DRIVING FORCE BEHIND ELECTROCHEMICAL PERFORMANCE OF MICROBIAL FUEL CELLS FED WITH DIFFERENT SUBSTRATES

Abstract

The performance of miniaturized microbial fuel cells operating with five different substrates (acetate, lactate, glucose and octanoate) were studied with the aim to identify the reason for its different performance. In all cases, the COD removal rate was about 650 mg COD L⁻¹ d⁻¹. However, the bio-electrochemical performance of the MFC was very different, showing the MFC fed with acetate the best performance: 20 A m⁻² as maximum current density, 2 W m⁻² of maximum power density, 0.376 V of OCV and 12.6% of CE. In addition, the acetate showed the best bio-electrochemical performance in the polarization curves and cyclic voltammetries. These polarization curves were modelled and the key to explain the better electrical performance of acetate was its lower ohmic losses. When working with acetate, its ohmic losses were one log-unit below those attained by the other substrates. These lower ohmic losses were not associated to the electrolyte conductivity of the fuel but to the lower ohmic losses of the biofilm generated.

Keywords

Microbial fuel cell; Mathematical modelling; Wastewater; Bioconversion

1. INTRODUCTION

The ability of microorganisms to interact with electrodes is the key to understand bioelectrochemical systems (BES) (Santoro et al., 2017). Amongst the BES, the Microbial Fuel Cells (MFCs) stand up as a sustainable technology for energy production, because MFCs are able to oxidize substrates while producing electricity (Logan, 2008). Based on the discovery of exo-electrogenic bacteria by Potter in 1991 (Pant et al., 2010), only recent studies has shown the way to develop MFC with real applications (Qian and Morse, 2011).

In the MFCs, it is used an anodic electrode as the immediate electron acceptor, instead of any chemical compound such as molecular oxygen. Once the electrons are collected on the anodic electrode, they are driven through an external electrical circuit to reach the cathode. Simultaneously, the cations travels from the anodic to the cathodic throughout the electrolyte. At the cathode, oxygen is usually reduced to form water by mean of its combination with the electrons driven through the external electrical circuit and the diffused protons. Oxygen is commonly used because of its availability, sustainability and high standard redox potential (Mateo et al., 2017b).

Electrogenic bacteria are the key component of MFCs and are present in most of the ecosystems (Chabert et al., 2015). The most commonly used inoculum sources are activated and anaerobic sludge (Lobato et al., 2012), domestic wastewater and marine sediments (Liu and Logan, 2004; Rezaei et al., 2007; Erable et al., 2009) but their occurrence has also been reported garden compost (Niessen et al., 2006) and soils (Parot et al., 2008), among other environments. In the literature, it has been described that the electrogenic microbial consortia developed in the anodic chamber is influenced by the source used as inoculum (Ieropoulos et al., 2010), the enrichment procedure (Wang et al., 2012) and the substrate (Pant et al., 2010). However, the substrate used as carbon

source has a clear impact in the metabolisms of the electrogenic organisms and consequently into the electricity generation (Khater et al., 2017; Zhang et al., 2017).

A wide variety of chemicals can be used as carbon source, including, among others, simple molecules like carboxylic acids or alcohols (Kim et al., 2007; Song et al., 2009; Song et al., 2010; Fernandez et al., 2016; Zhang et al., 2017) and complex organic molecules such as those contained in meat processing wastewater (Heilmann and Logan, 2006), paper recycling wastewater (Huang and Logan, 2008), urine (Ieropoulos et al., 2013), brewery wastewater (Feng et al., 2008), chocolate industry wastewater (Patil et al., 2009), rice straw hydrolysate and potato wastewater (Phung et al., 2004), etc.

From these works, it can be drawn that the biodegradability of the substrate seems to be the key to develop biological cultures rich in electrogenic bacteria (D'Angelo et al., 2017; Khater et al., 2017): electrogenic bacteria are able to completely oxidise readily biodegradable substrates; however, when dealing with complex substrates, the electrogenic microorganisms require the cooperation of hydrolytic and fermentative microorganisms, which generate short-chain fatty acids and fermentation products that can be easily consumed by the electrogenic bacteria (Lovley, 2006; Lovley, 2008; Mateo et al., 2018).

In the literature, synthetic wastewater has been intensely used in research studies mainly to ensure that pure cultures are not externally contaminated. It is also very common the addition of acetate and glucose (Khater et al., 2017) to the synthetic wastewater as main substrate and, sometimes, it has been also used lactate and xylose (Huang et al., 2008). Performance attained depends strongly on the substrate (fuel) dosed to the MFC (Catal et al., 2011). Thus, glucose has been reported to produce maximum power densities in two-chamber MFCs of 40.3 mW m^{-2} using carbon paper as anode and *Geobacter SPP*

as inoculum (Jung and Regan, 2007), 16 mW m^{-2} with graphite as anode and *Saccharomyces cerevisiae* and 283 mW m^{-2} using graphite plates as anode and mixed culture in two-chamber air breathing MFC (Rahimnejad et al., 2009). Feeding acetate to a double chambered MFC with carbon paper in a single chamber MFC, in which the anode was carbon cloth and the bacteria inoculated were mixed culture, results in 506 mW m^{-2} (Catal et al., 2008). In the case of the lactate in a double chambered MFC whose anodic electrode was carbon paper and the bacteria enriched was *Geobacter SPP*, the maximum power obtained was 52 mW m^{-2} (Jung and Regan, 2007). These results point out that it is difficult to compare performances reported in the literature when the studies were carried out at different environmental and operational conditions, with different material of the electrodes, different surface area and enriched with different inoculum sources. Moreover, most of the comparison reported in the literature are based on phenomenological observations without going deep inside the real cause of the performance.

For this reason, the aim of this work is to study the influence of different substrates over the performance of MFC operating in identical set-ups and under the same operational conditions. The organic substrates studied were selected following a criteria of increasing the number of carbon atoms in the molecule: acetate (C2), lactate (C3), glucose (C6) and octanoate (C8). Additionally, with the aim to elucidate the main driving force of the electrochemical performance of the different substrates, the polarization curves describing the steady-state performance of the MFC operating with different substrates were fitted using an empirical model and fitting parameters were discussed in order to shed light on the mechanism of the processes happening in the electrochemical cell.

2. Material and methods.

2.1. Experimental set-up.

The set up consist on four identical MFCs built in methacrylate. The MFCs were operated simultaneously during 105 d in order to avoid variations in the external factors and to ensure the comparison and reproducibility of the results. The anode was made of carbon felt ($35.3 \text{ m}^2 \text{ g}^{-1}$ of specific area, 500 g m^{-2} of area weight, thickness of 0.4 cm and 95% of porosity). The volume of the anodic chamber was 0.346 mL. Cathodic electrode was made of carbon paper with a 10% of Teflon content (Freudenberg C2) modified with the deposition of a layer of $0.5 \text{ mg Pt cm}^{-2}$ (Mateo et al., 2017b). Anode and cathode were placed as close as possible but separated by a Nafion N117 proton exchange membrane (DuPont™ Nafion PFSA Membrane) and connected externally by a load of 120Ω . The active area of the electrodes and the dimensions of the membrane were 0.866 cm^2 . The anode was connected to an auxiliary tank of 115 mL, which contained the medium, and the cathode was open to the atmosphere using the oxygen from the air (Mateo et al., 2015). More information describing the set-up used can be found elsewhere (Mateo et al., 2017a).

2.2. Experimental operation.

The MFCs were started-up following the same criteria: auxiliary tank seed with aerobic sludge from the Wastewater Treatment Plant of Ciudad Real, previously described in the literature (Rodríguez Mayor et al., 2004), ensuring a proportion of 50% of decanted sludge and 50% of real wastewater to facilitate the flow in the system. The second and the third days, the 50% of the volume was removed and replaced by the same fresh mixture. The fourth day, a Hydraulic Retention Time (HRT) of 2.5 d was established in the MFCs, so 46 mL were removed from the system and replaced by fresh synthetic wastewater (D'Angelo et al., 2017; Mateo et al., 2017a). This procedure was carried out

in a daily basis during the operation time of the MFCs. The medium was continuously pumped to the anodic chamber at 3 mL min^{-1} and then recirculated to the auxiliary tank. This tank was completely mixed to ensure the homogeneity.

2.3. Wastewater.

The wastewater used in this study was synthesised in the laboratory to ensure reproducible characteristics. Four different carbon sources were used: Acetate, lactate, glucose and octanoate. Regardless the carbon source, the composition and the concentration of the trace minerals remained unchanged at these values: 1.85 g L^{-1} of ammonium sulphate, 2.77 g L^{-1} of sodium carbonate, 0.92 g L^{-1} of hexahydrate magnesium chloride, 1.11 g L^{-1} of potassium dihydrogen phosphate, 1.25 g L^{-1} of calcium chloride and 0.07 g L^{-1} of ferric ammonium sulphate. The carbon sources were added to get a concentration of $10,000 \text{ mg COD L}^{-1}$ in the corresponding wastewater. To do that, the following amounts of carbon source were added: 16 g L^{-1} of sodium acetate, 25.5 g L^{-1} of calcium lactate, 35.1 g L^{-1} of glucose and 32 g L^{-1} of sodium octanoate. All the reagents of analytic grade were purchased from Sigma Aldrich and Panreac. Before its use in the MFC, the synthetic wastewaters were autoclaved at 120°C during 15 minutes in order to preserve its characteristics (Fernández et al., 2011).

2.4. Analytical methods

The pH was measured using a GLP22 Crison pH-meter. The removal of organic matter was followed by measuring the COD in the purge daily removed. Before COD determination, the samples were filtered by means of a $0.45 \mu\text{m}$ glass fibre filter. The COD determination was carried out according literature (American Public Health Association, 2005). The determination of the intermediates were carried out from filtered samples using a HPLC (Agilent Technologies) with an UV–DAD detector at a

detention wavelength of 210/8 nm and a reference wavelength of 360/80 nm, and a Zorbax SB-Aq (4.6 x 150 mm 5-microm) column. The oven temperature was 25 ° C, the mobile phase was a buffer solution of 20 mM of NaH₂PO₄ and H₃PO₄. Calibrates were carried out each time the mobile phase was replaced.

2.5. Electrochemical methods.

Voltage was monitored continuously with a Keithley 2000 voltmeter data logger. Polarization curves were carried out in a weekly basis using AUTOLAB PGSTAT30 (Ecochemie, The Netherlands) at a scan rate of 0.001 V s⁻¹ from the OCV to 0.001 V with the GPES software. Cyclic voltammetries were carried out with the same AUTOLAB PGSTAT30 and with the GPES software at a scan rate of 0.001 V s⁻¹ and a step potential of 0.005 V. The scan ranged from -0.5 to 0.5 V starting the scans at a mid-point of 0.005. The reference electrode used was Ag/AgCl. The same potentiostat/galvanostat (Autolab PGSTAT30) using the frequency response analyser module was used to carry out electrochemical impedance spectroscopy (EIS). EIS measurements were run for the full cell at open circuit conditions, in a frequency range of 10 kHz–1 mHz and with an AC signal of 10% of OCV. The cathode was used as the working electrode and the anode as the counter and the reference electrode (Ramasamy et al., 2008).

3. Results and discussion

3.1. Electricity generation

Fig. 1 shows the evolution of the current density generated under closed circuit conditions by the MFCs fed with the different substrates. They were operated simultaneously in order to avoid changes in the operating conditions which may affect their performance and, therefore, which could make more difficult the comparison of performances.

Fig. 1. Evolution of the current density during the operation time. Data is an average of three replicates with 2% of error.

In all cases, the electricity generation started after a lag phase. The MFC using acetate required 9 d to start the electricity production while lactate took about 10 d. Regarding

to the more complex substrates, glucose and octanoate, the length of the lag phase was about 15 d. Likewise, a steady state electricity production was reached after about 50 d in the four MFCs tested. The exerted current density values obtained with acetate, lactate, glucose and octanoate were 5.451 ± 0.690 , 0.163 ± 0.027 , 0.051 ± 0.009 and $0.015 \pm 0.001 \text{ A m}^{-2}$, respectively. From these results, it seems that the higher the number of carbons contained in the substrate, the lower is the current density exerted. This could be explained because the nature of the substrate determines the kind of metabolic reactions taking place in the MFC. The best performance was achieved with acetate. This molecule is the simplest one as it consists of two carbons and it is known to be very easily oxidised by electrogenic bacteria (D'Angelo et al., 2017). Current density exerted when feeding lactate was one log-unit below of current density exerted when feeding acetate. This lower electrogenic activity must be due to the transformation of the lactate into acetate. In the tests with glucose and octanoate, the low current density exerted could be explained by a scarce electrogenic metabolism outcompeted by the predominant fermentative and/or methanogenic metabolisms (Pant et al., 2010). These results confirm that the complexity of the carbon source affects strongly to the performance of a MFC, presenting the substrates containing the largest number of carbons, the higher probability for growing up non electrogenic microorganisms cultures and, hence, the higher probability for secondary reactions to occur.

3.2. COD removal

The evaluation of the mixed liquor in the anodic auxiliary tank was evaluated on a daily basis by studying daily cycles. The evolution of the COD consumption with the time is presented in Fig. SM-1.

In all the cases, the COD in the auxiliary tank presents a very similar trend, regardless of the type of fuel (substrate) consumed. During the first days, the COD was almost completely removed. In particular, the first day around 97.81%, 90.20%, 90.01% and 92.67% of the initial COD was removed when the MFCs were fed with acetate, lactate, glucose and octanoate, respectively. The high initial removal can be explained because of the source of the sludge, the aerobic reactor of a conventional WWTP, and the enrichment procedure followed. Under these conditions, the initial concentration of active microorganisms was very high, leading to a superb performance of the biological system in terms of COD removal. However, the COD degradation capacity of the system decreased until achieving an analogous consumption steady state, which is in agreement with the expected performance of biological reactors operated under the same sludge age. The COD consumption rate once reached the steady-state was very similar in all the cases: 625 ppm COD d⁻¹ for the acetate, 667 ppm COD d⁻¹ for the lactate, 520 ppm COD d⁻¹ for the glucose and 786 ppm COD d⁻¹ for the octanoate. In comparing the information of the COD removal and the electricity production, it must be highlighted that, despite of a very similar COD removal, the current produced by the MFC operated with lactate, glucose and octanoate was very low. Thus, MFCs can degrade a wide variety of substrates regardless its complexity without producing electricity under a sludge age of 2.5 d. This behaviour leads to an inefficient process, as reflected the very low Columbic Efficiency (CE) values reached at the steady state (shown in Fig. 2). About 0.3% of CE was obtained in all the cases with the exception of the acetate, which achieved a CE of 12.6%. The acetate showed a CE about 40 times the values obtained with lactate, glucose and octanoate. This fact indicates that the acetate improved the electrogenic performance of the MFC. In the literature, acetate has been described by its capacity to stimulate electroactive bacteria (Min et al., 2005), but the reason has not been well-established yet.

Fig. 2. Evolution of the Coulombic Efficiency of the MFCs fed with Acetate, Lactate, Glucose and Octanoate.

3.3. Intermediates generation and pH evolution

One of the best parameters to evaluate quickly the type of microorganisms contained in a biological culture is the pH evolution. Over the experiments, in all cases the influent pH is within the optimal range for the development of electrogenic bacteria (within 6-8). However, at the end of the daily cycle, the pH registered was 7.4 for the acetate, 6.0 for the lactate, 4.0 for the glucose and 7.6 for the octanoate. These results suggest different microbial transformations taking place in each anodic chamber. Anyway, the daily addition of raw wastewater causes a slight increase of the pH, which serves to regulate the initial pH at the beginning of the cycle. From the pH profiles, it can be said that the

245 MFC fed with acetate and octanoate were operating under an optimal pH to favour the
 246 growth of electrogenic microorganisms, while the low pH when using glucose and lactate
 247 indicates that the system was dominated by acidogenic bacteria, which transforms the
 248 glucose into volatile fatty acids.
 249 With the aim to deep inside the fermentation processes taking place, the intermediates
 250 were detected by HPLC (Fig. 3).

252 Fig. 3. Intermediates detected in the daily cycles of the MFCs fed with the different
 253 substrates. Data is an average of three replicates.
 254 As it can be observed, the acetate evolves partially into formate, which is known to be
 255 consumed by acetogenic bacteria (Ha et al., 2007). This explains the low concentration

detected. In addition, it is important to point out that the acetate usually acts as a final product because it is the end product of several metabolic pathways (Pant et al., 2010). On the other hand, lactate is almost completely converted into acetate and formate, pointing out the importance of hydrolytic microorganisms in the understanding of the performance of the MFC fed with this organic. In turn, glucose is known to undergo the cycle of carbohydrates, in which two molecules of pyruvate are generated. This cycle ends with the formation of lactate. However, any of the two species are detected and this has to be explained in terms of their subsequent oxidation in the liquid bulk. What it was identified in the electrolyte are acetate and formate molecules. Finally, it is important to point out that the formation of lactate was appreciable when the substrate used was octanoate, which also go forward into formate and lactate, being their concentration not relevant.

3.3. *Electrochemical Characterization of Anodic Biofilm*

Cyclic Voltammetry. In Fig. 4, the cyclic voltammetry of the MFCs working with the different substrates is shown. As known, the shape of the cyclic voltammeteries may give information about the processes taking place on the electrode surface, although in complex systems like the MFCs studied in this work, the information has to be evaluated very carefully, because of the large number of processes that may occur simultaneously. In each case, the black line corresponds to the abiotic reference (cyclic voltammetry of the systems in absence of microorganisms). The MFC growing with acetate fuel produced a significantly opened bioelectrochemical signal (difference between the direct and the reverse scan) while the curves were much closed for glucose, lactate and octanoate. Initially, this difference may be related with the electroactivity of the biofilm and, in fact, it has been proposed that a closed curve indicates a poor

formation of electrogenic biofilm (Khater et al., 2018). It is interesting to observe the voltammogram obtained in the case of the octanoate, which not show a similar pattern and which suggests more complex processes with this substrate.

Fig. 4. Cyclic voltammetries at steady state of the MFCs fed with (A) Acetate; (B) Lactate; (C) Glucose; (D) Octanoate. Black lines corresponds to the abiotic reference.

Polarization curves. The evaluation of the performance of MFCs requires of additional electrochemical measurements. Thus, polarization curves were carried out on a weekly basis since the start-up until the stationary phase. Figure SM-2 presents the polarization and power curves obtained. Table 1 summarizes the main results obtained from the polarization curves when operating under steady-state conditions with the different substrates studied.

Table 1. Main electrochemical parameters at the steady state for the MFCs working with different substrates at the anodic chamber.

Carbon source	OCV (V)	$j_{\max}$ (A m ⁻²)	$P_{\max}$ (W m ⁻²)
Acetate	0.376	19.506	1.809
Lactate	0.360	2.255	0.117
Glucose	0.259	0.347	0.035
Octanoate	0.294	0.443	0.037

From the power curves, it can be highlighter the good performance of the MFC fed with acetate. This MFC reached an OCV of 0.376 V, a maximum power density of approximately 2.0 W m⁻² and a maximum current density of 20 A m⁻². With regard to the lactate, the OCV was very similar to that of the acetate 0.360 V, while its maximum current density was 2.225 A m⁻², a value about one magnitude order below that of the acetate. Finally, the OCV and maximum current density obtained in the glucose and the octanoate experiments were very similar in both cases, about 0.30 V and 0.4 A m⁻².

With regard to the shape of the polarization curves, they reflect the good performance of the acetate as compared with the rest of the substrates. It is important to highlight that the cells fed with acetate and lactate showed the three stages of the polarization curves, whereas those fed with glucose and octanoate do not show the characteristic shape of the polarization curve.

With the aim to deep inside on the electrochemical behaviour of the systems, and to quantify the relevance of the main driving forces of the electrochemical performance of the MFC, the power curves were modelled by using an empirical polarization curve model developed by Kim et al., (2007) and presented in Eq. (1) and (2).

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$$315 \quad E_{cell} = E_0 - b \cdot \log(i) - R \cdot i - m \cdot \exp(ni) \quad [1]$$

316 In this equation, the parameter E_0 can be described as where $b \log(i)$ represents
 317 activation losses, $R \cdot i$ the ohmic losses, and $m \cdot \exp(ni)$ is an empirical term that
 318 approximates mass transfer loss.

$$319 \quad E_0 = E_{OCV} + b \cdot \log(i_0) \quad [2]$$

320 Results from modelling the polarization curves are shown in Table 2 and in Fig. 5. It
 321 can be observed an accurate adjustment, with correlation coefficients higher than 0.95 in
 322 all the cases.

323

324 Fig. 5. Modelling of the polarization curves.

325

326

Table 2. Fitting values of the parameters of the empirical model corresponding to the polarization curves.

	Substrate			
Parameter	<i>Acetate</i>	<i>Lactate</i>	<i>Glucose</i>	<i>Octanoate</i>
E_0 (V)	0.376	0.350	0.290	0.259
b (V dec ⁻¹)	-0.005	-0.005	-0.005	-0.005
R_i (Ω)	-228.42	-2850.26	-7276.41	-6073.74
m (A)	-0.011	-0.015	-0.013	-0.016
n (A ⁻¹)	1.00	1.00	1.00	1.00

By analyzing the results, it can be highlighted that the E_0 value slightly decreased with the complexity of the substrate, presenting the higher value the acetate and the lowest the octanoate. This indicates that performance of the MFC is enhanced when operating with the most biodegradable substrates. With regards to the b parameter, as it was mentioned previously, experimental results were fitted with a -0.005 V dec⁻¹ suggesting that no appreciable electrocatalytic effects can be highlighted.

The values calculated by the fitting procedure for m and n were almost the same in all the cases. These results are interesting because the parameter n is related to the smallest current density that causes the voltage to deviate from linearity, due to mass transfer limitations. In this sense, when the current density is less than the threshold value, the term of mass transfer losses is negligible in the low and moderate current density ranges. Because of that, the constant value of n presented in Table 3 indicates an identical behavior in terms of mass transfer limitations in all the cases. On the other hand, m coefficient is related to the relevance of the mass transfer phenomenon occurring over the entire range of current densities. As can be seen in Table 2, the m values were not identical although also very similar in all the cases. Because of that,

similar performance effects were expected when the threshold current density is overcome.

On the other hand, it is very remarkable the significant differences of the R value when operating with the different substrates, presenting the lowest value of R the acetate. The low value of the acetate could be explained by the development of a more electroactive biofilm when operating with this substrate or due to a higher conductivity of the fuel solution fed. Regarding to the conductivity, it can be considered negligible in the initial sludge used to enrich the process ($1.83 \pm 0.4 \text{ mS cm}^{-1}$). However, despite of preparing the synthetic wastewater with the same nutrients composition, the conductivity of the stock solutions containing different organic substrates presented different conductivities: 14.50 mS cm^{-1} for the acetate wastewater, 10.80 mS cm^{-1} in the lactate wastewater, 10.50 mS cm^{-1} for the octanoate and 6.40 mS cm^{-1} for the glucose wastewater. At the steady state, the values of the conductivity in the anodic reservoirs remained very similar to the conductivity of the wastewater (MFC fed with acetate $14.31\% \pm 1.33$, lactate $10.31 \pm 0.56\%$, octanoate 9.00 ± 0.56 and glucose $5.50 \pm 0.24\%$). It is important to remark the highest conductivity of the MFC fed with acetate, which enhances the electron transfer, and the low conductivity values in MFCs fed with more complex substrates. Because of that, the poor performances when operating with lactate, glucose and octanoate can be explained in terms of a higher internal resistance, ranging from 2.2 to $7.5 \text{ k}\Omega$, at least one magnitude order higher than the internal resistance experienced when acetate was used. These results indicate that the activation losses, kinetics limitation and mass transfer seemed to be very similar, being the main limitation that rules the system the ohmic losses. The acetate was the substrate yielding the minimal internal resistance and, therefore, the best performance.

4. Conclusions

This work demonstrates the robustness and competitiveness of miniaturized MFCs and the critical role that plays the substrate in their performance. Acetate was the more electroactive substrate, achieving 20 A m^{-2} as maximum current density, 2 W m^{-2} of maximum power density, 0.376 V of OCV and 21.6% of CE, values much better than those obtained by other substrates. By modelling the polarization curves it was found that the better performance was due to the lower ohmic losses experienced, which are not associated to the electrolyte conductivity but to the lower ohmic losses of the biofilm formed.

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