

1           **Influence of the fuel and dosage on the performance of double-**  
2                               **compartment microbial fuel cells**

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8  
9    **Abstract**

10 ~~In the search for novel application of bio-electrochemical devices technology.~~ This  
11 manuscript focuses on the evaluation of the use of different types and dosages of fuels in  
12 the performance of double-compartment microbial fuel cell equipped with carbon felt  
13 electrodes and cationic membrane. Five types of fuels (ethanol, glycerol, acetate,  
14 propionate and fructose) have been tested for the same organic load (5000 mg L<sup>-1</sup>  
15 measured as COD) and for one of them (acetate), the range of dosages between 500-  
16 20000 mg L<sup>-1</sup> of COD was also studied. Results demonstrate that production of electricity  
17 depends strongly on the fuel used. Carboxylic acids are much more efficient than alcohols  
18 or fructose for the same organic load and within the range 500-5000 mg L<sup>-1</sup> of acetate the  
19 production of electricity increases linearly with the amount of acetate fed but over these  
20 concentrations a change in the population composition may explain a worse performance.

21  
22    **Keywords**

23    Microbial fuel cells; fuel; organic load; substrate; acetate

24  
25    **Highlights**

- 26 - Production of electricity increases linearly with COD in the range 500-5000 ppm
- 27 - Carboxylic acids are more efficient fuels for the production of electricity than
- 28 alcohols
- 29 - Fructose behaves as a very low-efficient fuel for MFC
- 30 - Maximum power density attainable in the range 20-500 mW m<sup>-2</sup> depending on the
- 31 composition of fuel
- 32 - Propionic acid are slightly more efficient than acetic acid as fuel

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## 42 **Introduction**

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44 For a very long time, microbial fuel cells (MFC) have been studied as promising  
45 substitutive technologies for the biological reactor of conventional municipal wastewater  
46 treatment plants (Logan et al. 2006, Rodrigo et al. 2007). In a parallel application, they  
47 have also been considered for the treatment of highly-loaded industrial effluents ( Logan  
48 et al. 2006, Huang et al. 2009, Cusick et al. 2010). Results were promising at the lowest  
49 scale, but unfortunately, the scale-up of this bio-electrochemical technology is a huge  
50 handicap nowadays and much work has to be carried out in the next years in order to  
51 overcome it successfully (Virdis et al. 2008).

52 This bio-electrochemical technology is not easy and researchers are now realizing it. This  
53 point can be explained taking into account that, in fact, it consists of the combination of  
54 two very different technologies: fuel cells based on the electrochemistry and organic  
55 biodegradation based on biotechnology. The difficult coupling of these two different  
56 disciplines can help to explain the necessity of further research and the need for great  
57 efforts in order to find a ready-to-use technology. ~~Currently,~~ There is a great deal of  
58 papers focused on trying to improve the performance of microbial fuel cell and,  
59 particularly, their efficiency (Rabaey et al. 2003). Topics of interest include the electrode  
60 materials, fuel cell mechanical design and use of different types of synthetic solutions or  
61 real wastewater as fuel (Kim et al. 2015, Lee et al. 2015).

62 This last topic is of a great relevance because, perhaps, the environmental application  
63 looked for in the last years is not the best choice for this type of energy conversion  
64 devices. In turn, the power supply to systems with a low energy requirement in remote  
65 applications could have greater real opportunities. Within this context, the perspective of  
66 feeding the MFC with a synthetic fuel, manufactured only to harvest energy from organic  
67 matter can be a way to optimize these devices. Obviously, due to the metabolic

68 requirements, the fuel used should be a solution containing not only a carbon source but  
69 also nutrients in ratios enough to do not become the limiting reagents of the process, such  
70 as happens naturally with the wastewater types typically fed to these systems (Rodrigo et  
71 al. 2009a).

72 There are many types of potential fuels for MFC (Oh and Logan 2005, Viridis et al. 2010).  
73 Obviously, the simpler the molecule the easier and more effective is the resulting process,  
74 because complex molecules should be hydrolyzed before they can be oxidized by  
75 microorganisms. Metabolism of sugars, alcohols and carboxylic acids proceeds through  
76 very different pathways, for which the redox transfer enzymes and/or redox mediators  
77 involved can be very different and these differences should reflect on the performance of  
78 a MFC. It is important to know which of this carbon sources provides a higher efficiency,  
79 in the search for new applications of the technology. Hence, although typically, MFC  
80 have been studied as alternative for wastewater treatment processes, this application,  
81 although promising, is perhaps not the best choice because of the low power yielded by  
82 these devices. In the search for new applications of the technology, it is interesting to  
83 evaluate their performance with different synthetic fuels and in different concentrations,  
84 trying to determine the fuel that produces the highest efficiency.

85 The substrate also plays another important role in MFC (Lobato et al. 2012) helping to  
86 select population and hence to the development of optimal biofilms (Chae et al. 2009, Liu  
87 et al. 2009). A great variety of substrates can be used in MFCs for electricity production  
88 ranging from pure compounds such as glucose, acetate, butyrate, lactate, ethanol (Rabaey  
89 and Verstraete 2005) to complex mixtures of organic matter present in wastewater (  
90 Rodrigo et al. 2009a, Pant et al. 2010). The electrogenic bacteria are only capable of  
91 completely oxidizing non-fermentable substrates such as acetate by electricity

92 production, while the fermentative bacteria convert carbohydrates into short-chain fatty  
93 acids such as acetate (Lovley 2008).

94 Careful control of the substrate feed can thus be used to optimize the biofilm and in turn  
95 the electricity generation. These substrates include pure and non-fermentable ones such  
96 as acetate and lactate (Bond and Lovley 2003; Rabaey et al. 2003) in addition to  
97 fermentable ones such as glucoses and xylose (Huang and Angelidaki 2008, Ishii et al.  
98 2008, Rezaei et al. 2009, Makinen et al. 2013). Mixed substrates present in mixed  
99 inoculum feeds such as domestic and industrial wastewater, which contain fatty acids,  
100 protein, and carbohydrates, have also been studied (Feng et al. 2008).

101 With this background, the goal of this work has been to evaluate the best organic load,  
102 within the range 500-5000 ppm of acetate, and the best type of fuel, within a set that  
103 includes carboxylic acids, alcohols and sugars, in order to get information for the  
104 application of the technology in different fields, not only wastewater treatment but also  
105 in sensors and other devices.

106

## 107 **Materials and methods**

108 *Microbial fuel cell.* The set-up used in this work consisted of a MFC with two chambers  
109 (4 cm<sup>3</sup> volume each one) separated by a proton exchange membrane, PEM (Sterion®),  
110 which has a high ionic conductivity (0.9-0.02 meq g<sup>-1</sup>) and low electronic conductivity (8  
111 x 10<sup>-2</sup> S cm<sup>-1</sup>). MFC is formed by two HDL (high pressure laminate) plates and two silicon  
112 plates to improve the mechanical properties and avoid liquid losses. Carbon felts (KFA10,  
113 SGL Carbon Group®) were used as electrodes in both chambers (3 cm<sup>2</sup> each). The  
114 electrode spacing between the anode and the cathode was minimized in order to reduce  
115 as much as possible the internal electrical losses from the system. The two electrodes  
116 were connected by an external resistance (R<sub>ext</sub>) of 120 Ω; this low value was chosen to

117 prevent activation losses and facilitate electron transfer during the acclimation period  
 118 (Rodrigo et al. 2009b). The MFCs were operated simultaneously in semi-continuous  
 119 mode and at room temperature ( $25 \pm 3$  °C). The cathode compartment of the MFC was  
 120 connected to a water reservoir of 100 cm<sup>3</sup> and a peristaltic pump was used to circulate an  
 121 HCl solution (pH 3.5) from the reservoir through the cathode chamber of the MFC at 25  
 122 cm<sup>3</sup> min<sup>-1</sup>. A fishery compressor that can provide a flow rate of 1.6 L min<sup>-1</sup> and a  
 123 maximum pressure of 1.2 m of water-column was connected to the cathode to supply  
 124 oxygen to the cathodic chamber (Penteado et al., 2016).

125

126 *Inoculum and synthetic wastewater.* Activated sludge from a wastewater treatment plant  
 127 (Ciudad Real, Spain) was used as the inoculum for the anodic compartment. The activated  
 128 sludge of the biological reactors was placed in the anodic chamber for three days in a 1:2  
 129 ratio without aeration to favor the formation of a mixed culture of anaerobic  
 130 microorganisms. In this period, no synthetic wastewater was supplied to feed the culture.  
 131 After this period, different carbon based fuels were study to evaluate the performance of  
 132 the MFC. In all cases, inorganic compounds of the synthetic wastewaters were the same.

133 **Table 1:** Inorganic compounds in wastewater composition

134

Composition	Inorganic nutrients (g L)
Sodium bicarbonate	2.77
Ammonium sulfate	1.85
Potassium phosphate	1.11
Magnesium chloride	0.92
Calcium chloride	1.25
Ammonium iron (II) sulfate	0.07

139

140 Three types of carbon based fuels were studied: alcohols (ethanol and glycerol), a volatile  
 141 fatty acid (acetic and propionic acid) and a monosaccharide sugar (fructose).

142

143 Table 2: COD (mg O<sub>2</sub> L<sup>-1</sup>), concentration (g L<sup>-1</sup>), volume (cm<sup>3</sup>) and type of fuel used in

144

the synthetic wastewater

COD (mg O <sub>2</sub> L <sup>-1</sup> )	Fuel	[ ] (g L <sup>-1</sup> )	V (cm <sup>3</sup> L <sup>-1</sup> )
500	Sodium acetate	0.805	-
1000	Sodium acetate	1.61	-
2500	Sodium acetate	4.025	-
5000	Sodium acetate	8.05	-
10000	Sodium acetate	16.1	-
20000	Sodium acetate	32.2	-
5000	Ethanol	-	3.04
5000	Propionic acid	-	3.34
5000	Glycerol	-	3.26
5000	Fructose	4.69	-

145

146 It is worth to remind that although the MFC was fed only once a day, its operation mode

147 can be considered as semi-continuous within long periods of time. HTR was 3.16 days in

148 the different experiments. In all case no changes were made in the rest of parameters that

149 may affect the performance of the cell, and even the nutrient solution was kept the same

150 in the five test (concentrations of nutrients were checked to be high enough to not become

151 limiting reagents).

152

153 *Electrochemical and chemical measurements.* A digital multimeter (Keithley 2000

154 multimeter) was connected to the system to monitor continuously the value of the cell

155 voltage at the value of the external load (120Ω). Chemical oxygen demand (COD) was

156 determined using a Velp ECO-16 digester and a Pharo 100 Merck spectrophotometer

157 analyzer and pH, conductivity and dissolved oxygen were measured with a GLP22 Crison

158 pH meter, a Crison Cm 35 conductivity meter and an Oxi538 WTW oxy meter,

159 respectively. Polarization curves have been done in MFC. Three important parameters

160 were evaluated: the open circuit voltage (OCV) or the maximum allowable MFC voltage,

161 the maximum intensity and the maximum power density of the MFC. In addition, the  
162 shape of curves gives important information about the limiting processes, which control  
163 the performance of the cell. Polarization curves can be divided into three zones: a decrease  
164 of the current due to the activation losses, a linear decrease due to ohmic losses, and a third  
165 zone that corresponds to the region controlled by mass-transfer (concentration losses).

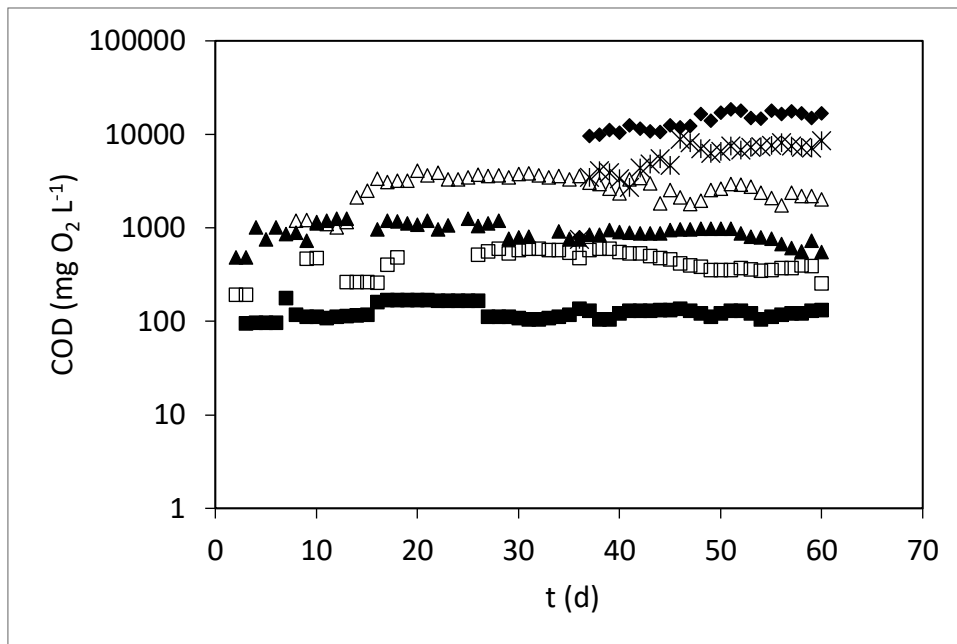
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## 167 **Results and discussion**

168

169 Figure 1 shows the changes in the COD monitored in the anode chamber of a divided  
170 MFC during 2.5-month tests in which different fuels, made up with acetate at different  
171 concentration, are fed in order to check the effect of the organic load on the performance  
172 of the cell. Over the tests, the other inputs are kept constant, including hydraulic retention  
173 time, temperature and external electric load of the cell. Likewise, the nutrient solution  
174 was the same and it was checked that concentrations of all nutrient were high enough to  
175 not become the limiting reagent in the performance of the MFCs. Hence, changes in these  
176 experiments are expected to be only consequence of the organic load of the influent fed  
177 to the MFCs.





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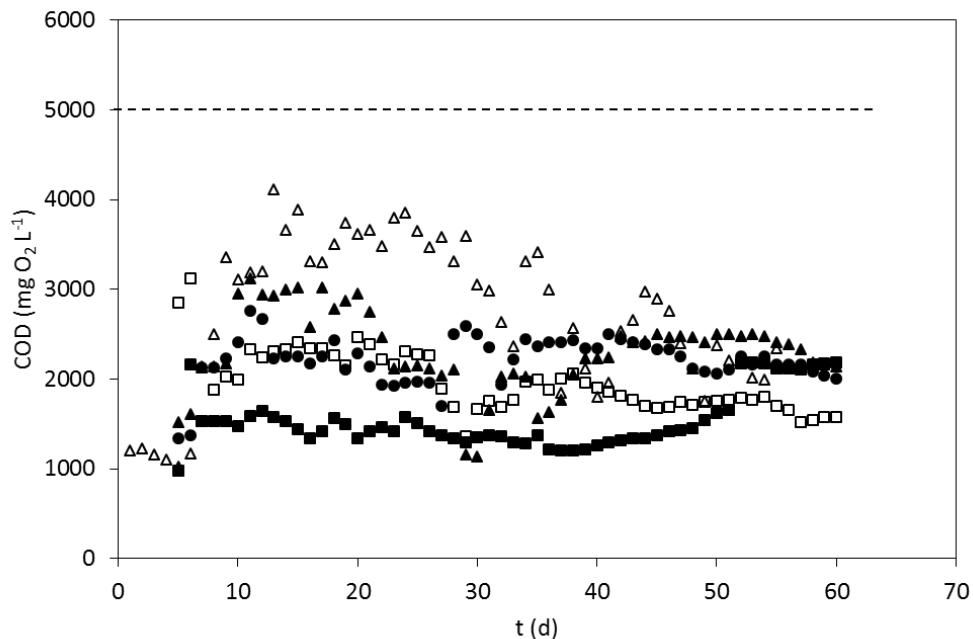
179

180 **Figure 1.** Changes in the COD during the tests. Operational conditions: Sodium acetate  
 181 as feed (■) COD 500 mg O<sub>2</sub> L<sup>-1</sup>, (□) COD 1000 mg O<sub>2</sub> L<sup>-1</sup>, (▲) COD 2500 mg O<sub>2</sub> L<sup>-1</sup>,  
 182 (Δ) COD 5000 mg O<sub>2</sub> L<sup>-1</sup>, (\*) COD 10000 mg O<sub>2</sub> L<sup>-1</sup>, (◆) COD 20000 mg O<sub>2</sub> L<sup>-1</sup>.  
 183 Average temperature: 27°C

184

185 It is worth to remind that although the MFC was fed only once a day, its operation mode  
 186 can be considered as semi-continuous within long periods of time. This is confirmed by  
 187 results obtained in this work. Thus, the Figure 1 shows how the COD remaining in the  
 188 anode compartment changes up to a steady-state value that it is related to the  
 189 concentration fed, in agreement to what it could be expected for a semi-continuous  
 190 system. At this point, It is important to point out that the COD changes over the day in  
 191 the cell because the cell is fed daily but what it is compared in the Figure is the value of  
 192 COD taken at the end of the day before the replacement with fresh solution of acetate. It  
 193 is also important to point out that the higher the concentration fed, the higher is the value  
 194 of COD contained in the anode compartment

195 Influence of the characteristics of the fuel for the same organic (5000 ppm) load are shown  
196 in Figure 2.



197

198 **Figure 2. Changes in the COD during the tests.** Operational conditions:  $[\text{COD}]_0 = 5000$   
199  $\text{mg O}_2 \text{ L}^{-1}$ , Carbon felt as electrode material. (■) Ethanol, (□) Glycerol, (▲) Propionic  
200 acid, (△) Sodium acetate, (●) Fructose. Average temperature:  $27^\circ\text{C}$

201

202 In comparing the output of the tests, it can be observed a different time-course of the COD  
203 over the experiments, although at long times, the value of COD in the reactor tends to  
204 stabilize around 2000 ppm in the five tests. It is important to point out how concentration  
205 of COD during the first days increases importantly in the tests in which the MFC were  
206 fed with carboxylic acids while it **remains constant only increases more slowly** with the  
207 fructose and the alcohols fuels. This increase may suggest a more difficult degradation of  
208 the substrate by the raw microorganism initially seeded to the anode chamber (which were  
209 obtained in the biological reactor of a municipal WWTP and hence were acclimated to  
210 urban wastewater that is very different from a carboxylic acid solution). This behavior  
211 reverses when the population was fully acclimated from the urban wastewater to the new

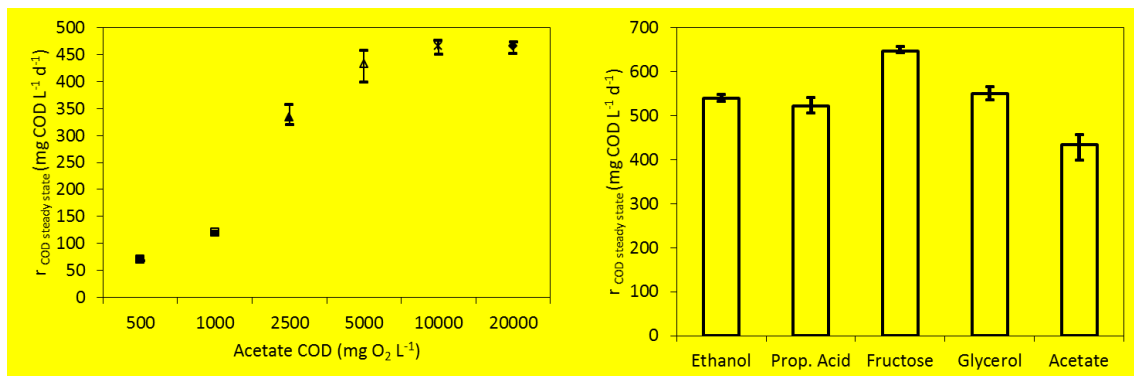
212 substrate solution used as fuel (after several days of running the MFCs). Under those  
 213 conditions, the concentration of COD in the anode chamber decreases down to almost the  
 214 same value attained in the test in which the MFC was fed with other types of substrate.  
 215 From the effluent COD, it can be obtained the COD consumption rate using a mass  
 216 balance. To do this, it should be taken into account that the MFC is  
 217 a semi-continuous reactor. This fact means that changes within long periods, such as the  
 218 applied in the tests carried out in this work, have to be evaluated as if the MFC behaves  
 219 as a continuous tank reactor (eq. 1)

$$qC_0 - qC_1 + rV = V \frac{dC_1}{dt} \quad (1)$$

222 Opposite, to evaluate performance of short periods (time scale of hours), such as the daily  
 223 changes of the COD, it is better to fit the performance of the cell to a discontinuous reactor  
 224 model (eq 2)

$$rV = V \frac{dC_1}{dt} \quad (2)$$

227 Taking into account this fact, reaction rates calculated by applying model shown in eq. 1  
 228 are plotted vs organic load and type of substrate in Figure 3.



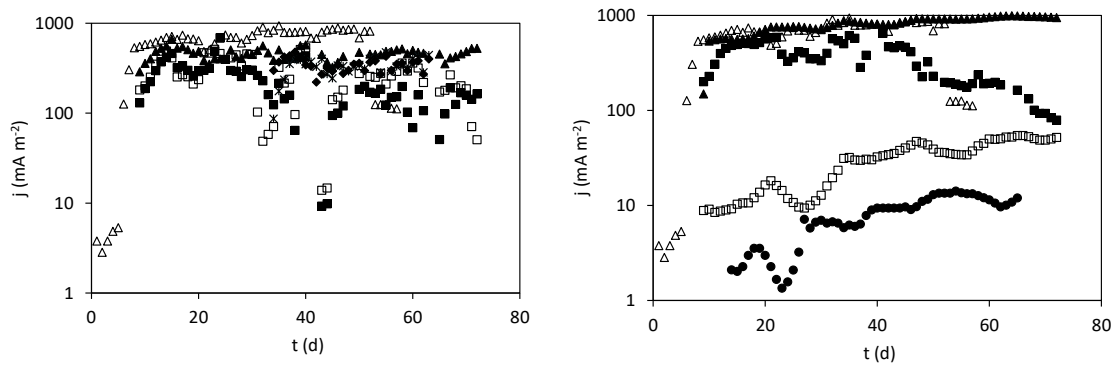
230 **Figure 3.** COD consumption rate achieved in the steady state (a) at different COD  
 231 concentrations of Sodium acetate, (b) with different types of substrates. Carbon felt used

232 as electrode material. Organic load in part a: (■) COD 500 mg O<sub>2</sub> L<sup>-1</sup>, (□) COD 1000  
233 mg O<sub>2</sub> L<sup>-1</sup>, (▲) COD 2500 mg O<sub>2</sub> L<sup>-1</sup>, (Δ) COD 5000 mg O<sub>2</sub> L<sup>-1</sup>, ( ) COD 10000 mg O<sub>2</sub>  
234 L<sup>-1</sup>, (◆) COD 20000 mg O<sub>2</sub> L<sup>-1</sup>. Organic load in part b: COD: 5000 mg O<sub>2</sub> L<sup>-1</sup>. Average  
235 temperature: 27°C

236 In comparing the effect of organic load, it is observed a perfect Monod-type behavior  
237 with a maximum COD removal rate slightly over 450 mg COD L<sup>-1</sup> d<sup>-1</sup>, which is obtained  
238 for concentrations over 10000 mg L<sup>-1</sup>. For lower dosages, up to 5000 mg COD L<sup>-1</sup>, the  
239 higher the amount of fuel, the higher is the degradation rate observed, with a clear first  
240 order kinetic behavior, which points out the linear dependence of the consumption of  
241 organics with the influent COD concentration. Regarding the reaction rates evaluated in  
242 the last stage of the test with different substrates, it is worth to point out that steady-state  
243 rates are within the range 450-650 mg COD L<sup>-1</sup> d<sup>-1</sup>. Values obtained for carboxylic acids  
244 are slightly lower and the highest rate is attained with the use of fructose as fuel.

245 It is important to remind that the MFCs were seeded with the same aerobic sludge and  
246 that conditions in the anodic chamber are anaerobic, because concentration of oxygen is  
247 nil, although metabolisms of aerobic microorganism adapted to use other sinks of  
248 electrons (both, directly on electrode surface or mediated processes) should still be  
249 aerobic.

250 COD changes inform about the globalized metabolism of the microorganisms contained  
251 in the anode compartment but not about the behavior of electrogenic microorganisms,  
252 because both electrogenic and non-electrogenic microorganisms are expected to consume  
253 COD. In order to evaluate the performance of electrogenic microorganisms, the electric  
254 current produced is the best indicative parameter. This parameter is shown in Figure 4,  
255 where it can be clearly seen how the current produced increases over the test up to reach  
256 approach a steady state, for which the values remain constant.

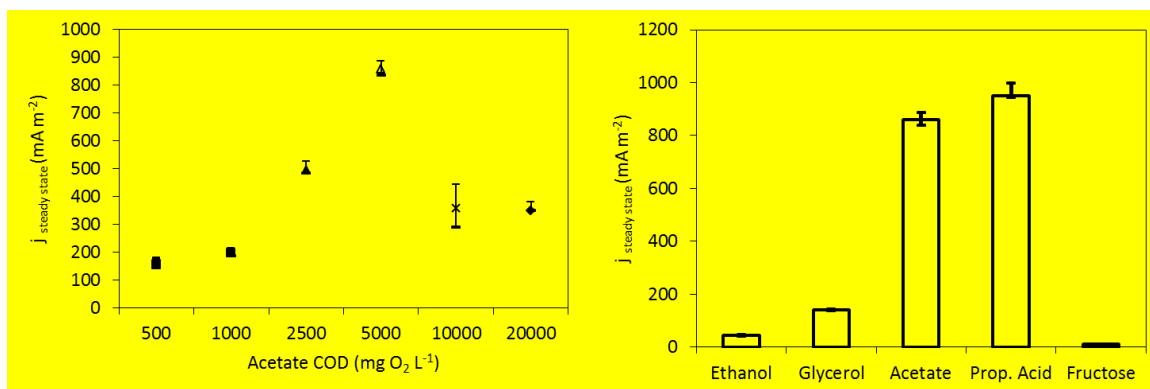


258

259 **Figure 4.** Current density produced in the MFC over the different tests carried out in this  
 260 work. **Part a.** Current densities profiles observed in MFC. (■) COD 500 mg O<sub>2</sub> L<sup>-1</sup>, (□)  
 261 COD 1000 mg O<sub>2</sub> L<sup>-1</sup>, (▲) COD 2500 mg O<sub>2</sub> L<sup>-1</sup>, (Δ) COD 5000 mg O<sub>2</sub> L<sup>-1</sup>, ( ) COD  
 262 10000 mg O<sub>2</sub> L<sup>-1</sup>, (◆) COD 20000 mg O<sub>2</sub> L<sup>-1</sup>. **Part b.** Current densities profiles observed  
 263 in MFC under different types of feed nutrients. (■) Ethanol, (□) Glycerol, (▲) Propionic  
 264 acid, (Δ) Sodium acetate, (●) Fructose. COD: 5000 mg O<sub>2</sub> L<sup>-1</sup>. Average temperature:  
 265 27°C.

266

267 This general behavior is obtained in all tests, regardless of using different organic loads  
 268 with the same substrate (part a) or different substrates with the same organic load (part  
 269 b). Differences become more important in the comparison of substrates, which clearly  
 270 points out that carboxylic acids are more efficient fuels than alcohols and fructose. This  
 271 is observed in Figure 5, where the steady-state values reached in each test are compared  
 272 and it is an indication that carboxylic acids are better fuels than less oxidized molecules  
 273 such as alcohols or sugars.



274

275 **Figure 5.** Steady-state electric current density produced in double-compartment MFC fed  
 276 with (a) different COD concentrations of Sodium acetate, (b) different types of fuels.  
 277 Operational conditions: Carbon felt as electrode material. Part a: (■) COD  $500 \text{ mg O}_2 \text{ L}^{-1}$   
 278  $^1$ , (□) COD  $1000 \text{ mg O}_2 \text{ L}^{-1}$ , (▲) COD  $2500 \text{ mg O}_2 \text{ L}^{-1}$ , (Δ) COD  $5000 \text{ mg O}_2 \text{ L}^{-1}$ , (\* )  
 279 COD  $10000 \text{ mg O}_2 \text{ L}^{-1}$ , (◆) COD  $20000 \text{ mg O}_2 \text{ L}^{-1}$  Part b: COD  $5000 \text{ mg O}_2 \text{ L}^{-1}$ .  
 280 Average temperature:  $27^\circ\text{C}$

281

282 Another interesting information that can be drawn from this Figure is the effect of the  
 283 organic load, because opposite to what it was shown with the COD consumption rate  
 284 (which fitted well to a Monod Type behavior), the changes observed in this  
 285 electrochemical parameter are different. There is a linear increase in the current density  
 286 within the range  $500\text{-}5000 \text{ mg L}^{-1}$  and then, for the two highest organic loadings, the  
 287 current produced decrease substantially. Both changes behaviors have an equivalent  
 288 region in the  $r_{\text{COD}}$  vs operation time plot, although in the comparison it was expected a  
 289 higher value of the current density in the tests fed with concentrations over  $5000 \text{ mg L}^{-1}$ .  
 290 Thus, the linear increase in the COD and  $j$  for concentrations under  $5000 \text{ mg L}^{-1}$  can be  
 291 explained by assuming the same biological culture is operating in all test, because the  
 292 ratio current produced/COD consumed is kept constant. Opposite, higher concentrations  
 293 of COD should lead to a change in the microorganisms composition, with a lower  
 294 population of electrogenic microorganisms and hence, despite the COD consumption is

295 maintained in the highest rate, the production of electricity can become significantly  
 296 lower. **It is important to take in mind that** Current density informs directly about the rate  
 297 of the electrochemical processes. As COD is also a quantification of the organic content  
 298 and it requires four electrons to be transformed into carbon dioxide, the ratio in the same  
 299 units of  $j$  and  $r_{\text{COD}}$  is an efficiency measurement **(in fact, it is the well-known coulombic**  
 300 **efficiency)**, which is also directly related with the population of bioelectrogenic  
 301 microorganism, at least from the viewpoint of **COD consumption**.

302

303 
$$efficiency = \frac{j \left( \frac{mA}{m^2} \right) \cdot A (m^2)}{r \left( \frac{mg \text{ COD}}{L \cdot d} \right) \cdot V (L) \cdot \left( \frac{1 \text{ mmol COD}}{32 \text{ mg COD}} \right) \cdot \left( \frac{4 \text{ mmol } e^-}{1 \text{ mmol COD}} \right) \cdot \left( \frac{96500 \text{ mC}}{1 \text{ mmol } e^-} \right) \cdot \left( \frac{1 \text{ d}}{86400 \text{ s}} \right)}$$
 (3)

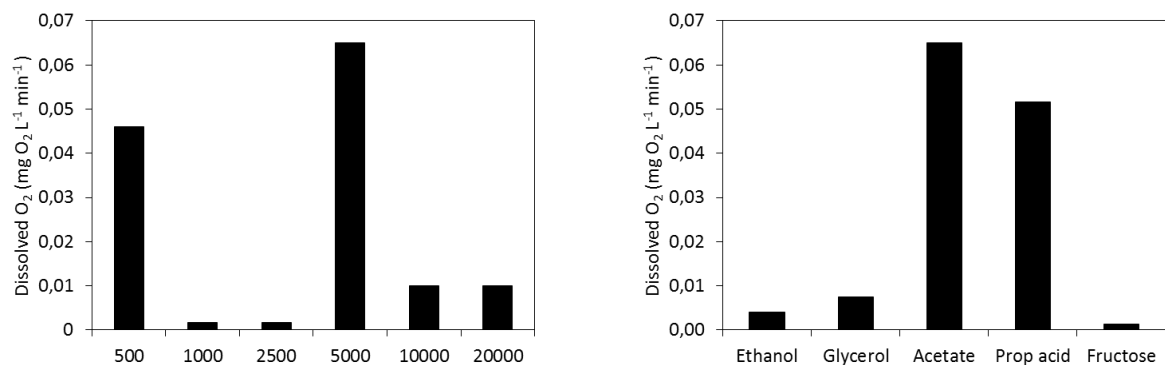
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305 This value is approximately kept constant within the range 500-5000 mg L<sup>-1</sup> in a value of  
 306 9.24% and c.a. 3.82% for higher doses, **suggesting indicating** that concentration of  
 307 electrogenics is three times higher for the cultures acclimated at the lowest range of COD.  
 308 Regarding the influence of the type of fuel (for the same loading rate of 5000 mg L<sup>-1</sup>),  
 309 application of eq. 3 results in values near 10% for both carboxylic acids. These values are  
 310 reduced to 1.31, 0.41 and 0.09%, respectively for glycerol, ethanol and fructose,  
 311 respectively, pointing out the prevalence of non-electrogenic populations with those  
 312 substrates.

313 ~~Another important point to be studied in the performance of MFC is the cathodic~~  
 314 ~~reactions.~~ **The cathodic reactions play an important role in the performance of the MFC.**

315 **They** consists of the reduction of oxygen to water on the surface of the cathode and hence  
 316 it is not a biological but an abiotic process. Membrane used in the electrochemical cell  
 317 prevents the crossing of oxygen molecules to the anode compartment (Lobato et al. 2012),  
 318 improving in this way the efficiency of the MFC because the only sink of electrons  
 319 available for the oxidation of the COD in the anode chamber is the anode surface. Hence,

320 initially it could be expected than the rate of the anode and cathode processes should be  
 321 related, in particular if a pure electrochemical process is developed in the cell and other  
 322 side processes do not affect oxygen. In order to evaluate this important point, oxygen  
 323 consumption rates were evaluated in the cathode chamber. This measurement was carried  
 324 out by turning off the oxygen flow fed to the cathode chamber and measuring the oxygen  
 325 decay (Rodrigo et al. 2010). Results are shown in Figure 6, where it can be seen that  
 326 except for the tests carried out in the MFC fed with the lowest organic load, the oxygen  
 327 consumption rate varies in a similar way than the current intensity produced in the cell.  
 328 Discrepancies can be related to non-electrochemical oxygen consumptions such as  
 329 desorption of oversaturated oxygen or the growth of microorganism in the cathode  
 330 chamber and to side electrochemical processes like corrosion of the carbon felt or  
 331 production of hydrogen peroxide instead of water as a reaction product of the cathode  
 332 reaction.



333  
 334 **Figure 6.** Oxygen consumption rate in the cathode chamber. (a) different COD  
 335 concentrations of sodium acetate, (b) different types of fuel. Average temperature: 27°C

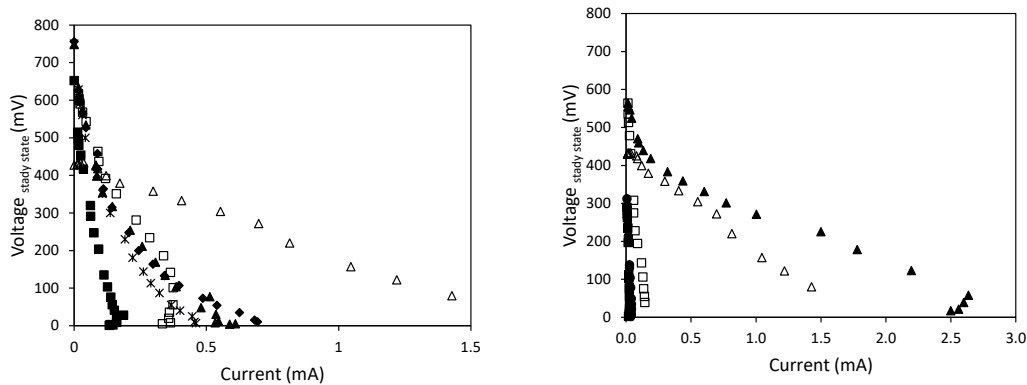
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337 The polarization curves give To know more details about the electrochemistry of the  
 338 microbial fuel cells, it is important to focus on the results of a very important test: the  
 339 polarization curves. The plots obtained during the 55<sup>th</sup> day of operation (when the system



340 is completely stabilized) are compared in Figures 7 (voltage vs current plot) and 8 (power  
341 vs current plot).

342 As it can be observed, there are only two zones in the polarization curves. This is a  
343 common observation in most MFC which contrast to the three zones typically observed  
344 in conventional fuel cells (polarization, ohmic and mass transfer losses). The open circuit  
345 potential is not the same despite the electrode materials were the same in all test, pointing  
346 out the differences in the oxidation and reduction reactions that develop in each cell. The  
347 most important remark is the much better performance of the cells fed with propionic and  
348 acetic acid, which according to the slopes of the second zone can be explained by a much  
349 lower ohmic resistance of the cell.

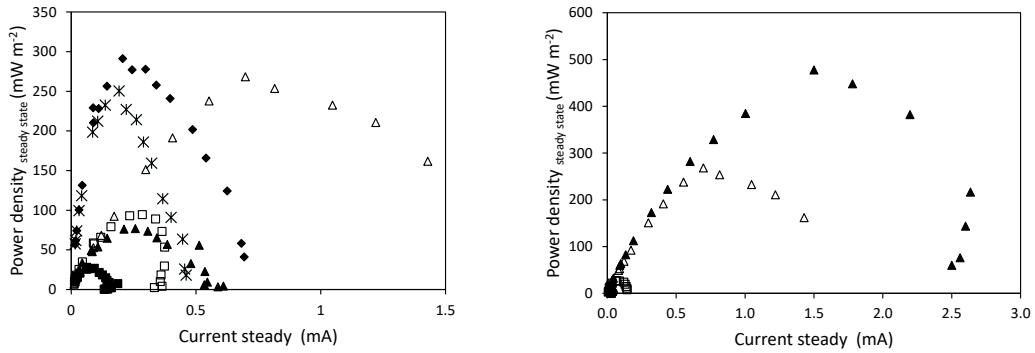


350

351 **Figure 7.** V vs current polarization curves obtained in the steady state. Operational  
352 conditions: Carbon felt as electrode material. Part a: (■) COD 500 mg O<sub>2</sub> L<sup>-1</sup>, (□) COD  
353 1000 mg O<sub>2</sub> L<sup>-1</sup>, (▲) COD 2500 mg O<sub>2</sub> L<sup>-1</sup>, (Δ) COD 5000 mg O<sub>2</sub> L<sup>-1</sup>, (○) COD 10000  
354 mg O<sub>2</sub> L<sup>-1</sup>, (◆) COD 20000 mg O<sub>2</sub> L<sup>-1</sup>. Part b: (■) Ethanol, (□) Glycerol, (▲) Propionic  
355 acid, (Δ) Sodium acetate, (●) Fructose. Average temperature: 27°C

356

357



358

359 **Figure 8.** Power vs. current polarization curves in the steady state. Operational  
 360 conditions: Carbon felt as electrode material. Part a: (■) COD 500 mg O<sub>2</sub> L<sup>-1</sup>, (□) COD  
 361 1000 mg O<sub>2</sub> L<sup>-1</sup>, (▲) COD 2500 mg O<sub>2</sub> L<sup>-1</sup>, (Δ) COD 5000 mg O<sub>2</sub> L<sup>-1</sup>, (\*) COD 10000  
 362 mg O<sub>2</sub> L<sup>-1</sup>, (◆) COD 20000 mg O<sub>2</sub> L<sup>-1</sup>. Part b: (■) Ethanol, (□) Glycerol, (▲) Propionic  
 363 acid, (Δ) Sodium acetate, (●) Fructose. Average temperature: 27°C

364

365 Regarding the maximum power attainable by these devices, it can be seen that in each  
 366 case they are within the range 40-500 mW m<sup>-2</sup>. Maximum values are obtained for the two  
 367 carboxylic acids, being higher in the case of the microbial fuel cell fed with propionic  
 368 acid. It is important to point out that the maximum power obtained in the three reactors  
 369 fed with organic loads over 5000 mg L<sup>-1</sup> of acetate are nearly the same. However, they  
 370 are obtained for lower current intensities at higher concentration.

371 To compare these results with those shown in the literature, Table 3 presents a  
 372 comprehensive list of substrates that have been used in MFC studies together with the  
 373 reported maximum power density. ~~At this point,~~ It is important to point out that it is  
 374 difficult to compare MFC performances from literature, due to different operating  
 375 conditions, surface area and type of electrodes and different microorganisms involved  
 376 and hence only relative comparison within the same manuscript should be carried out  
 377 (Dumas et al. 2008).

379 **Table 3.** Comparison of maximum power density and type of feeding solution.

	<b>Type of substrate</b>	<b>Power density (mW/m<sup>2</sup>)</b>
Liu et al., 2005 (Liu et al. 2005)	Acetate	506
	Glucose	494
	Butyrate	305
	Ethanol	488
	Domestic waster	146
Oh and Logan, 2005 (Oh and Logan 2005)	Acetate	88
	Propionate	67
Ahn and Logan, 2010 (Ahn and Logan 2010)	Acetate	549
	Butirate	487
	Domestic water	302
Catal et al., 2008 (Catal et al. 2008)	Glucose	2160
	Galactose	2090
	Fructose	1810
	Fucose	1760
	Rhamnose	1320
	Mannose	1240
	Xylose	2330
	Arbinose	2030
	Ribose	1520
	Galacturonic acid	140
	Gucuronic acid	2770
	Gluconic acid	2050
Sun et al., 2015(Sun et al. 2015)	Acetate	450
	Acetate /Xylose 1:1	300
	Xylose	280
	ethanol(xylose)	80
Kim et al., 2007 (Kim et al. 2007)	Ethanol (single-chamber)	40
	Ethanol (two-chamber)	488
Yu et al., 2012(Yu et al. 2012)	Glucose (HTR 12 h)	191
	Glucose (HTR 10 h)	351
	Glucose (HTR 8 h)	557
	Glucose (HTR 6 h)	753

This study	Acetate	268
	Propionic acid	478
	Ethanol	100
	glycerol	40
	Fructose	20

380

381 Accordingly, our results are in agreement with those shown in the literature and points  
382 out that carboxylic acids are the best choice as substrate for MFC. However, opposite to  
383 several of the works reported, propionic improves **slightly significantly** the performance  
384 obtained by acetic acid. Both propionic and acetic acid can be easily obtained by  
385 biodegradation processes which can be easily coupled to MFC and this points becomes a  
386 promising approach for future MFC developments.

387

### 388 **Conclusions**

389 From this work the following conclusions can be drawn:

- 390 - There is a clear effect of the type of organic fed to MFC on the production of  
391 electricity. For the same organic load, carboxylic acids are the most efficient fuels  
392 in comparison to single sugars (like fructose) or alcohols (like ethanol or glycerol)
- 393 - For a system fed with acetate solution, there is a linear increase in the production  
394 of electricity with the organic load fed up to 5000 ppm of COD. The ratio between  
395 the consumption of COD and the production of electricity is kept approximately  
396 constant in a value that indicates that around 9.24 % of the COD is used to produce  
397 electricity, which can be interpreted in terms of a constant ratio of the  
398 bioelectrogenic population in the sludge. For higher COD fed, the ratio decreases  
399 to 3.82% indicating that extremely high loads lead to a change in the population  
400 of adapted microorganisms with a lower weight of bioelectrogenic  
401 microorganisms.

402

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406

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