	-	-	2.5	2.5	1.5	0.85	0.087	0.075	2.81	1
LS/HC	-	-	0.179	0.179	0.107	0.85	0.087	0.075	2.81	1
HS/LC	-	-	2.5	2.5	1.5	0.0607	0.00625	0.00537	0.2	1
Ac/HC*	8.37	-	-	-	-	-	0.49	0.42	-	-
Su/HC*	-	6.8	-	-	-	-	0.49	0.42	-	-
MS/HC**	-	0.22	0.046	-	-	0.52	1.38	0.69	0.83	1

* Concentración del influente después de mezclarlo con el agua de dilución.

**también contiene (g/L): MgSO₄7H₂O 0.26 and MnSO₄7H₂O 0.26

Inicialmente se alimentó la MEC-1x con agua residual sintética con una carga orgánica de 6 g L_R⁻¹ d⁻¹ y 7 mS cm⁻¹ (HS/HC), con la cual se logró una eliminación de 4.3 g-DQO L_R⁻¹ d⁻¹, lo cual equivale a una tasa depurativa del 71% y un consumo energético de 1.5 kWh kg-DQO⁻¹ bajo una tensión optimizada de 0.91V. Posteriormente y con el fin de analizar el efecto de la concentración de la fuente de carbono sobre la tasa de eliminación de DQO y la producción de hidrógeno se redujo la concentración del influente a 0.7g L_R⁻¹ d⁻¹ (LS/HC), esta disminución en la materia orgánica administrada se tradujo en un notable descenso tanto en la producción de hidrógeno como en la capacidad depurativa la cual descendió hasta un 57%, al igual que la tensión aplicada que bajó hasta 0.58V. Una vez analizado el efecto se

restablecieron las condiciones iniciales y tanto la corriente como la producción de hidrógeno y la tasa de eliminación de DQO alcanzaron valores, aunque si algo inferiores, semejantes al primer ensayo en el que se administró una carga de $6 \text{ g L}_R^{-1} \text{ d}^{-1}$ y 7 mS cm^{-1} (HS/HC). Esto parece indicar que el factor limitante es la concentración de la fuente de carbono, lo cual fue confirmado en el siguiente ensayo en el que se mantuvo la misma carga de $6 \text{ g L}_R^{-1} \text{ d}^{-1}$ pero con una conductividad de 1.5 mS cm^{-1} (HS/LC). Los resultados obtenidos indicaron que la conductividad tiene un efecto insignificante sobre el funcionamiento de la MEC, siendo entonces la carga orgánica el factor limitante sobre la actividad de los microorganismos electrógenos.

Tras esta confirmación el siguiente paso fue analizar el efecto de la hidrólisis y la fermentación de la materia orgánica sobre la eficiencia de la MEC. Para esto se operó la celda MEC-1x con acetato con una carga de $0.5 \text{ g L}_R^{-1} \text{ d}^{-1}$ (Ac/HC), para así eliminar la etapa de hidrólisis y fermentación ofreciendo un sustrato directamente asimilable por los microorganismos electrógenos. Se observó una mejoría en términos de producción de hidrógeno, generación de corriente y eliminación de DQO en comparación con los ensayos con agua residual sintética de baja carga, sin embargo los resultados fueron inferiores a los valores logrados con agua residual sintética de alta carga. Con lo cual se puede concluir que en este caso o bien la baja carga orgánica administrada a la celda o la baja concentración de DQO en el influente fueron los factores limitantes. Un hecho notable fue el incremento en la producción de metano en el cátodo lo que actuó en detrimento de la producción de hidrógeno debido a la actividad de los metanógenos hidrogenotróficos.

Para comprobar el efecto de la fermentación en el proceso bioelectroquímico se administró un influente con sacarosa con una carga de $0.5 \text{ g L}_R^{-1} \text{ d}^{-1}$ manteniendo la conductividad anterior (Su/HC). Al administrar

sacarosa estamos eliminando directamente el proceso de hidrólisis por lo cual se disminuye la complejidad de la materia orgánica y se facilita su degradación y asimilación por parte de los electrógenos en comparación con el agua residual sintética ensayada anteriormente. Investigaciones previas han demostrado el crecimiento de estos microorganismos en medios con sacarosa y glucosa (Chaudhuri and Lovley, 2003). Tanto la corriente generada como el hidrógeno fueron superiores a los obtenidos en los ensayos con el agua residual sintética de baja carga (0.6 g L^{-1} (LS/HC) pero por debajo de los obtenidos con acetato (Ac/HC), sin embargo la tasa de eliminación de DQO fue superior a ambos, lográndose una tasa depurativa del 90% con un consumo energético notablemente inferior ($1.0 \text{ kWh kg-DOQ}^{-1}$). Aunque, hay que destacar que este incremento en la capacidad depurativa podría deberse en parte a la actividad metanógena en la cámara anódica, ya que la eficiencia coulombica fue inferior a la obtenida con acetato pero superior al agua residual sintética.

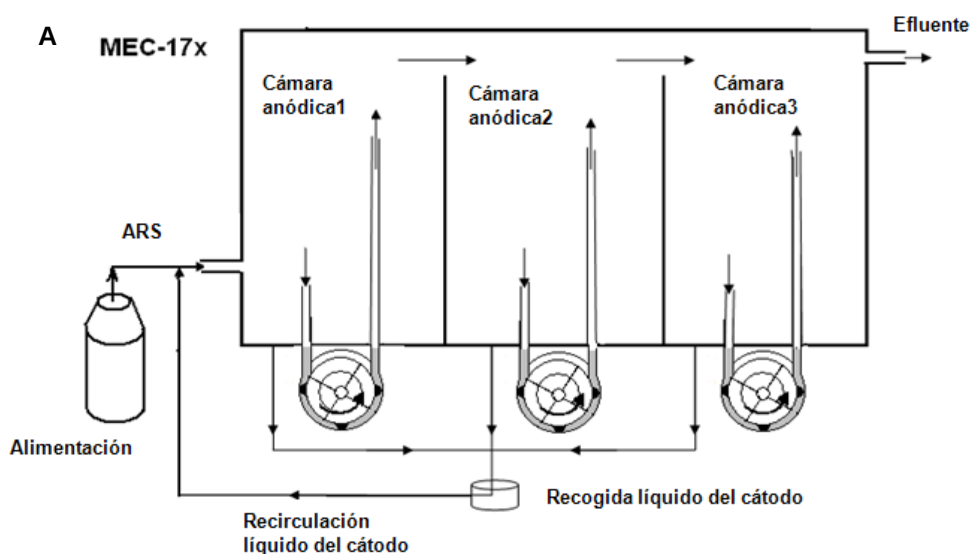
A modo de conclusión se puede decir que un influente con baja conductividad no tiene un gran impacto en el funcionamiento de la celda con lo cual se puede demostrar la viabilidad del tratamiento de aguas residuales urbanas, las cuales en la mayoría de los casos suelen tener conductividades muy bajas, incluso por debajo de 1 mS cm^{-1} . En efecto, se puede confirmar que tanto la concentración como la composición del influente juegan un papel muy importante en el funcionamiento de la MEC, tanto desde el punto de vista energético como depurativo, siendo así la baja carga orgánica, la baja concentración de la fuente de carbono y el proceso de hidrólisis factores limitantes (Johansen JE, 2006; Zhu et al., 2009).

9.4.2. Resultados de los ensayos en la MEC de 855mL (MEC-17x)

Según los resultados mostrados en la sección anterior, el funcionamiento de la MEC de 50 mL para las cargas y las concentraciones de materia orgánica mencionadas, no se puede demostrar su eficiencia para el

escalado mediante un simple aumento del volumen del compartimento anódico. Un simple incremento volumétrico supondría un aumento del grosor del ánodo lo que incrementaría la resistencia al transporte de protones, además también sería necesario un aumento de la recirculación en esta cámara lo que podría derivar en una disminución de la producción de hidrógeno y el aumento de la cantidad de metanógenos hidrogenotróficos. Teniendo en cuenta esto, una solución factible sería la utilización de MECs en serie, donde para el caso del tratamiento de aguas residuales llegaría la mayor carga orgánica a las primeras celdas, utilizándose las siguientes como una etapa de pulido pudiéndose así lograr los niveles de los parámetros requeridos por la normativa vigente en materia de evacuación de aguas residuales.

Así para la siguiente serie de ensayos se operó una celda MEC (MEC-17x) con tres compartimentos anódicos y uno catódico común, con un volumen anódico total de 855 mL como la que se muestra en la Figura 9.3.



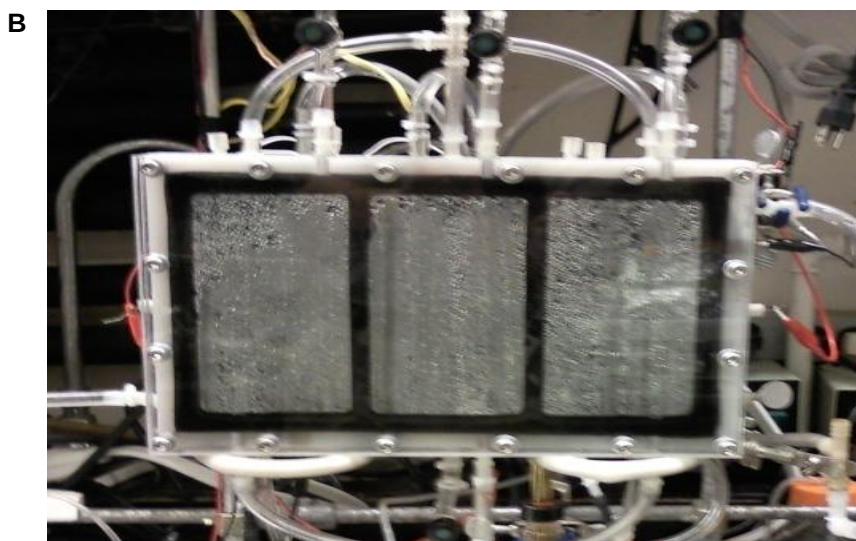


Figura 9.3. Vista de la MEC plana en serie de 855 mL (MEC-17x) utilizada en los ensayos. (A) Diagrama; (B) Foto de la MEC-17x.

En esta serie de ensayos se evaluaron varias cargas orgánicas e influentes de agua residual sintética de distinta concentración (Tabla 9.1). Para las aguas residuales sintéticas de alta carga con un TRH de 41 horas ($3.4 \text{ g L}_R^{-1} \text{ d}^{-1}$) se lograron corrientes de 176.1 mA aunque hay que destacar que la mayor parte de la corriente fue generada en los dos primeros compartimentos al igual que la tasa de eliminación de la DQO, por lo que se puede suponer que la mayoría del hidrógeno generado ($0.98 \text{ L L}_R^{-1} \text{ d}^{-1}$) fue en los dos primeros compartimentos. Al disminuir el TRH a 27 horas ($8.3 \text{ g L}_R^{-1} \text{ d}^{-1}$) como se muestra en la tabla 9.2 se observó un aumento significativo en la corriente, que alcanzó los 256.3 mA, al igual en la producción de hidrógeno y en la tasa de eliminación de DQO que alcanzó el 69%, sorprendentemente en este caso la corriente en el primer y tercer compartimento fueron comparables, sin embargo la depuración en el tercer compartimento fue mínima no correspondiendo así con la corriente generada, lo cual podría explicarse debido a la escasez de sustrato

asimilable por los electrógenos, sin poder descartar la presencia de un cortocircuito en este compartimento.

Tabla 9.2. Resultados de los ensayos de MEC -17x en función del TRH.

test	TRH h	WW	OLR $\text{g L}_R^{-1} \text{d}^{-1}$	DQOin g L^{-1}	I mA	DQOe $\text{g L}_R^{-1} \text{d}^{-1}$	E_{coul} %	Ef.H ₂ mol / mol	Econs Wh/L H ₂	Econs kWh/ kg COD
2-1	41	HS/HC	3.4	5.7	176.1	1.4	104	1.01	5.3	3.7
2-2	27	HS/HC	8.3	9.8	256.3	5.7	37	0.61	3.2	1.4
2-3	27	MS/HC	1.2	1.3	64.1	0.9	64	0.13	23.4	2.2
2-4	18	MS/HC	1.2	0.9	36.5	0.8	26	na/a*	n/a	1.4

* Flujo de H₂ no pudo ser contabilizado debido a una fuga de gas.

Posteriormente se disminuyó la carga del influente (MS/HC) para asemejarlo a las características del agua residual urbana real. En efecto el resultado fue una disminución en la producción de hidrógeno y en la generación de corriente para un TRH de 27 horas.

En un intento de mejorar las producciones se disminuyó el TRH para así aumentar la carga orgánica administrada a la celda. Sin embargo, este incremento no supuso ningún efecto positivo en la producción de hidrógeno, únicamente ocasionó un incremento en la producción de metano en el cátodo. Por lo tanto las conclusiones obtenidas en la MEC de 50 mL se pueden confirmar con estos resultados, donde vemos el claro efecto de la concentración de materia orgánica del influente y su carga orgánica en el funcionamiento de la MEC. Al igual que en la celda de 50 mL se contabilizaron pérdidas de hidrógeno que podrían ser debidas a la actividad de los metanógenos hidrogenotróficos, generalmente observados a bajas cargas orgánicas y cuya generación podría ser debida a la baja concentración de acetato en el líquido anódico (Rozendal et al., 2007; Cusick et al., 2011; Gil-Carrera et al., 2011), así como a la recirculación de

hidrógeno sugerida ya anteriormente por otros investigadores (Rittmann et al., 2006; Ditzig et al., 2007; Lee and Rittmann, 2010).

Desde el punto de vista de la tasa de eliminación de DQO, esta fue menor que en los ensayos anteriores a lo que hay que añadir que la mayoría fue eliminada en el primer compartimento, actuando el segundo como una etapa de pulido, mientras en el tercero apenas se observó degradación de materia orgánica.

Para confirmar estos resultados se analizó la cantidad proteica en cada uno de los ánodos de los tres compartimentos, y como era de esperar las mayores densidades de proteínas se encontraron en el primer compartimento donde se obtuvo la mayor tasa de eliminación de DQO en todos los ensayos y por consiguiente la mayor corriente, seguido del segundo compartimento. Por el contrario en el tercer compartimento no se encontró ninguna biomasa. La correlación entre la media de corriente y la concentración de proteínas en cada compartimento confirma que las proteínas están relacionadas con la biomasa activa y no solo con la acumulación de compuestos orgánicos no degradados (Gil-Carrera et al., 2011).

En cuanto al consumo energético se lograron valores que estarían por debajo de los asociados a los sistemas convencionales, así para los TRH menores se obtuvieron consumos energéticos de $1.4 \text{ kWh kg-DQO}^{-1}$ y si se tiene en cuenta que el último compartimento apenas funcionó, se podrían incluso reducir aún más esos consumos con una configuración de dos compartimentos.

Partiendo de los resultados obtenidos con la celda MEC de 855 mL, se procedió al escalado con la finalidad de diseñar una planta piloto.

9.4.3. Resultados de los ensayos en la MEC de 10L (MEC-200x)

Se diseñó un reactor constituido por dos celdas MEC de 5L cada una, con un volumen total de MEC de 10L (Figura 9.4). Dichas celdas fueron operadas en serie para así alcanzar una eliminación máxima de materia orgánica. MEC-200x fue alimentada con agua residual urbana real ($150\text{--}180\text{ mg-DQO L}^{-1}$) obtenida de una planta de tratamiento de aguas residuales y se ensayaron cuatro tiempos de retención hidráulica para comprobar la viabilidad de este diseño e incrementar tanto la eficiencia energética como depurativa de la MEC.

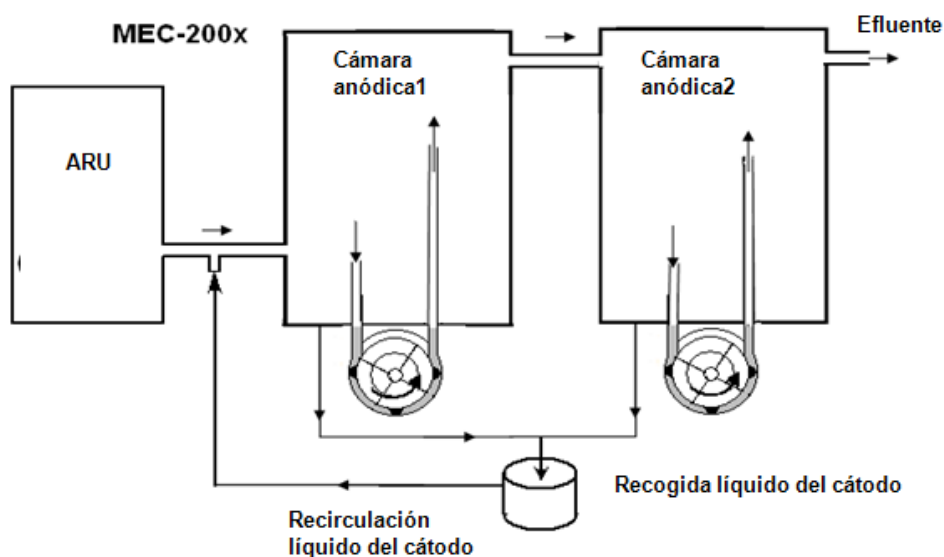


Figura 9.4. Diagrama MEC-200x de 10 L.

Tras comenzar con un TRH de 32 horas ($0.13\text{ g L}_R^{-1}\text{ d}^{-1}$) se consideró que la carga administrada no era suficiente ya que la concentración de DQO a la salida de ambas celdas fue muy semejante, de 70 y 80 mg-DQO L^{-1} respectivamente. Con lo cual se redujo el TRH a 24 horas para así aumentar la carga orgánica. Aún así los consumos energéticos fueron muy elevados, siendo los más bajos de $3.6\text{ kWh kg-DQO}^{-1}$ para una tasa de

eliminación de DQO del 66%. Al igual que ocurrió con la configuración de la MEC de 855 mL, la mayor parte de la materia orgánica fue degradada en la primera celda, llegándose a valores de 40 mg-DQO L⁻¹ en el efluente.

Con TRH de 16 y 10 horas, 0.23 y 0.65 g-DQO L⁻¹ d⁻¹ respectivamente, se lograron producciones de hidrógeno de 0.04 L L_R⁻¹ d⁻¹, resultados que se pueden equiparar a los obtenidos en la MEC de 50 mL, además de una tasa de eliminación de DQO del 76% con un consumo energético de 0.9 kWh kg-DQO⁻¹ (Tabla 9.3), dicho consumo está por debajo de la energía requerida en tratamientos aerobios de aguas residuales que se considera de 1.5 kWh kg-DQO⁻¹ (Tchobanoglous, G. et al., 2003).

Tabla 9.3. Resultados de los ensayos de MEC 200x en función del TRH.

test	HRT h	OLR g L _R ⁻¹ d ⁻¹	DQOin g L ⁻¹	I mA	DQOe g L _R ⁻¹ d ⁻¹	E _{coul} %	Ef.H ₂ mol / mol	Econs Wh/L H ₂	Econs kWh/ kg COD
3-1	32	0.13	0.17	108.9	0.06	129	0.36	23.2	7.4
3-2	24	0.10	0.99	90.7	0.07	103	0.12	79.3	5.2
3-3	16	0.23	0.16	62.7	0.1	42	0.13	22.8	1.7
3-4	10	0.66	0.27	186.3	0.5	23	0.19	8.7	0.9

Estos consumos energéticos en la MEC de 10 L son consistentes con los obtenidos previamente en las MEC de 50 y 855 mL, y similares a los logrados en un estudio previo en el cual se operó una MEC de 250 mL con agua residual urbana (Escapa et al., 2012a).

En base a estos resultados se puede concluir que para lograr cantidades significantes de hidrógeno serían necesarias cargas orgánicas superiores a 1g-DQO L⁻¹ d⁻¹, aunque para cargas de 0.66 g-DQO L⁻¹ d⁻¹ y el diseño propuesto ya se obtienen tasas de eliminación que satisfacen la normativa vigente además de lograr consumos energéticos relativamente bajos de 0.9 kW kg-DQO⁻¹. Por lo cual se demuestra la viabilidad del escalado de la

MEC con diseño plano y configuración en serie, aunque sería necesario investigar más en la producción de hidrógeno para evitar pérdidas por recirculación y/o por el método de captura así como en la mejoraría de la eficacia de su generación en el cátodo.

9.5. EMPLEO DE UNA MEC TUBULAR BIMODULAR PARA EL TRATAMIENTO DE AGUAS RESIDUALES URBANAS

A partir de los resultados obtenidos en los ensayos anteriores y para comprobar que diseño de MEC es más eficaz, se decidió evaluar otro tipo de configuración que pudiera además reducir los consumos energéticos y mejorar la capacidad depurativa del proceso.

En contraste con el diseño plano de las celdas ensayadas anteriormente, en este estudio se optó por un diseño bimodular tubular de las celdas MEC, y al igual que en el estudio anterior se alimentaron con agua residual urbana y se operaron en serie para aumentar su capacidad depurativa.

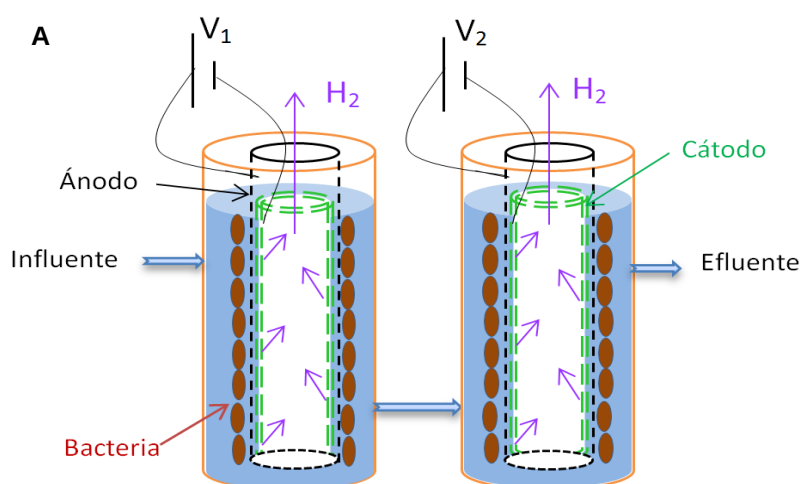
En este estudio se han evaluado los siguientes factores para el tratamiento de aguas residuales urbanas (ARU): (i) parámetros de operación como el tiempo de retención hidráulica (TRH) y la carga orgánica (OLR); (ii) la eficiencia de celdas tubulares operadas en serie.

Se eligió un diseño tubular puesto que hay estudios anteriores que muestran que esta configuración tiene una distribución óptima del ánodo y del cátodo (Kim et al., 2010). Uno de los primeros estudios fue en un reactor MFC tubular alimentado con acetato, el cual alcanzó potencias de $5\text{--}90\text{ W m}^{-3}$ con unas eficiencias coulombicas de $\sim 70\%$ (Rabaey et al., 2005; Kim et al., 2009). También se han logrado valores de eliminación de DQO del 80% en MFCs tubulares para el tratamiento de aguas (Liu et al., 2004). Así mismo una configuración de dos MFCs tubulares en serie ha sido propuesta como una etapa depuradora para los efluentes de digestiones

anaerobias (Hu et al., 2009; Kim et al., 2010). MECs tubulares también han demostrado una alta eficacia alcanzando unas tasas de producción de hidrógeno y una eficiencia energética de $2.3 \text{ L L}_a^{-1} \text{ d}^{-1}$ y 240%, respectivamente, a valores de tensión medios-bajos y alimentados con una solución sintética (Hu et al., 2009).

Por consiguiente se propuso una configuración con dos reactores puesto que los resultados anteriores han demostrado que el funcionamiento de estas celdas se ve notablemente afectado cuando son alimentadas con influentes de baja carga, por lo cual la utilización de celdas en serie permitiría mejorar el tratamiento de aguas.

Con el diseño de una celda como la que se muestra en la Figura 9.5 con un volumen total de 4 litros y constituida por dos módulos tubulares conectados en serie se pretende maximizar la eliminación de DQO así como incrementar la producción de hidrógeno, especialmente, en el primer módulo ya que suele recibir mayores cantidades de materia orgánica y así el segundo actuaría puliendo e incrementando la eficacia depurativa.



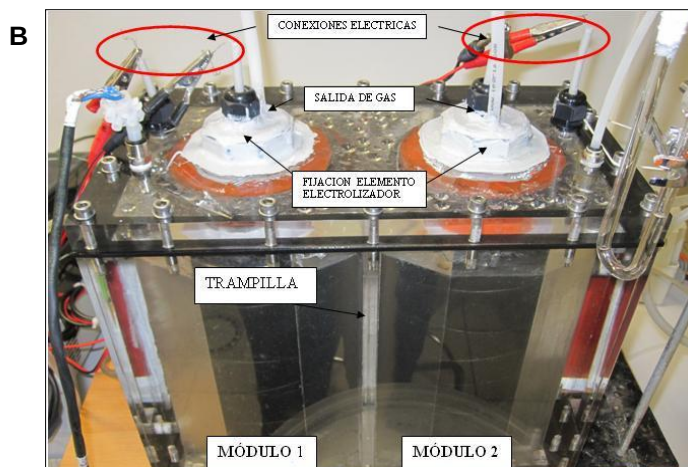


Figura 9.5. Vista de la MEC tubular bimodular utilizada en los ensayos. (A) Diagrama (M_A , módulo 1; M_B , módulo 2; V_1 , V_2 , tensión aplicada en cada módulo); (B) Foto.

El objetivo de estos ensayos ha sido estimar el efecto del tiempo de retención hidráulica sobre el funcionamiento de la MEC, identificar la carga orgánica que hace viable el uso de esta tecnología así como los límites de carga orgánica que justifican el uso de dos módulos en serie. Así mismo se realiza una evaluación de dicha configuración, para lo cual se observa su efecto en las tasas de eliminación de materia orgánica, de consumo de energía y de producción de hidrógeno.

En los ensayos cuyos resultados se muestran a continuación, se empleó como fuente de carbono agua residual urbana del decantador primario procedente de una depuradora municipal con una concentración de DQO que varió temporalmente entre 54.0 y 118.2 mg-DQO L^{-1} . La tensión aplicada se mantuvo en 1 voltio en ambos módulos durante todo el ensayo y se fueron variando los tiempos de retención hidráulica desde 25, 23, 15, 11, 7 hasta 4 horas. Estos tiempos de retención se corresponden con unas cargas orgánicas desde 0.10 a 1.32 g-DQO $L_a^{-1} d^{-1}$ en el módulo A (primer módulo) y de 0.05 a 0.62 g-DQO $L_a^{-1} d^{-1}$ en el módulo B (segundo módulo).

Los resultados muestran que para cargas orgánicas que podemos clasificar como medias-bajas de 0.67-0.10 g-DQO $L_a^{-1} d^{-1}$ y tiempos de retención hidráulica de 7-25 horas, la fuente de carbono fue uno de los factores limitantes afectando a la producción de hidrógeno y a la capacidad depurativa de la MEC. Aun así se alcanzaron tasas de eliminación de DQO del 86%, siendo el 90% de esta eliminación llevado a cabo en el primer módulo. Sin embargo, la corriente en este módulo representa solamente el 50-60% de la corriente total, lo que resulta en grandes diferencias en la eficiencia coulombica en ambos módulos. Especialmente en el módulo B donde esta fue superior al 100% para casi todos los tiempos de retención excepto el de 4 horas. Estas diferencias implican que en el módulo B gran parte de la corriente no está relacionada con la eliminación de la DQO, pudiéndose deber entonces a la recirculación de hidrógeno que hace que este sea difundido hacia el ánodo generando un incremento artificial en los valores de corriente (Lee et al., 2009; Parameswaran et al., 2009), aunque tampoco se puede descartar la presencia de cortocircuitos. Otra de las explicaciones podría ser la llegada al módulo B de compuestos orgánicos ya fermentados lo que implica que son más fácilmente asimilables por las bacterias electrógenas. Esto ya ha sido comprobado por otros autores que obtuvieron mejores resultados en MECs alimentadas con compuestos ya fermentados que con aguas residuales más complejas (Ditzig et al., 2007). Además hay que señalar que la baja eficiencia coulombica alcanzada en el módulo A sugiere que parte de la eliminación de DQO ha sido llevada a cabo por otros microorganismos en combinación con los electrógenos (Lefebvre et al., 2011) .

En lo referente a la tasa de producción de hidrógeno (Figura 9.6), esta aumentó a medida que incrementamos la carga orgánica haciéndose más notable para cargas por encima de 0.37 g-DQO $L_a^{-1} d^{-1}$, es decir para TRH menores a 11 horas. Por el contrario, para cargas mayores a 1.32 g-DQO $L_a^{-1} d^{-1}$ no se observó ningún incremento en la producción de hidrógeno en

el módulo A, en cambio se produjo un aumento en la producción de metano pudiendo deberse al aumento en la cantidad de compuestos orgánicos lo cual proporcionó unas condiciones favorables para el desarrollo de los microorganismos metanógenos. Esto ya ha sido descrito por otros autores que observaron un incremento de la actividad metanógena con mayores cantidades de sustrato y especialmente con compuestos ya fermentados (Parameswaran et al., 2009; Wang et al., 2009).

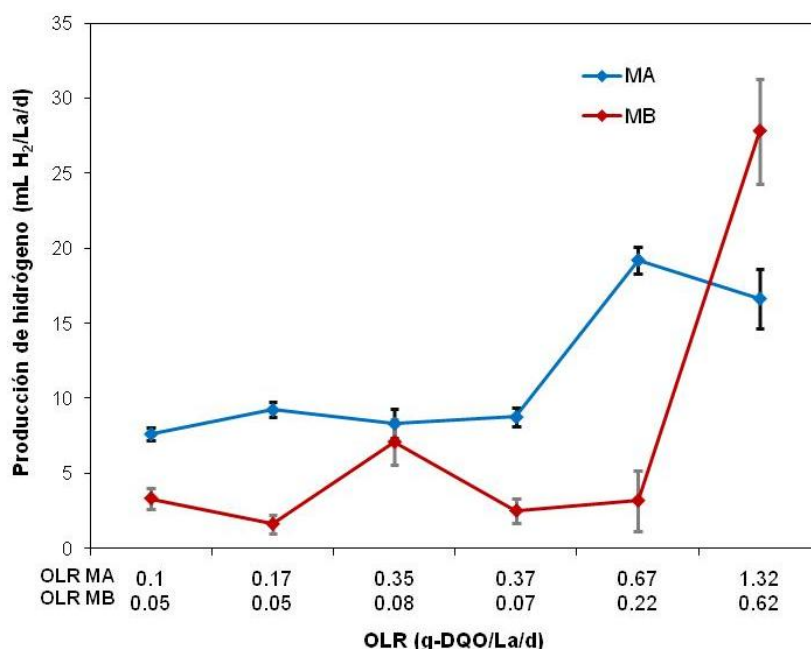


Figura 9.6. Producción de hidrógeno en función de cada carga orgánica administrada.

En cuanto a la eficiencia en la producción de hidrógeno, los resultados muestran que aumenta a medida que aumenta el TRH lo cual se debería a una mayor tasa de hidrolización y fermentación de los compuestos orgánicos. Además esto explicaría las eficiencias mayores en el segundo módulo donde los compuestos ya llegan más degradados, al igual que las menores proporciones de metano ya que bajas concentraciones de fuente de carbono limitan el crecimiento de estos, favoreciendo las condiciones

para el desarrollo de los microorganismos electrógenos (Lawrence and McCarty, 1969; Esteve-Núñez et al., 2005).

La eficiencia catódica fue muy baja, entre 9-24%, si se compara con otros estudios realizados en MECs alimentadas con agua residual urbana (Ditzig et al., 2007). Esto indicaría pérdidas de hidrógeno a través del sistema de recogida y/o debido a la recirculación de este, como ya se ha mencionado.

Con los resultados obtenidos se puede afirmar la viabilidad de este diseño con dos módulos en serie para cargas mayores de $0.67 \text{ g-DQO L}_a^{-1} \text{ d}^{-1}$, actuando así el segundo módulo como etapa de pulido. En cambio para cargas menores no se puede justificar el uso de un segundo módulo, principalmente debido al elevado consumo energético como se observa en la figura 8.7 ($27\text{-}7 \text{ kWh kg-DQO}^{-1}$), a la escasa tasa de depuración y la limitada producción de hidrógeno en dicho módulo.

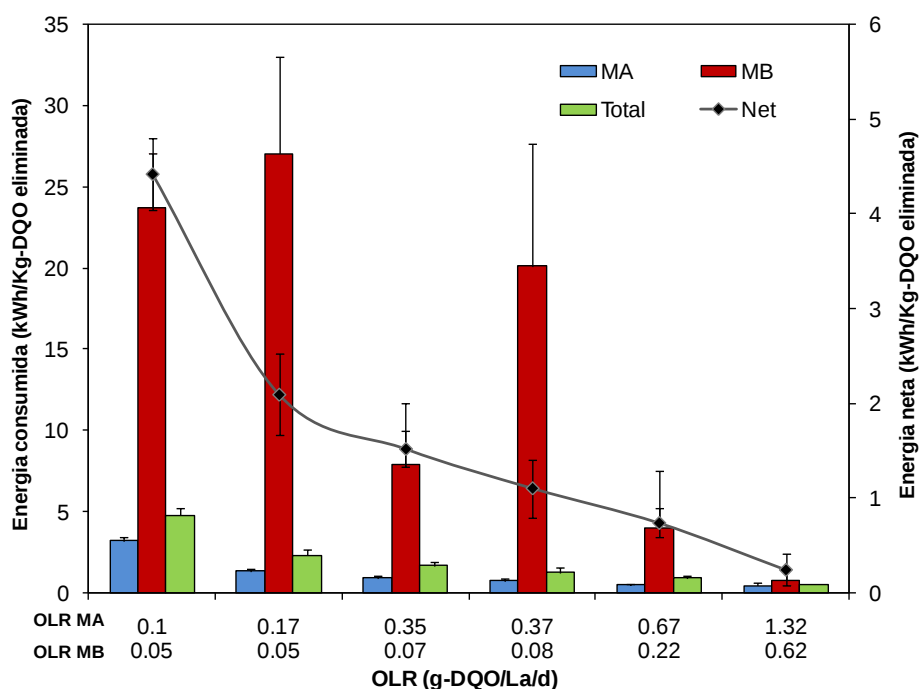


Figura 9.7. Consumo energético en cada módulo y consumo total y neto en función de la carga orgánica administrada a la celda.

Para cargas orgánicas por encima de $0.17 \text{ g-DQO L}_a^{-1} \text{ d}^{-1}$, el consumo energético en el módulo A fue inferior a $1.3 \text{ kWh kg-DQO}^{-1}$, el cual ya estaría por debajo de los consumos asociados al tratamiento aerobio de aguas residuales urbanas $1.5 \text{ kWh kg-DQO}^{-1}$ (Metcalf& Eddy Inc., 2003). Además la buena calidad del efluente a la salida del primer módulo para cargas orgánicas inferiores a las señaladas anteriormente, indica que en términos energéticos y de capacidad depurativa un solo módulo es competitivo con los tratamientos tradicionales. Así existen estudios recientes que afirman que la tecnología MEC, al menos considerando el consumo energético, sería competitiva incluso para cargas tan bajas como $0.12 \text{ g-DQO L}^{-1} \text{ d}^{-1}$ (Heidrich et al., 2012). Estos autores lograron consumos energéticos de $0.64 \text{ kWh kg-DQO}^{-1}$ en el tratamiento de aguas residuales urbanas en una MEC de 120L.

Por último, habría que descartar el uso de MECs para cargas de $0.10 \text{ g-DQO L}_a^{-1} \text{ d}^{-1}$, ya que a pesar de lograr un efluente con baja concentración de DQO, los consumos energéticos fueron tan elevados que sería imposible competir con los tratamientos aerobios, impidiendo así la viabilidad de este sistema.

9.6. OPTIMIZACIÓN DE LOS PARÁMETROS DE OPERACIÓN DE LA MEC TUBULAR BIMODULAR PARA AUMENTAR LA EFICIENCIA ENERGÉTICA Y LA CAPACIDAD DEPURATIVA DEL AGUA RESIDUAL URBANA

Basándonos en los resultados anteriores se procedió a optimizar el tiempo de retención hidráulica y la tensión aplicada, todo ello con el objetivo de disminuir el consumo energético y aumentar la capacidad depurativa del sistema para proceder a su posterior escalado.

Para esta serie de ensayos se utilizó la misma MEC bimodular tubular. Tomando como base los resultados anteriores, la celda fue operada con

una carga orgánica desde 0.46 a 1.59 kg-DQOL_a⁻¹ d⁻¹, lo que corresponde a tiempos de retención hidráulica de 10, 7 y 4 horas. La tensión aplicada fue siempre de 1 voltio en el segundo módulo y en el primero se modificó entre 0.6 y 1 voltio como se detalla a continuación.

En lo que se refiere a la capacidad depurativa (Figura 9.8) de la MEC, como sucedió anteriormente, el tiempo de retención hidráulica afectó ligeramente y, supuso una disminución en la tasa de depuración de 79 a 64% cuando la tensión aplicada fue de 1 voltio en cada módulo. Así mismo, la mayoría de la DQO fue eliminada en el módulo A.

Teniendo en cuenta que la mayoría de la depuración se realizó en el primer módulo, se disminuyó la tensión aplicada en este módulo para tratar de lograr que llegase una mayor cantidad de materia orgánica al segundo módulo, siendo así más equilibrada la carga orgánica en ambos módulos y permitiendo que la tasa de eliminación de DQO aumentase con consumos energéticos aceptables. Sin embargo, los resultados obtenidos no satisficieron el principal objetivo puesto que aunque se produjo una mejoría en la capacidad depurativa en el segundo módulo, la eliminación de DQO se vio negativamente afectada en el primer módulo y en consecuencia la tasa de eliminación de DQO global solo mejoró para el tiempo de retención hidráulica de 4 horas. Analizando los valores de eficiencias coulombicas, observamos que fueron muy bajas teniendo en cuenta la materia orgánica degradada, lo que indicaría que un elevado porcentaje de electrones no fue transformado en corriente. Esto sugiere que parte de la eliminación fue llevada a cabo por otros microorganismos además de los electrógenos. Al igual que ocurrió en el estudio anterior, se obtuvieron grandes discrepancias en los valores de eficiencia coulombica entre ambos módulos.

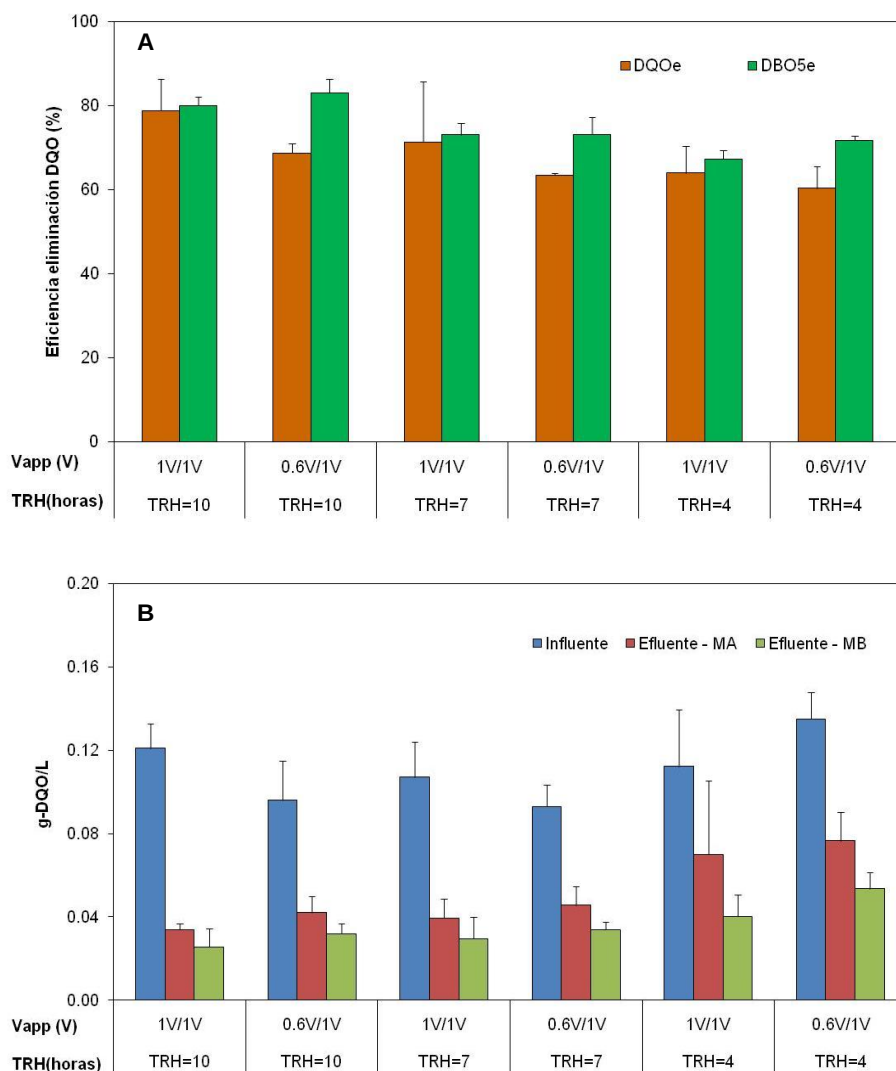


Figura 9.8. (A) Tasa de depuración de DQO y DBO₅ para cada TRH y Vapp. (B) Concentración de DQO en el influente y efluente en cada módulo en función del TRH y Vapp.

Una vez concluidos los ensayos se desmontaron ambos módulos para tomar muestras de las capas de ánodo y se observó una acumulación de lodos en la base de la celda, la cual podría deberse a la presencia de procesos fermentativos. Así mismo, gracias a la fotografía SEM (Figura 9.9), se observaron cúmulos de microorganismos en el biofilm del ánodo, lo

cual podría explicar las discrepancias en la eficiencia coulombica así como la eliminación de materia orgánica por parte de otros microorganismos no electrógenos. Previamente otros autores han observado agregados de bacterias muertas y de bacterias no electrógenas acumuladas en el biofilm del ánodo; estos agregados no contribuyeron al metabolismo eléctrico pero si consumieron parte de la materia orgánica que llegaba a la celda (Ren et al., 2011). Por otra parte Lefebvre et al., (2011) confirmaron la acumulación de lodos en la base del compartimento anódico de reactores verticales, favoreciendo estos la proliferación de microorganismos competidores de los electrógenos, lo que explicaría los valores de eficiencias coulombicas obtenidos.

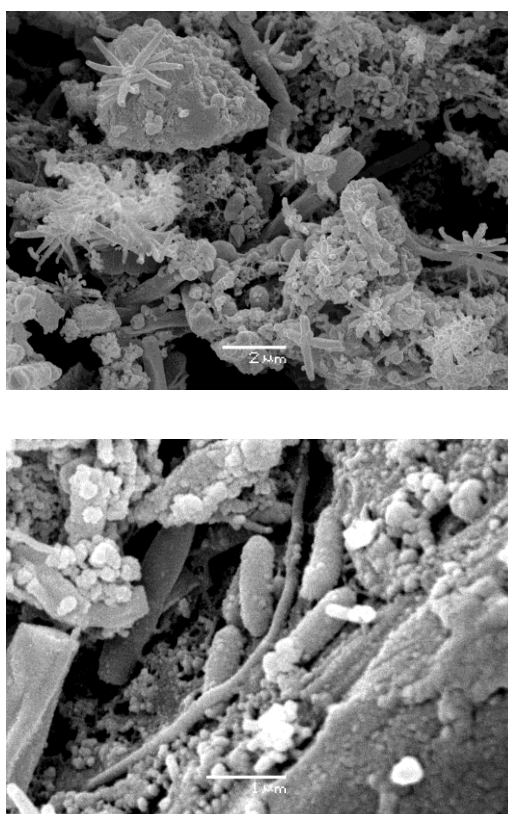
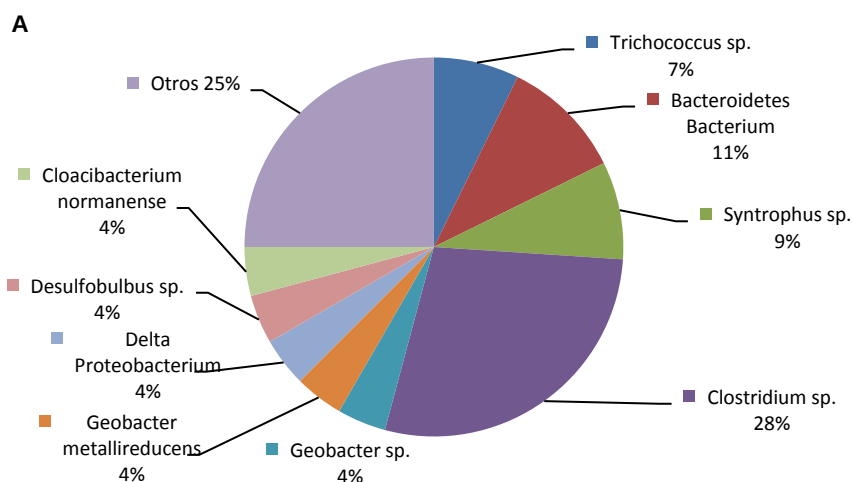


Figura 9.9. Vista de las aglomeraciones de biomasa del ánodo de la celda. SEM (400x) de las muestras del ánodo.

Una vez extraídas las muestras del ánodo se llevó a cabo una electroforesis en gel con gradiente desnaturalizante y una clonación de los microorganismos del ánodo. En la figura 9.10 podemos observar los resultados obtenidos que mostraron la presencia de los siguientes microorganismos no electrógenos: *Syntrophus sp.* (bacterias anaerobias que degradan ácidos grasos en asociación con microorganismos consumidores de hidrógeno (Hatamoto et al., 2007)), *Trichococcus sp.* (microorganismos que son normalmente encontrados en fangos activados participando en los procesos fermentativos por lo que podrían degradar la materia orgánica en MECs (Bergey and Holt, 1994)), *Chlorobium limicola* (consumidora de hidrógeno generado en fermentaciones (Silke et al., 1999)) y por último *Clostridium sp.* (este microorganismo al igual que las anteriores participa en procesos fermentativos). Este último fue encontrado mayoritariamente en la capa externa del ánodo, la cual está en contacto directo con el agua residual alimentada al reactor. En cambio en la capa interna se encontró la mayoría de la comunidad electrógena, lo cual sugiere que los compuestos más complejos fueron transformados en compuestos más simples en la capa externa mientras los electrógenos utilizaron los compuestos más asimilables para generar electricidad.



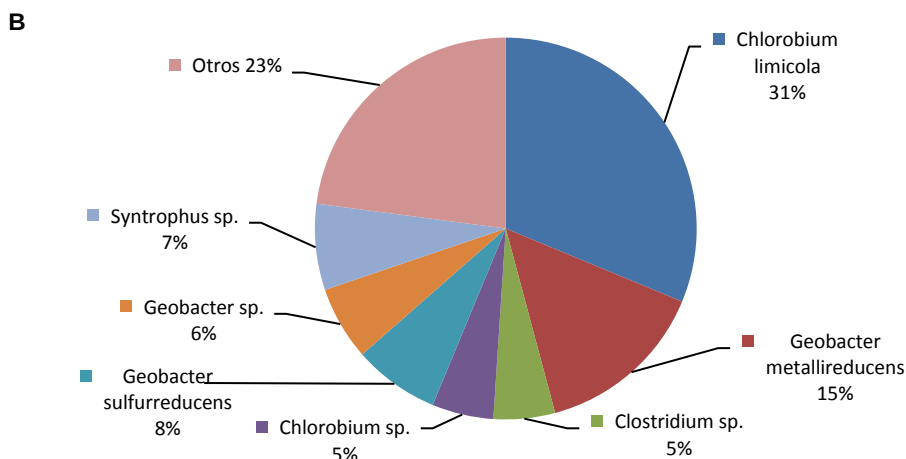


Figura 9.10. Comunidades bacterianas del ánodo según los ensayos de clonación de las secuencias de rRNA16S. (A) Ánodo externo, (B) Ánodo interno.

En relación a la producción de hidrógeno (Tabla 9.4) cuando se mantuvo la tensión aplicada en 1 voltio en cada módulo, se observó un aumento de la producción de hidrógeno a medida que el TRH disminuyó, debido al incremento de la materia orgánica suministrada a la celda. Considerando cada módulo por separado, la producción fue mayor en el segundo módulo para tiempos de retención hidráulica de 4 y de 7 horas, independientemente de la tensión aplicada en cada módulo. Estos resultados indicarían que mayor cantidad de productos ya fermentados alcanzaron el segundo módulo siendo así más accesibles para los electrógenos. En cambio al disminuir la tensión aplicada a 0.6 voltios en el módulo A, la producción de hidrógeno se vio notablemente afectada especialmente en el primer módulo, donde apenas se observó producción de gas. En líneas generales la producción de gas estuvo limitada por la baja carga del agua residual suministrada, lo que sugiere que existen otros fenómenos que están afectando negativamente a la cantidad de gas generado. Estos pueden ser desde fugas en el sistema de captura del gas, así como recirculaciones de los protones y/o del hidrógeno generado, el cual sería consumido por microorganismos homo-acetógenos, hidrogenotróficos metanógenos o ARB

(Lee and Rittmann, 2010; Parameswaran et al., 2011), hasta pérdidas de electrones que se ven envueltos en otras reacciones en vez de en la generación de hidrógeno (Parameswaran et al., 2009), aumentando así la corriente pero no la eficiencia en la producción de hidrógeno (Lee and Rittmann, 2010).

Tabla 9.4. Producción de gas en función del TRH y V_{app} .

TRH (h)	V M_A/M_B (V)	H ₂ (mL d ⁻¹)			CH ₄ (mL d ⁻¹)		
		Total	M _A	M _B	Total	M _A	M _B
10	1/1	37.6	28.9	8.7	1.5	1.5	0.0
	0.6/1	11.5	<0.1	11.5	0.6	<0.1	0.6
7	1/1	32.7	12.7	21.8	2.0	0.8	1.2
	0.6/1	52.7	<0.1	51.9	5.0	0.0	5.0
4	1/1	89.4	33.2	55.6	19.6	6.4	13.2
	0.6/1	31.1	<0.1	30.0	5.5	<0.1	5.5

Junto con el hidrógeno también se generó metano, el cual siguió una tendencia similar a la del hidrógeno, sin embargo apenas se vio afectado por la tensión aplicada, por lo que se puede considerar que este metano deriva del incremento de materia orgánica y no del hidrógeno. Por otra parte analizando los efectos del tiempo de retención hidráulica y la tensión aplicada en el consumo energético (Figura 9.11), se detectó una notable influencia de ambos sobre la energía necesaria para desarrollar el proceso de electrólisis biocatalítica. El consumo energético disminuyó con el TRH, así al bajar el TRH de 10 a 4 horas, la energía consumida descendió de 1.02 a 0.56 kWh kg-DQO⁻¹ (kilowatios hora por kilogramo de DQO eliminada), mientras que un aumento de 0.6 voltios a 1 voltio en el primer módulo supuso un incremento del consumo en este módulo de 0.12 y 0.28 kWh kg-DQO⁻¹ para TRH de 10 y 4 horas, respectivamente. Los valores de consumo energético obtenidos están por debajo de la energía consumida en procesos aerobios de tratamiento de aguas residuales urbanas (0.7-2

kWh kg-DQO⁻¹) (Pant et al., 2011), lo cual intensifica la viabilidad de esta tecnología. Hay que destacar que se lograron consumos energéticos tan bajos como 0.23 kWh kg-DQO⁻¹, el cual está por debajo de los valores teóricos necesarios para la producción de hidrógeno en una MEC (0.47 kWh kg-DQO⁻¹, suponiendo una tensión aplicada de 0.11 V). Estudios previos encontraron resultados similares, donde una MEC alimentada con ácidos grasos volátiles con un TRH de 12 horas y una tensión aplicada de 0.6 voltios funcionó con consumos energéticos de 0.11 kWh kg-DQO⁻¹ (Escapa et al., 2012b). Estas discrepancias podrían explicarse teniendo en cuenta la contribución en la eliminación de DQO de otros microorganismos aparte de los electrógenos. Esta hipótesis se apoyaría en los resultados obtenidos en los análisis microbiológicos, en los cuales se encontró una gran cantidad de microorganismos no electrógenos como ya se ha comentado anteriormente. Con lo cual resulta complicado estimar el consumo energético real.

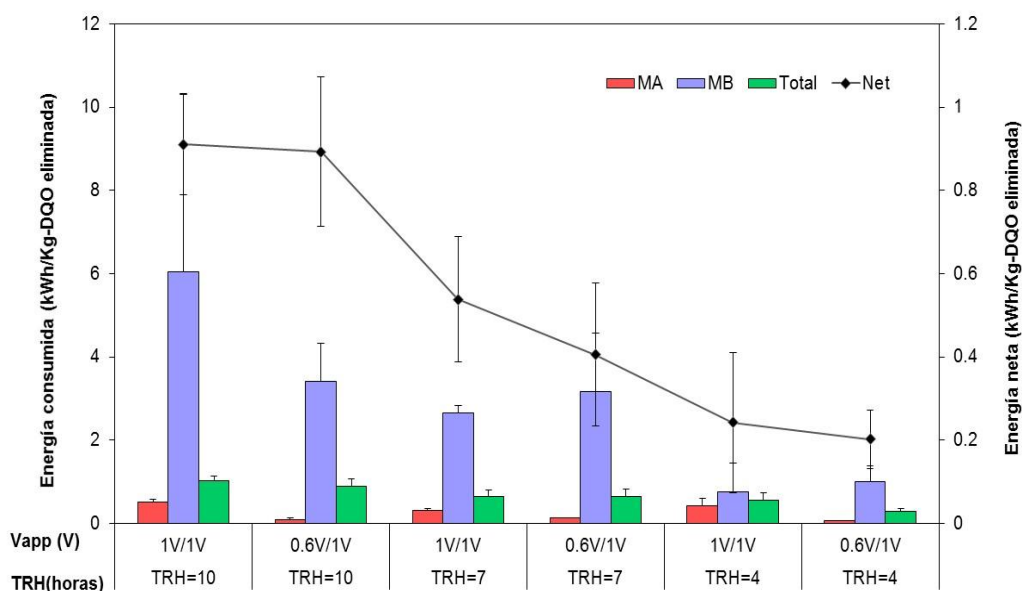


Figura 9.11. Consumo energético en cada módulo, consumo total y neto en función del TRH y Vapp.

Con estos resultados se pudieron estimar el TRH y la tensión aplicada más adecuados para el funcionamiento de la MEC tubular. Así se puede establecer el TRH de 4 horas como el límite para el cual se recomienda utilizar un segundo módulo como etapa de pulido de la DQO. En cambio, para TRHs superiores a 4 horas no sería necesario este segundo módulo ya que apenas se observó eliminación en este, además del elevado consumo energético alcanzado. Así para TRH de 10 a 7 horas el efluente se puede considerar que estaba dentro de los parámetros legales en cuanto a concentración de DQO, los cuales se alcanzaron con un consumo de energía relativamente bajo.

Sin embargo esto solo sería una estimación ya que estas condiciones de operación podrían variar dependiendo de los límites legales de calidad de aguas así como de la composición del agua.

En cuanto a la tensión aplicada en cada módulo, los resultados obtenidos indican que sería necesario una tensión aplicada superior a 0.6 voltios para obtener una buena tasa de eliminación de DQO a bajos consumos energéticos, ya que por el contrario una tensión aplicada de 0.6V no tuvo ninguna ventaja, afectando negativamente a la tasa de eliminación de DQO así como a la producción energética.

A modo de conclusión se puede considerar que tanto el TRH como la tensión aplicada juegan un papel importante en la eficiencia energética y en la producción de hidrógeno de la MEC tubular bimodular para el tratamiento de aguas residuales urbanas de baja carga. Por el contrario, la capacidad depurativa del sistema no se vio tan afectada debido a la presencia de microorganismos no electrógenos, los cuales contribuyeron a la degradación de materia orgánica pero no a la producción de hidrógeno ni corriente. Al igual que en el ensayo del apartado anterior, podríamos establecer un TRH de 4 horas (cargas orgánicas por encima de 0.64-0.74 g-DQO $L_a^{-1} d^{-1}$) para que la configuración de dos módulos fuese viable y se

lograsen efluentes con una buena calidad en términos de concentración de DQO. Mientras que para alcanzar tasas depurativas por encima del 70% solo sería necesario un módulo operando a un TRH de 10 horas, obteniéndose unos consumos energéticos por debajo de los necesarios en tratamientos aerobios, demostrando así el gran potencial de la tecnología MEC para el tratamiento de aguas residuales urbanas a lo que habría que añadir otro factor de gran importancia que es la disminución en la generación de fangos.

9.7. CÁLCULO DE LA HUELLA DE CARBONO DE LAS INSTALACIONES DE UNA PLANTA DE TRATAMIENTO DE AGUA RESIDUAL URBANA (EDAR) CON UN SISTEMA BE INTEGRADO EB Y SU COMPARACIÓN CON UNA EDAR CONVENCIONAL

El tratamiento de aguas residuales contribuye a la producción de gases de efecto invernadero a través del metano y del dióxido de carbono del propio tratamiento en sí, así como de los gases generados a partir de los requerimientos energéticos. Estudios previos han estimado que una planta de tratamiento de aguas residuales podía incrementar en un 5% la producción global de metano (El-Fadel and Massoud, 2001). Como se ha comentado anteriormente los sistemas bioelectroquímicos son una nueva tecnología capaz de generar energía a partir de las aguas residuales con baja carga orgánica y capaz de reducir los consumos energéticos en dicho proceso al igual que la generación de gases de efecto invernadero.

El análisis de la huella de carbono permite evaluar el efecto de un proceso sobre el medio ambiente analizando su contribución al incremento de los gases de efecto invernadero. Especialmente en el desarrollo de nuevas tecnologías es esencial analizar su huella de carbono antes de la aplicación de dichas tecnologías a gran escala. Así mismo es importante llevar a cabo

dicho estudio principalmente en las tecnologías que usan distintos tipos de residuos o biomasa para generar compuestos químicos, combustible u otros productos, ya que a primera vista puede parecer que solo conllevan efectos positivos sobre el medio ambiente, sin embargo cuando se realiza un estudio exhaustivo de todo el proceso, en ocasiones, aparecen significantes efectos nocivos que ponen en peligro el potencial de dicha tecnología. Así, un claro ejemplo es el análisis de los impactos ambientales de una planta productora de algas para la generación de biocombustible. Clarens et al. (2010) llevó a cabo dicho análisis y concluyó que este proceso requiere seis veces más energía que el proceso a partir de plantas productoras de biocombustible, además de emitir una mayor cantidad de gases de efecto invernadero a la atmósfera, en contradicción a lo que se creía inicialmente considerando que las algas podían ser un gran recurso para la generación de biocombustibles. Esto demuestra la importancia de llevar a cabo un análisis completo de la huella de carbono en nuevas tecnologías antes de llevar a cabo su comercialización.

Para este análisis se ha utilizado la herramienta O2CTM basada en el análisis de ciclo de vida y las emisiones de efecto invernadero (ISO 14040). Esta herramienta integra la metodología de Bilan Carbone definida por ASTEE (Scientific and Technical Association for Water and the Environment). En este estudio se llevó a cabo el análisis de la huella de carbono de dos escenarios. El escenario uno consistió en una planta de tratamiento de aguas residuales para una población de 73600 habitantes con una capacidad de $12696 \text{ m}^3 \text{ d}^{-1}$, 1595 t-DBO₅ eliminada anualmente y 215 t-N eliminado anualmente. Se trata de una planta convencional con un sistema de tratamiento biológico aerobio (fangos activados) que permite obtener un efluente con cargas de 695 g-DQO m^{-3} , $48.15 \text{ g-TNK m}^{-3}$ y 13.04 g m^{-3} de fósforo. En el segundo escenario se consideró la integración de una MEC como parte del tratamiento biológico, cuyo efluente sería posteriormente tratado aeróbicamente como un paso de pulido logrando así

cargas de 15-125 g-DQO m⁻³. Esta planta además requiere un compresor y un tanque de almacenamiento del gas producido.

Utilizando la metodología indicada anteriormente, la huella de carbono para el escenario uno fue estimada en 17525 t CO₂e generadas por la infraestructura y 6164 t CO₂e anualmente generadas por la operación de la planta. En cuanto a la infraestructura, el 62% de las emisiones están asociadas con los materiales para la construcción (11179 t CO₂e) siendo el cimentado y pavimento el mayor contribuidor a dicho porcentaje con 7328 t CO₂e, seguido por acero de estructura con 1196 t CO₂e y el pavimento con 942.8 t CO₂e. El consumo eléctrico contribuye con un 34% al total de las emisiones a partir del consumo de 20000 MWhe. El transporte contribuye con un 2% (424 t CO₂e) y la maquinaria solo contribuye en 1% (243 t CO₂e). La maquinaria tiene un menor impacto, siendo las tuberías de PVC (85.5 t CO₂e) y las de acero (17.3 t CO₂e) los mayores generadores de CO₂.

En cuanto a la operación de la planta, los residuos generados en el proceso emiten 4004 t CO₂e anuales, siendo el secado de los fangos (7040 t DS año⁻¹) y su posterior traslado y evacuación, los mayores generadores de CO₂. Los productos químicos utilizados en el proceso así como el resto de servicios generan 991 t CO₂e anuales, siendo el mayor contribuidor el uso de productos químicos. El consumo energético solo contribuyó en un 10% con 588 t CO₂e anuales y el transporte un 1%, debido principalmente al transporte de residuos (42 t CO₂e año⁻¹). Teniendo en cuenta la generación de CO₂ debida al tratamiento se puede estimar en 330 t CO₂e año⁻¹ para la eliminación de 2990 t-DQO año⁻¹ y 170 t-N año⁻¹.

En el escenario dos (WWTP+MEC) la huella de carbono fue estimada en 17765 t CO₂e generadas por la infraestructura y 5504 t CO₂e anuales por la operación de la planta. En cuanto a la construcción de la planta, los materiales de construcción tuvieron el mayor impacto, al igual que ocurría

en el caso anterior, generando 11395 t CO₂e, lo cual corresponde al 63%, al igual que en el escenario anterior la cimentación es el mayor generador de emisiones con 7288 t CO₂e, seguido por el acero de estructura con 1177 t CO₂e. En este caso hay que destacar la elevada emisión del uso del carbón activado que es necesario para el cátodo y el ánodo de las MECs. El consumo energético genera 6120 t CO₂ en lo que supone un 34% del total. La maquinaria contribuye solo en un 1% (249 t CO₂e) y el transporte en un 2% (425 t CO₂e). En cuanto a la operación de la planta se estimó en 5500 t CO₂e, lo cual es principalmente producido por el secado del fango y la evacuación de residuos (fangos) (3353.2 t CO₂e año⁻¹) en relación a la generación de 5914 t DS anuales. Los consumibles generan 1087 t CO₂e anuales y el principal emisor son los servicios (493 t CO₂e anuales) y el consumo de 40% solución de FeCl₃ (284 t CO₂e anuales). La energía requerida en el proceso genera 759 t CO₂e año⁻¹ a partir de los 1416.2 MWh consumidos anualmente. El transporte solo contribuye con un 1% y es mayoritariamente debido al transporte de residuos. Por otra parte, si se tiene en cuenta la cantidad de biogás generado en el proceso (1 373 568 Nm³) que podría ser inyectada en la red, se evitaría la emisión de 2267 t CO₂e anuales. Teniendo en cuenta la eliminación en valores de DQO y N, se estima en 54.3 t CO₂e año⁻¹ para 2990 t-DQO eliminada año⁻¹ y 170 t-N año⁻¹.

A partir de los resultados obtenidos podemos concluir que la construcción de la planta de tratamiento de aguas residuales tiene un elevado impacto teniendo en cuenta la cantidad de CO₂ liberado a la atmósfera, siendo los materiales de construcción como el cementado y el acero de refuerzo los mayores contribuidores. Sin embargo, las emisiones son mayores en el segundo escenario debido al uso de carbón activado en el ánodo y cátodo de las MECs. Este material incrementa sustancialmente la cantidad de CO₂ liberado, siendo este factor un aspecto negativo que hay que tener en cuenta a la hora de comercializar esta tecnología. Por lo tanto será

necesario la implementación y búsqueda de nuevos materiales que tengan menos efectos nocivos en el medio ambiente. Así existen previos estudios en los cuales se ha investigado en el uso de nuevos materiales más eficientes y capaces de reducir las emisiones a la atmósfera (Kipf et al., 2013; Lanas and Logan ,2013).

En cuanto al funcionamiento de ambas plantas, la planta de tratamiento con la MEC integrada genera 660 t CO₂e anuales menos que la planta convencional, esto supone una importante reducción en la cantidad de emisiones a lo largo de la vida de la planta. Esta reducción en las emisiones es principalmente debida a la disminución de residuos como los lodos que pasaron de 7040 t DS anuales en el primer escenario a 5914 t DS en el segundo. La disminución en la producción de lodos ya ha sido confirmada por otros autores (Villano et al., 2013) y es una de las principales ventajas de las celdas bioelectroquímicas. Otro factor a destacar es la cantidad de emisiones que se evitan en el segundo escenario, gracias a la reducción en la generación de lodos así como en el consumo energético si consideramos que se podría utilizar la energía contenida en el hidrógeno generado que podría ser utilizada para el abastecimiento del propio proceso.

9.8. CONCLUSIONES

1. Se demuestra la viabilidad del paso de escala de laboratorio de una MEC de 50 mL alimentada con agua residual sintética a una MEC de 10 L alimentada con agua residual urbana.
2. El diseño de MECs operadas en serie fue identificado como el más factible para alcanzar unas producciones de hidrógeno adecuadas y cumplir con la legislación vigente sobre tratamiento de aguas residuales. Además se identificó la necesidad de administrar cargas orgánicas superiores a 1-2 g L_R⁻¹ d⁻¹ para poder alcanzar producciones de hidrógeno aceptables.

3. Para reducir los consumos de energía en una MEC, la tensión aplicada debe controlarse individualmente en cada celda y optimizándose a tiempo real en función de la carga orgánica en cada momento.
4. La celda bioelectrolítica tubular consiguió reducir un 85% de la demanda química de oxígeno del agua residual urbana con unos consumos energéticos por debajo de los consumos asociados a los tratamientos aeróbicos de aguas residuales.
5. La MEC tubular es capaz de competir con los tratamientos aeróbicos en términos de consumos energéticos y calidad del efluente cuando se le administra a la celda una carga orgánica de $0.10 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$. Así mismo podemos establecer una carga de $0.67 \text{ g-COD L}_a^{-1} \text{ d}^{-1}$ como el umbral por encima del cual es necesario añadir otro módulo operando en serie para así mejorar la depuración y alcanzar los valores establecidos por la legislación actual en España, referido a la DQO.
6. Se demostró la viabilidad de una celda bioelectrolítica formada por módulos en series para el tratamiento de aguas residuales urbanas con una carga orgánica suficientemente elevada para garantizar el funcionamiento y la eficacia de las celdas en series.
7. El tiempo de retención hidráulica y la tensión aplicada mostraron significantes influencias en la producción de hidrógeno y el consumo de energía en la celda tubular semi piloto alimentada con agua residual urbana. Sin embargo, la tasa de eliminación de DQO fue menos afectada debido a la presencia de microorganismos que aunque no contribuyen al metabolismo electrogénico si consumen una parte de la carga orgánica que es alimentada a la celda.

8. Las emisiones durante el funcionamiento de la planta serían menores en la planta con el electrolizador biocatalítico, a lo cual si sumamos las emisiones que se evitan si se utilizase el hidrógeno generado para el propio abastecimiento de la planta o bien para su inyección en la red, los beneficios serían aún mayores.

9.9. BIBLIOGRAFÍA

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