

1 **MICROALGAE AS SUBSTRATE IN LOW COST TERRACOTTA-BASED MICROBIAL FUEL CELLS:**
2 **NOVEL APPLICATION OF THE CATHOLYTE PRODUCED**

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16 **HIGHLIGHTS**

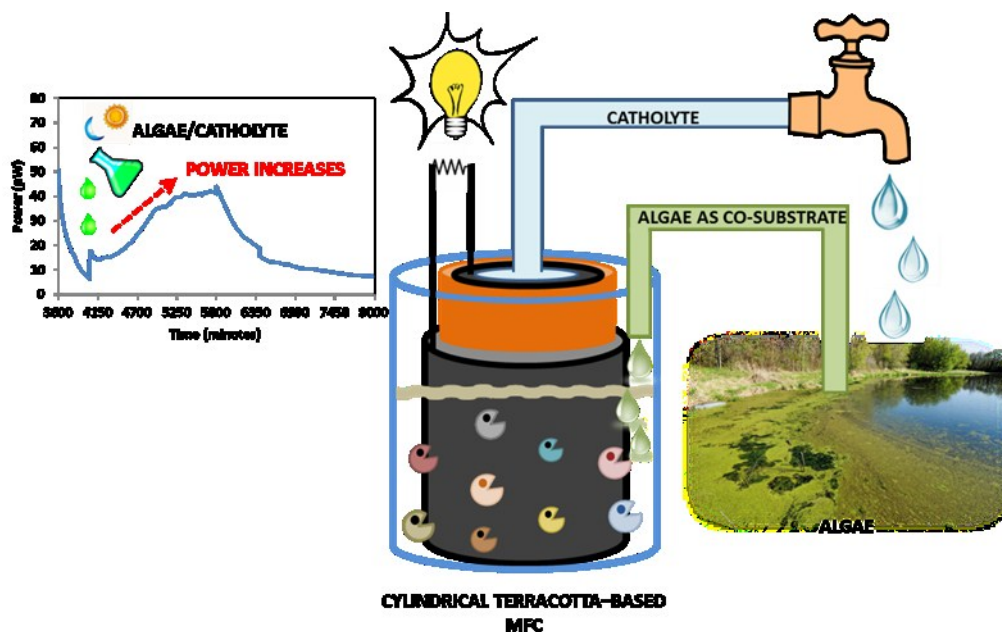
- 17 ▪ Novel application of the catholyte produced in ceramic MFCs to lyse
- 18 microalgae.
- 19 ▪ Microalgae as a potential low cost co-substrate for MFCs.
- 20 ▪ Light/dark cycle assisted microalgae digestion in the presence of catholyte.

21 **ABSTRACT**

22 In this work, the by-product generated during the operation of cylindrical MFCs, made
23 out of terracotta, is investigated as a feasible means of degrading live microalgae for
24 the first time. In addition to the low cost materials of this design, the reuse of the
25 solution produced in the cathode renders the technology truly green and capable of
26 generating bioenergy. In this study, the effect of a light/dark cycle or dark conditions
27 only on the digestion of live microalgae with the catholyte is investigated. The results

28 show that a combination of light/dark improves the degradation of algae and allows
29 them to be used as substrate in the anode. The addition of 12.5 mL of a 1:1 mix of
30 catholyte and microalgae (pre-digested over 5 days under light/dark) to the anode,
31 increases the power generation from 7 μ W to 44 μ W once all the organic matter in the
32 anode have been depleted.

33 GRAPHICAL ABSTRACT



34

35 **Keywords:** *Microbial fuel cells; ceramic membrane; catholyte; microalgae; bioenergy.*

36 **1. INTRODUCTION**

37 The ongoing energy crisis and global warming have challenged the scientific
38 community to develop alternative sources of energy [Creutzig *et al.*, 2015; Guo *et al.*,
39 2015; Heinimö & Junginger 2009; Mao *et al.*, 2015]. A wide range of materials has
40 been investigated to produce bioenergy such as industrial or crop waste [Deublein &
41 Steinhauser, 2008; Ho *et al.*, 2014]. However, algae have received increased attention
42 in recent years as an alternative option to conventional materials. The use of algae

43 presents many advantages due to their high growth rates in relatively confined spaces,
44 compared to other terrestrial plants. Algae can be grouped into two large categories:
45 microalgae and macroalgae. The main characteristic of microalgae is that they are
46 unicellular green plants that contain proteins, lipids and carbohydrates in different
47 proportions, depending on the strain but not cellulose or lignin. Moreover, they are
48 rich in chlorophyll and can be used for feeding aquatic organisms [Schenk *et al.*, 2008;
49 Velasquez-Orta *et al.*, 2009]. On the other hand, macroalgae do not contain lignin but
50 have low values of cellulose, which makes them more resistant to some predators.
51 They mainly consist of polysaccharides and unsaturated fatty acids [Velasquez-Orta *et*
52 *al.*, 2009; Vergara-Fernández *et al.*, 2008]. Microalgae and macroalgae can be
53 cultivated in different aqueous environments such as rivers, seas or wastewater, and
54 both types have been studied for the production of energy, via different pathways:
55 macroalgae have been used in the production of methane and microalgae are more
56 suitable for producing a wide range of energy products, such as bio-oil, methane,
57 methanol, hydrogen or even electricity. The main drawback is the need for a resource-
58 intensive infrastructure to support the transformation of microalgal biomass into
59 electricity (storage, transport and processing) [Bahadar & Khan 2013; Velasquez-Orta
60 *et al.*, 2009]. In this regard, microbial fuel cells (MFCs) have played a key role in recent
61 years as a technology that can directly produce electricity from different sources of
62 organic waste, and perhaps algae. MFCs use microorganisms to degrade organic
63 matter contained in different types of waste, producing electrons and protons. The
64 electrons go through an external circuit to the cathode while protons go through a
65 separator, usually a proton exchange membrane, to reach the cathode. In the cathode,

66 incoming electrons and protons react with oxygen to produce water [Hernández-
67 Fernández *et al.*, 2015; Oliveira *et al.*, 2013; Potter 1911].

68 Many advances have been achieved in terms of new materials and designs in the field
69 of MFCs to improve their performance and reduce the cost [Hernández-Fernández *et*
70 *al.*, 2015]. The use of ceramic materials as a separator is amongst the most important
71 since commercial membranes like Nafion® are expensive [Ghadge & Ghangrekar 2015;
72 Winfield *et al.*, 2013]. Ceramics have been previously reported as membrane/electrode
73 combinations (Park & Zeikus, 2003) and as separators (Behera *et al.*, 2010), and more
74 recently, Gajda *et al.* (2015) reported a low-cost ceramic cylinder as the membrane
75 and chassis of MFCs [Gajda *et al.*, 2015]. In this latter work, carbon veil was used as the
76 anode electrode, wrapped around the outside of the cylinder, and the same carbon
77 veil, covered with activated carbon was used as the cathode, on the inside of the
78 cylinder. The group reported a maximum power of 2.86 mW.m^{-2} , enough energy to
79 power a LED over 7 days, with a concomitant 92% reduction in chemical oxygen
80 demand (COD). In addition to the low cost and high power output of this assembly, the
81 production of an alkaline solution inside the terracotta cylinder (cathode) was also
82 reported as a function of the electrical performance. The catholyte was colourless and
83 odourless containing carbonate and bicarbonate salts, and high levels of pH and
84 conductivity. All of these chemical properties of the catholyte suggest opportunities
85 for exploitation in a range of future applications [Gajda *et al.*, 2015].

86 This current work investigates for the first time the application of the alkaline
87 catholyte solution, produced in the aforementioned cylindrical terracotta MFCs, to lyse
88 live microalgae and then feed the lysed cells as substrate, in the anode for the

89 microbes. This takes advantage of the chemical potential produced by the MFC by
90 using the alkaline catholyte as an external digester to provide organics to the anode
91 microbes that would be difficult or impossible for them to break down directly. The
92 performance of these MFCs is then compared with that from MFCs using live non-lysed
93 microalgae. This study shows a novel and promising application of the by-product
94 generated during the operation of ceramic MFCs, which opens up further avenues for
95 exploration and exploitation.

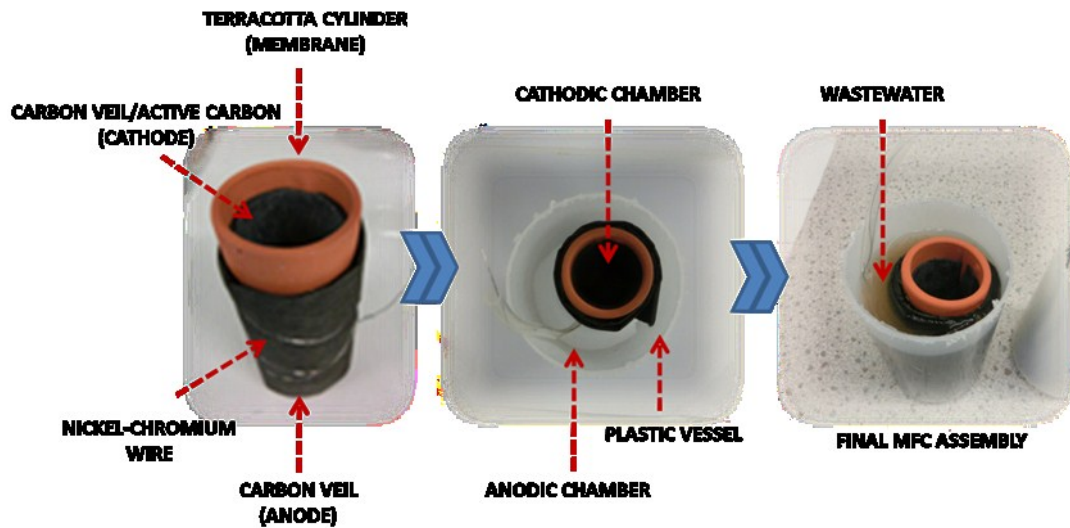
96 **2. MATERIALS AND METHODS**

97 ***Microbial fuel cell configuration***

98 The microbial fuel cells used consist of a 10 cm tall terracotta cylinder sealed at the
99 bottom with an internal and external diameter of 3.5 cm and 4 cm, respectively
100 (Weston Mill Pottery, Nottinghamshire, UK). This structure acts as separator between
101 the anodic and cathodic chamber. The anode is made out of carbon veil (20 g.m^{-2})
102 folded and wrapped around the outside surface of the terracotta cylinder. A nickel-
103 chromium wire is used to hold this electrode in place and also serves as the current
104 collector and connection point. The cathode is formed by the same carbon veil (90
105 cm^2 , substratum/diffusion layer) coated with a mixture of (conductive layer) activated
106 carbon and polytetrafluoroethylene (PTFE 30%_{V/V}). It is placed inside the terracotta
107 cylinder with the conductive layer facing the separator. The cathode electrode is held
108 against the inner wall of the cylindrical ceramic body, using a plastic ring and the
109 cathode compartment is open to the air, in order to allow the oxygen reduction
110 reaction to take place. Finally, an external resistance of 100Ω is used to load the circuit

111 and two stainless steel crocodile clips connect both electrodes to the data logger.

112 Figure 1 describes the main components of the MFCs studied.



113

114 Figure 1. Main components of the MFCs studied.

115

116 **Analytical Method**

117 A 16-channel ADC-24 Picolog recorder data logger (Pico Technology Ltd,
118 Cambridgeshire, UK) was used to monitor the voltage vs. time. The polarisation and
119 power curves were measured by changing the external resistive loads, from 999999 to
120 0 Ω (including open circuit voltage), for 3 minute intervals for each load, using an
121 automatic resistorstat tool [Degrenne *et al.*, 2012]. Data sampling (i.e. recording
122 capacity) during the polarisation run was 30 second intervals.

123 The catholyte and anolyte solutions were characterised by measuring pH and
124 conductivity during the experiment with a handheld pH meter (Hanna 8424, Hanna
125 Instrument, UK) and 470 Jenway conductivity meter (Camlab, UK), respectively.

126

127 **Operation Mode**

128 The terracotta cylinders were placed inside a plastic container, which serves as the
129 anode chamber, and filled with 170 mL of substrate. The fuel is a solution consisting of
130 10%_{V/V} of sludge provided by Wessex Water Scientific Laboratory (Cam Valley, Saltford,
131 UK), 90%_{V/V} of deionised water and supplemented with sodium acetate anhydrous
132 (Fisher Chemical, Loughborough, UK) with a final concentration of 20 mM. Prior to
133 starting the experiments, the MFCs were matured during 14 days using a solution
134 composed of sludge supplemented with 100 mM acetate as the substrate. At this
135 starting point, the cathode chamber was completely empty and dry, in order for
136 catholyte to be actively produced, as a direct result of the MFC operation. During this
137 process, MFCs generated sufficient amount of catholyte to be subsequently used for
138 microalgal lysis. MFCs were studied in batch mode under the 4 different conditions
139 detailed below. Each one was carried out in triplicate and they were applied
140 sequentially in the same reactors in the following order:

141 ▪ **Condition A:** Following the maturing of the MFCs, the anodes were filled with
142 170 mL of substrate (20 mM of acetate and 10%_{V/V} of sludge). The power increased
143 initially due to the bacterial metabolism, then stabilised and finally started to decrease
144 due to the depletion of the organic matter. When the power is below 10 μ W, 12.5 mL
145 of substrate (10% of the volume at that moment) is removed and replaced by 12.5 mL
146 of a 1:1 mix consisting of microalgae and deionised water, to maintain the liquid
147 volume of the reactor. The microalgae culture was collected from a pond at Frenchay
148 Campus (University of the West of England, Bristol, UK) and grown in the laboratory in
149 the cathodic chamber of a separate MFC. It is a wild mixed algal culture with an optical

150 density of 1.77 (4.8 g.L⁻¹), which was measured using a Jenway 6300
151 spectrophotometer (Wolflabs, UK) at a wavelength of 678 nm. The effect of adding the
152 catholyte/deionised water solution in the systems was evaluated in terms of power
153 generation.

154 ▪ **Condition B:** Following the completion of Condition A, the MFCs were rinsed
155 and carefully cleaned with deionised water. Then, they were filled with 170 mL of the
156 same substrate as described for Condition A, but with the 12.5 mL added, consisting of
157 catholyte and deionised water (1:1) as a control, to investigate the effect on power
158 production. The catholyte is an alkaline solution that mainly contains carbonate and
159 bicarbonate, and traces of chloride, phosphate and sulphate (data not shown).

160 ▪ **Condition C:** Following the completion of Condition B, and before feeding, the
161 MFCs were cleaned with deionised water again. When the power reached a value
162 below 10 μW, 12.5 mL of a solution made from microalgae and catholyte (1:1) digested
163 over a period of 5 days under natural cycles of day and night, were added to the
164 anode.

165 ▪ **Condition D:** In this case, the procedure followed is the same as for the
166 previous conditions, but the 12.5 mL of mix added consisted of microalgae and
167 catholyte (1:1) but the micro-algal digestion occurred over 5 days in total darkness.

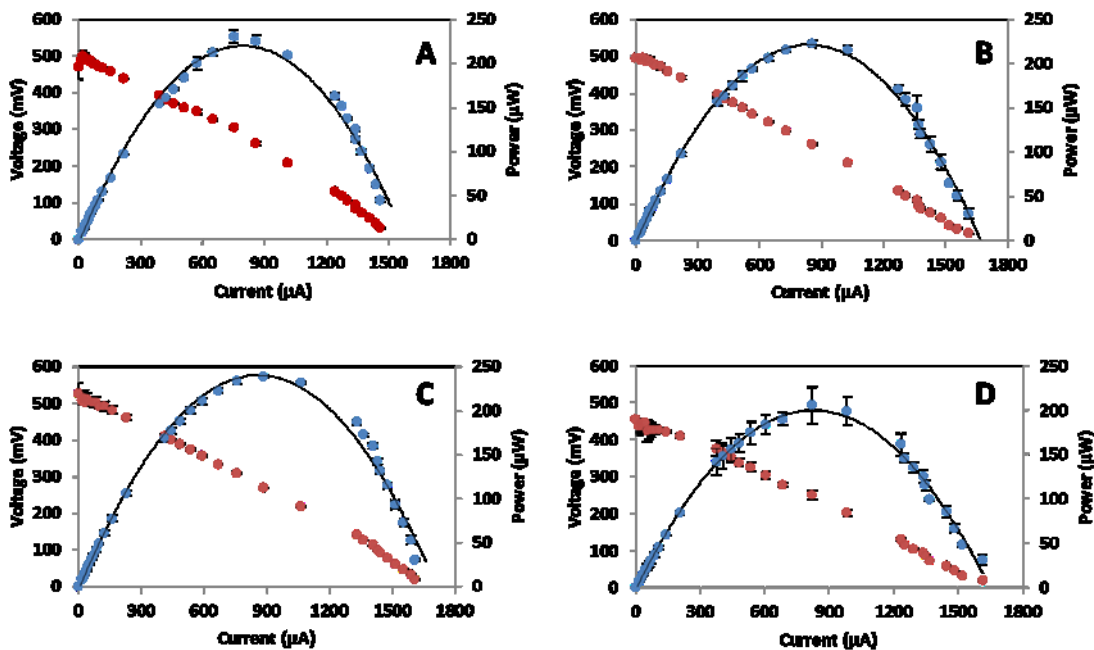
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169 **3. RESULTS AND DISCUSSION**

170 This work shows a novel application of the catholyte generated in ceramic tubular
171 MFCs. This catholyte is a colourless and odourless liquid with high values of pH and
172 conductivity. The amount of catholyte produced is a function of power performance
173 for this type of MFCs; in other words, the higher the performance of the microbial fuel

174 cells, the higher the production of catholyte and the higher the pH and conductivity
 175 levels [Gajda *et al.*, 2015]. The catholyte used in this study had a pH of 12.5 and a
 176 conductivity of 24.5 mS.cm^{-1} . As mentioned above, the main purpose of this work is to
 177 reuse the catholyte produced to lyse algae in such a way that the mixture can be used
 178 as low cost substrate for anodic microorganisms.

179 Figure 2 shows the power and polarisation curves from each group of three MFC
 180 replicates, once they become stable and before applying the Conditions described
 181 above. A maximum power of $230 \mu\text{W}$ was recorded by the four groups of MFCs. Then,
 182 the four types of assay were carried out under Conditions A-D.



183
 184 Figure 2. Power and polarisation curves of the three MFC replicates of each condition
 185 before adding the co-substrate mix.

186 ● Polarisation curve ● Power curve

187 Figure 3 shows the evolution of the average power produced by each group of
 188 microbial fuel cell triplicate vs. time. The decay in the power curves indicates the
 189 precise time point at which the solution investigated as a substrate is added to the

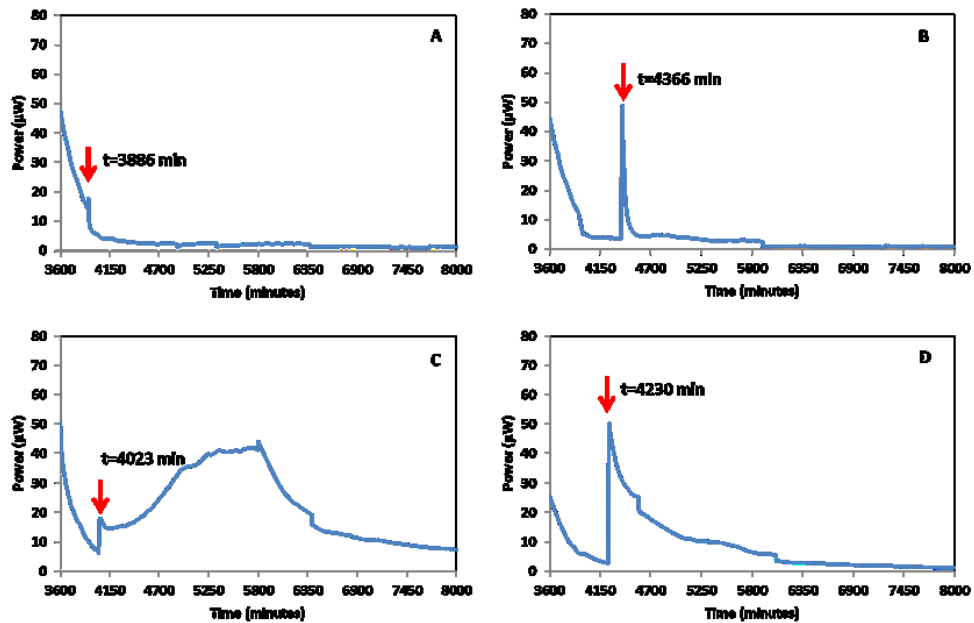
190 anodic chamber, indicated by the red arrows in the figure. As can be seen in Figure 3A,
191 when the solution of algae/deionised water was added, the power of the MFCs
192 continued decreasing. This suggests that the microorganisms are not able to directly
193 utilise this type of algal mixture.

194 In Figure 3B, a peak of approximately 50 μW was recorded, when 12.5 mL of
195 catholyte/deionised water (1:1) were added in the anode solution. However, this was a
196 short spike, since power decreased within 3 hours. The power increase is due to the
197 high conductivity of the catholyte although this effect disappears very quickly when
198 the charges are balanced between the cathode and the anode. This would imply that
199 the mixture added, does not contain bio-available compounds for the microorganisms
200 to utilise.

201 Figure 3C shows a higher increase, after adding the solution containing algae/catholyte
202 digested over 5 days under a light/dark cycle (natural diurnal). In contrast with the
203 previous cases, the power continued increasing for 33 hours until a maximum of 44
204 μW was reached. This result suggests that perhaps the bacteria in the anode chamber
205 could degrade better the substrate. This would mean that a natural cycle of light/dark
206 (16:8 hours) may be necessary to lyse algae in the presence of the synthesised
207 catholyte. Previous research shows that microalgae need light and darkness for
208 growth, since they use the light for photochemical reactions (generating adenosine
209 triphosphate (ATP), coenzymes, nicotinamide adenine dinucleotide phosphateoxidase
210 (NADPH)) and the darkness for synthesising essential molecules by biochemical
211 reactions (Calvin Cycle) [Al-Qasmi *et al.*, 2012]. This cycle is used by the photosynthetic
212 cells to transform the inorganic carbon into organic carbon using the energy stored in

213 the molecules synthesized during the light cycle, such as ATP. Some of the
214 photosynthesised organic compounds are excreted into the media as dissolved organic
215 carbon (DOC) that anodic microorganisms could degrade and use as carbon-energy
216 source. The rate of decomposition depends on the identity of the microbial species
217 present in the community, their affinity to the dissolved carbon source and their
218 growth kinetics [Kouzuma & Watanabe 2015]. In this case, the mix of algae/catholyte
219 was digested under a light/dark cycle, which allows microalgae to carry out the
220 photochemical and biochemical reactions, and excrete a wider range of organic
221 compounds into the media. On the other hand, during the growth process, algae will
222 neutralise the alkaline pH of the catholyte. In order to buffer the external pH,
223 microalgae also release acidic extracellular metabolites. These metabolites along with
224 DOC enrich the media. It is therefore assumed that a combination of these factors
225 renders the added mixture a better carbon-energy source that could be more readily
226 degradable by the anodic microorganisms, thereby improving the performance of the
227 MFCs.

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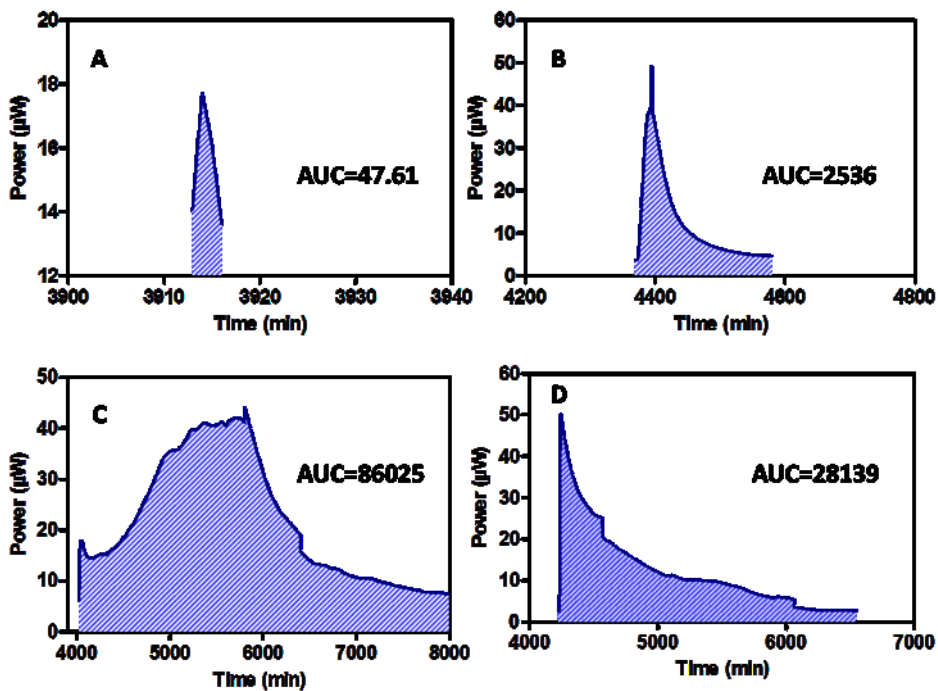
230 Figure 3. Power output of each MFC under the conditions studied. A) Condition A:
 231 Algae+Deionised water; B) Condition B: Catholyte+Deionised water; C) Condition C:
 232 Algae digested with catholyte under a cycle of light/darkness; D) Condition D: Algae
 233 digested with catholyte under darkness.

234

235 Finally, Figure 3D shows an immediate increase in power, when the mix of
 236 algae/catholyte digested under darkness was added. The magnitude of this peak is
 237 similar to that recorded in Figure 3B, when catholyte/deionised water was added. This
 238 implies that the increase in the power generated is caused by the high conductivity of
 239 the catholyte but perhaps not by the algal degradation itself. In this case, the mix was
 240 kept under dark conditions, so that algae could neither carry out their normal cycle of
 241 photochemical/biochemical reactions, nor adapt to the new conditions of the media
 242 (pH>12). This implies that they would not produce ATP or NADPH, which are required
 243 in the Calvin cycle to synthesise organic compounds necessary for their growth, such as
 244 sugars or starch. Hence, all the available biochemical material would be consumed for
 245 their survival. When this liquid mix is added into the MFC anode, it appears that there
 246 is not sufficient carbon-energy for the anodic microorganisms to consume [Kouzuma &

247 Watanabe 2015], apart perhaps from any lysed algae, which is results in the relatively
248 longer duration, of increased power, compared to Fig. 3B.

249 Figure 4 shows the area under curve of the power output (AUC) representation caused
250 by the addition of the substrates analysed. The energy produced by the addition of the
251 solution investigated was also determined and found to be: 0.003, 0.152, 5.162 and
252 1.688 Joules for Conditions A, B, C and D, respectively. As can be observed, the use of
253 algae digested with catholyte under light/darkness shows the highest effect on power
254 output, both in terms of magnitude and length (see Figure 4C). It is three times higher
255 than the effect caused when the digestion is performed under dark (Fig. 4D). 34 times
256 higher than the result after feeding with catholyte and deionised water (Fig. 4B) and
257 1.8 times higher than the power generated when the MFCs were fed with microalgae
258 and de-ionised water (Fig. 4A).



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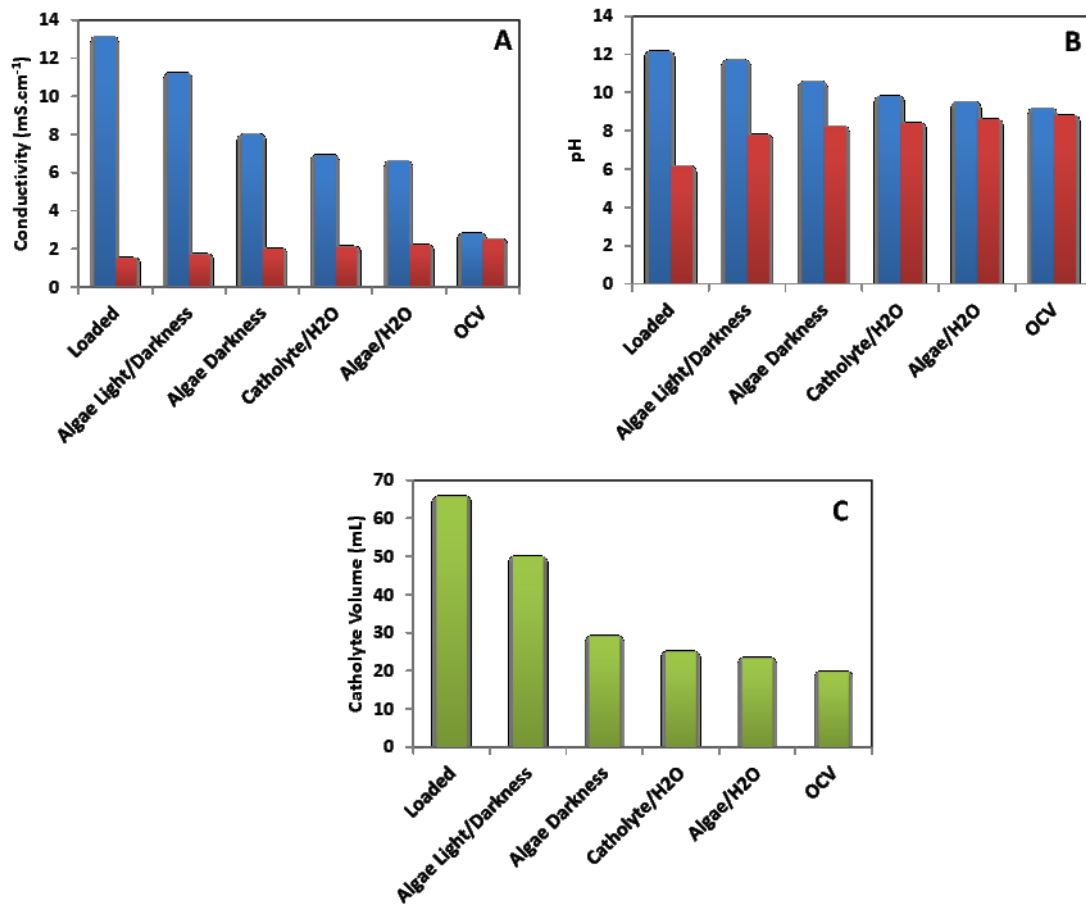
260 Figure 4. Area under curve representation of the effect of the conditions investigated
261 on the MFCs power output. A) Condition A: Algae+Deionised water; B) Condition B:
262 Catholyte+Deionised water; C) Condition C: Algae digested with catholyte under a
263 cycle of light/darkness; D) Condition D: Algae digested with catholyte under darkness.

264 Power output was related to the conductivity, pH and volume of the catholyte
265 produced in each MFC. All the results were compared with two types of MFCs, i.e. the
266 controls, which (i) contained sludge and acetate in the proportion described above and
267 were externally loaded with 100 Ω , and (ii) the open circuit voltage MFCs, which also
268 contained sludge and acetate at the same concentrations, but were not externally
269 loaded (no electrons transfer).

270 As can be seen in Figure 5, the better the MFCs perform, the higher the values of these
271 parameters for the catholyte. Figures 5A and 5B show the conductivity and pH
272 differences between the anolyte and catholyte for each set of conditions investigated.

273 These results reveal that the conductivity and the pH of the catholyte increased with
274 higher MFC performance, while decreasing in the anolyte. Moreover, the
275 catholyte/anolyte ratio is higher for the MFCs that worked better (MFCs loaded and
276 MFCs with algae and catholyte digested under a light/darkness cycle). In terms of
277 volume of catholyte produced, the trend is the same (see Figure 5C). As previously
278 mentioned, the volume of the catholyte is directly proportional to the level of MFC
279 performance, since it is the result of oxygen reduction reaction, electro-osmotic drag
280 and passive osmosis. In this regard, the MFCs with algae and catholyte digested under
281 a cycle of light/darkness, resulted in the highest values of conductivity, pH and volume
282 of catholyte, followed by the MFCs with algae and catholyte digested in dark

283 conditions, which was followed by the MFCs with catholyte and deionised water, the
 284 MFCs with algae and deionised water and finally by the MFCs under open circuit
 285 conditions. These results are in line with those obtained by Gajda *et al.* 2015, who
 286 related high MFC performance to high values of pH and conductivity of the catholyte
 287 and high volumes of catholyte produced in terracotta-based MFCs.



288 Figure 5. Physico-chemical parameters for the conditions investigated: A) conductivity
 289 levels in the catholyte and anolyte; B) pH levels in the catholyte and anolyte; C) volume
 290 of catholyte produced.
 291

292 ■ Catholyte ■ Anolyte ■ Catholyte Volume

293
 294 The results show that the unique properties of the MFC-generated catholyte such as
 295 the high values of pH and conductivity allow for a wild culture of microalgae to be
 296 lysed in five days under a natural cycle of light/dark in a kind of self-produced external

297 digester. A mix of 12.5 mL (1:1) of catholyte/microalgae resulted in a 6-fold power
298 increase – from 7 μ W to 44 μ W. These values could be explained by the light/dark
299 conditions (i.e. natural rhythm of algae) and the specific properties of the catholyte,
300 acting as an algal lyser. Moreover, higher power output led to higher values of pH,
301 conductivity and volume of the catholyte generated, and in this context the MFCs
302 using algae digested with catholyte under a cycle of light/darkness outperformed the
303 rest of the test conditions. Higher power output was also quantified as area under
304 curve, which revealed a significant improvement from feeding the MFCs with the
305 catholyte/microalgae mixture.

306

307 The main advantages of the process described above are the low cost of the materials
308 and the reuse of the by-product generated. Unlike previous work, the assembly
309 studied uses a terracotta cylinder as exchange membrane instead of a commercial
310 membrane, activated carbon as conductive layer in the cathode instead of platinum
311 and live algae from a natural habitat, in a way alluding to algal bloom reduction [Rashid
312 *et al.*, 2013; Velasquez-Orta *et al.*, 2009]. All of these materials reduce the cost of the
313 MFC mitigating the main drawback for the scaling up process of these systems.

314

315 **4. CONCLUSIONS**

316

317 This work reveals a novel application of the catholyte produced during the operation
318 of a terracotta tubular MFC. These results show great promise since they demonstrate
319 that algae can be used as natural carbon source in terracotta-based MFCs when

320 treated with the catholyte that has been synthesised *in-situ*. Further work is required
321 to better understand the lysing mechanisms as well as the process of nutrient
322 extraction optimise the catholyte and algal biomass ratio and improve the operating
323 conditions from batch to continuous flow.

324

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