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2 **A grey box model of glucose fermentation and**  
3 **syntrophic oxidation in Microbial Fuel Cells**

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24 **Abstract**

25 In this work, the fermentative and oxidative processes taking place in a microbial fuel  
26 cell (MFC) fed with glucose were studied and modeled. The model accounting for the  
27 bioelectrochemical processes was based on ordinary, Monod-type differential  
28 equations. The model parameters were estimated using experimental results obtained  
29 from three H-type MFCs operated at open or closed circuits and fed with glucose or  
30 ethanol. The experimental results demonstrate that similar fermentation processes  
31 were carried out under open and closed circuit operation, with the most important  
32 fermentation products being ethanol (with a yield of  $1.81 \text{ mol mol}^{-1}$  glucose) and lactic  
33 acid (with a yield of  $1.36 \text{ mol mol}^{-1}$  glucose). A peak in the electricity generation was  
34 obtained when glucose and fermentation products coexisted in the liquid bulk.  
35 However, almost 90% of the electricity produced came from the oxidation of ethanol.

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38 **Keywords:** Microbial fuel cell; glucose; fermentation; ethanol; modeling.

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## 40 **1. Introduction**

41 Due to increasing energy demands and environmental concerns, the interest in the  
42 development of renewable energy sources as alternatives to fossil fuels has increased  
43 in recent years (MacKie et al., 2013). At the same time, more stringent environmental  
44 requirements for wastewater treatment are pressing for the development of more  
45 efficient treatment techniques. This phenomenon is not only an environmental  
46 concern but also an economical issue because water and wastewater systems are  
47 significant energy consumers. For example, an estimated 3-4% of U.S. electricity is  
48 consumed by the water and wastewater industry (Chandrappa & Das, 2014). In such a  
49 scenario, a reduction in treatment costs is necessary. Currently, the most important  
50 operational cost in a conventional wastewater treatment plant (WWTP) is aeration  
51 (Fernández et al., 2011a). Thus, the development of a process allowing for the  
52 oxidation of the pollutants with lower aeration requirements is of crucial importance.  
53 Currently, the most adequate technology for solving the combined energetic and  
54 environmental problem seems to be bioelectrochemical technology. Because of that,  
55 bioelectrochemical systems, including Microbial Fuel Cells (MFCs) and Microbial  
56 Electrolysis Cells (MECs), have been investigated as alternative energy sources and  
57 wastewater treatment systems (Brillas & Martínez-Huitle, 2015).

58 MFCs are electrochemical devices that can directly convert organic and inorganic  
59 substrates into electricity by means of a microbial culture. MFCs mimic a biological  
60 system, with the only difference being that they do not transfer electrons directly to  
61 the electron acceptor. Instead, the MFC anodophilic bacteria transfer the electron to a  
62 solid electrode as part of their respiration pathway. Then, the electron is externally  
63 conducted over the anode to the cathode, which results in the production of electricity



64 (Rao et al., 1976). Because of this ability, the interest in MFC is increasing due to its  
65 dual benefit: the production of green electricity and the oxidation of wastewater  
66 components without an oxygen supply (Piciooreanu et al., 2008). The main advantages  
67 of these devices are the mild operational conditions (neutral pH and ambient  
68 temperature) and the unlimited range of potential fuels that could be used (Schröder,  
69 2007), including wastes and fuels for which catalysts are currently unavailable.

70 In the MFC, one of the most widely used fuels is glucose. Glucose has been proposed  
71 as an interesting renewable energy source because it is safe (non-flammable and non-  
72 toxic) and its energy density ( $16 \text{ Mj Kg}^{-1}$ ) is lower than that of methanol or gasoline but  
73 is still quite high. Moreover, glucose is a basic unit of organic compounds that  
74 abundantly exist in wastewater. Therefore, glucose seems to be a powerful and  
75 environmentally friendly option.

76 Glucose-rich wastewaters can be found in different industries, but the industry  
77 producing the highest amounts is the agro-food industry (De Lucas et al., 2005). Agro-  
78 food wastewaters are characterized by very high organic loads and biodegradability  
79 (Rodríguez et al., 2007). The development of a technology capable of efficiently using  
80 the glucose contained in agro-food wastewater is of crucial importance to developing  
81 real applications of the MFC technologies.

82 The main drawback of the use of glucose as a fuel in the MFC is that glucose-rich  
83 streams give rise to only a small current when they are used as fuel in the MFC (Kim et  
84 al., 2007; Lee et al., 2008). This small current occurs because glucose is a complex  
85 substrate, and glucose can be used as a substrate in a large number of non-  
86 electrogenic anaerobic processes (Freguia et al., 2008).

87 During the operation of MFCs, multiple/parallel bioelectrochemical reactions take  
88 place. One of these simultaneous processes is fermentation. Fermentation and  
89 electrogenesis are two primary steps in bioelectrochemical systems (Premier et al.,  
90 2013). The objective of fermentation is to transform organic materials into the end  
91 products of liquids and gases through a variety of bioconversion stages (Fernández et  
92 al., 2011b). Several MFC studies have shown that fermentation and anode respiration  
93 processes are often combined (Freguia et al., 2008). Fermentative bacteria are able to  
94 ferment complex organic substrates to short-chain fatty acids (SCFA), alcohols and  
95 other fermentation products. All of these products can subsequently be oxidized to  
96 produce electricity (Kim et al., 2007). Integrating both processes in the anodic chamber  
97 may have a positive influence on the overall efficiency of wastewater treatment and  
98 the generation of electricity. Therefore, the description of the fermentation process at  
99 the anodic chamber of MFCs is of great interest.

100 One of the most economical and appropriate approaches to investigating the microbial  
101 behavior of and to comprehending the bioelectrochemical interactions in both MFCs  
102 and MECs is through mathematical modeling (Karimi Alavijeh et al., 2015). Modeling  
103 efforts have also been directed towards several aspects related to MFCs, evolving in  
104 recent years from simple to complex models, such as multi-species, multi-dimensional  
105 or multiscale models (Ortiz-Martínez et al., 2015). In the literature, there are papers  
106 discussing generalized models describing wastewater treatment and the associated  
107 energy production on MFC (Karimi Alavijeh et al., 2015), models of MFC biofilms and  
108 suspended cultures (Picioreanu et al., 2010a), models of inorganic pollutants and pH  
109 effects on MFC (Picioreanu et al., 2010b) and papers reviewing and classifying the  
110 existing models (Ortiz-Martínez et al., 2015).

111 In this context, the objective of this study was to evaluate the performance of a MFC  
112 fed with glucose in order to study the influence of the fermentation process and the  
113 syntrophic oxidation of the fermentation products generated on the performance of  
114 the MFC.  
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116

## 117 **2. Materials and methods**

### 118 *2.1 MFC Model definition*

119 To clarify the glucose transformations in the MFC, the fermentation process and the  
120 subsequent oxidation of the fermentation products were modelled. The model  
121 definition was based on experimental observations when studying the fermentative  
122 and subsequent oxidative processes in the H-type MFCs used in this work. These  
123 equations are presented in the form of the Petersen matrix in Table 1, and the  
124 parameters of the model are presented in Table 2. All of the kinetics expressions are  
125 based on the classical, commonly-used Monod terms (Monod, 1949). **The equations**  
126 **address the soluble compounds involved anaerobic and electrogenic transformations.**  
127 **Thus, the effect of the concentration of the mediator in oxidized form was included in**  
128 **the electrogenic processes (Piciooreanu et al., 2010a).**

129 [TABLE 1 NEAR HERE]

130 [TABLE 2 NEAR HERE]

131 In the developed model, the reactions proposed included glucose fermentation to  
132 fermentation products (via non-electrogenic fermentation processes) and the  
133 oxidation of glucose and the fermentation products to produce electricity. The  
134 fermentation was considered to be non-electrogenic because the main fermentation  
135 products accumulated in the liquid bulk of both MFCs (open and closed circuit)  
136 correspond to those not generated by means of the electricity generation processes.  
137 On the other hand, based on the experimental results, the main electrogenic reactions  
138 were the electrogenic oxidation of glucose and the main fermentation product (i.e.,  
139 ethanol) to CO<sub>2</sub>.

140 When the anodic and cathodic chambers were connected via the external electrical  
141 circuit (i.e., closed circuit), the environmental conditions required for electrogenic  
142 metabolism by the organisms were obtained. In contrast, the fermentative processes,  
143 rather than the electrogenic ones, may be the predominant processes when working  
144 under open circuit conditions. Through the comparison of MFCs of both circuit types  
145 (closed and open), the fermentation and electrogenic metabolisms were  
146 discriminated.

147 Taking into account the similarities observed when working with the open and closed  
148 circuit MFCs, the fermentation equations proposed for the open circuit MFC were also  
149 used for the closed circuit MFC. The only difference was the existence of a lag phase  
150 when fermenting glucose to lactic acid under closed circuit conditions but not under  
151 open circuit conditions. To describe both behaviors, a switching function in the lactic  
152 acid production rate was included (see process B2 in Table 2). The different behavior of  
153 the closed circuit MFC could be explained because of the ability of the microorganisms  
154 to switch between glucose oxidation with electricity production and lactic acid  
155 generation from glucose fermentation. The switching function was based on an  
156 inhibition function proposed in the literature (Edwards, 1970). In this function, an  
157 additional term called  $I_x$  was included. This term is a Boolean function with a value of 0  
158 for open circuit and 1 for closed circuit. The inclusion of this term allowed us to  
159 describe the differences in the fermentation under open or closed circuit conditions.  
160 Additionally, the glucose and fermentation products generated could be oxidized to  
161 carbon dioxide, thereby generating electricity. The equations describing these  
162 processes in the model are processes E1 and E2 (Table 2). A reaction-scheme of the  
163 proposed model is depicted in Figure 1.



164 FIGURE 1 NEAR HERE

165 Neither hydrogen production by fermenters nor methane production by methanogens  
166 were included in the developed model because neither methane nor hydrogen were  
167 detected in the gas phase of the H-type MFC. The lack of methane in the gas phase  
168 could be explained because of the acidification of the anodic chamber, which inhibits  
169 the activity of the methanogenic microorganisms (Fernández-Morales et al., 2010).  
170 Regarding hydrogen, its absence could be related to the seed used for the inoculation  
171 of the MFC. Acetic and formic acids only appeared in trace concentrations, in both  
172 open and closed circuit experiments, and therefore they were not included in the  
173 model.

174 Taking into account the processes identified, the calibration was performed in three  
175 stages. In the first stage, the calibration was focused on the non-electrogenic  
176 fermentation processes. In the second stage the electrogenic ethanol oxidation was  
177 modeled. Finally, a third stage was used to simultaneously study the whole system,  
178 including the glucose fermentation and the electrogenic glucose and ethanol  
179 consumption, as well as the associated electricity generation.

180 To determine the best fit of the model, mathematical calculations were performed  
181 using the Solver Tool in MS Excel. These calculations required the calculation of the  
182 minimum residual sum of squared errors, which is associated with the difference  
183 between the experimental data and the theoretical predictions of the model (de Lucas  
184 et al., 2007).

185

## 186 *2.2 MFC Design and configuration*

187 Dual-compartment H-type MFCs were used. The MFCs held 0.7 L in each compartment,

188 and the compartments were separated by a tubular central compartment into which  
189 the separator was located (see Figure 2). The separator used in this work was a 5 cm  
190 thick microporous separator, including 16 cm<sup>3</sup> of compacted kaolin powder (with  
191 particle diameters less than 35 microns). This separator was supported by a glass fiber  
192 filter to avoid disintegration due to the action of bulk liquid at both anodic and  
193 cathodic sides of the separator. The separator was designed to avoid the transport of  
194 microorganisms from the anodic to the cathodic chamber and to avoid the transport of  
195 oxygen from the cathodic to the anodic chamber. It must be stated that the MFC used  
196 in this work was mainly limited due to the internal resistance of the separator;  
197 therefore, the results are not directly applicable to a single-chamber MFC. The  
198 electrodes used in both the anode and the cathode were porous graphite rods (1 cm  
199 OD x 10 cm L) without any surface treatment or catalytic addition. Before the  
200 experiments, the electrodes were first soaked in deionized water for a period of 24 h.  
201 The submersible external surface of each electrode was 25.9 cm<sup>2</sup>. The electrodes were  
202 placed at a distance of 20 cm on either side of the MFC. Cooper wires and a 120 ohm  
203 resistor were used as a contact from the electrodes. The cathode was continuously  
204 aerated by using an aquarium air pump. During the experiments, the air pump  
205 supplied 6 L min<sup>-1</sup> of air at 1.5 atmospheres of pressure to the cathodic chamber.  
206 Three identical H-type MFCs were used to study different processes, including  
207 electricity production from glucose, electricity production from the main fermentation  
208 product (ethanol), and the glucose fermentation process. In the latter case, the  
209 fermentation process was isolated from the electricity production process by  
210 disconnecting the external electrical circuit.

211 [FIGURE 2 NEAR HERE]

212

### 213 *2.3 MFC operation*

214 The anodic chamber of the MFCs were fed with a medium solution containing the  
215 organic substrate, 9 g L of glucose or 3.3 g L of ethanol (depending on the test) and the  
216 following trace minerals (in g L<sup>-1</sup>): **NH<sub>4</sub>Cl 3.0150**; KH<sub>2</sub>PO<sub>4</sub> 1.7550; NaCl 0.6570; Na<sub>2</sub>SO<sub>4</sub>  
217 0.1290; MgCl<sub>2</sub> 6H<sub>2</sub>O 0.2700; EDTA 0.1125; ZnSO<sub>4</sub> 7H<sub>2</sub>O 0.0072; FeSO<sub>4</sub> 7H<sub>2</sub>O 0.0070;  
218 MnCl<sub>2</sub> 4H<sub>2</sub>O 0.0056; CuCl 2H<sub>2</sub>O 0.0050; CoCl<sub>2</sub> 6H<sub>2</sub>O 0.0022; CaCl<sub>2</sub> 0.0014; NiCl<sub>2</sub> 6H<sub>2</sub>O  
219 0.0011; Na<sub>2</sub>MoO<sub>4</sub> 2H<sub>2</sub>O 0.225·10<sup>-3</sup>; and H<sub>3</sub>BO<sub>4</sub> 0.225·10<sup>-3</sup>. The carbon and mineral  
220 solution was sterilized in an autoclave at 110 °C for 20 min. **A high organic substrate**  
221 **concentration was used to identify non-electrogenic anaerobic reactions and also to**  
222 **maintain a longer energy production in the batch microbial fuel cell. The cathodic**  
223 **chamber was fed with demineralized water.**

224 The anodic chambers of the MFCs were seeded two days after the start-up of the  
225 MFCs with a selectively enriched mixed culture taken from the effluent of a working  
226 MFC (Gonzalez del Campo et al., 2013). By working in this way, the absence of  
227 electricity generation before the seed of the MFC was verified, serving these data as  
228 abiotic control data.

229 All of the experiments were conducted in batch mode. The experiments were  
230 continued until there was no significant change in the measured quantities. The  
231 contents in the anode and cathode chambers were continuously homogenized by  
232 means of magnetic stir bars rotating at 80 rpm. The power output was monitored by  
233 measuring voltage with an external resistor (120 ohms) connected between the  
234 electrodes.

235

236 *2.4 Analytical methods and calculations*

237 Aqueous samples were collected from each MFC and then immediately centrifuged  
238 (12000 rpm) and filtered through a 0.45  $\mu\text{m}$  membrane filter. Once filtered, the  
239 samples were analyzed or preserved frozen, according to the procedures described in  
240 the literature (Eaton et al., 2005).

241 Glucose concentrations were measured by HPLC (Agilent) with a refractive index  
242 detector (series 1200). A Zorbax Carbohydrate Column (4.6 x 150 mm, 5-micron) was  
243 used to separate the components at 35  $^{\circ}\text{C}$  using a mobile phase composed of 75%  
244 acetonitrile and 25% water v/v and a flow rate of 1.5  $\text{cm}^3 \text{min}^{-1}$ . Furthermore, lactic  
245 acid was determined from centrifuged and filtered samples by HPLC (Agilent) equipped  
246 with UV-DAD and Zorbax SB-Aq (4.6 x 150 mm, 5-micron). The mobile phase was a  
247 buffer at pH 2 (0.05 M phosphate). SCFA (acetic, propionic and butyric) and ethanol  
248 contents were determined from a centrifuged (12000 rpm) and filtered sample (0.45  
249  $\mu\text{m}$  membrane) by gas chromatography (Perkin Elmer) with a flame ionization detector  
250 (FID) using a Crossbond Carbowax Column (15 m x 0.32 mm ID, 0.25 mm df). The initial  
251 temperature of the oven was 140  $^{\circ}\text{C}$ , which held for for 1.5 min, and the temperature  
252 was raised at 25  $^{\circ}\text{C} \text{min}^{-1}$  until 190  $^{\circ}\text{C}$ , where it was maintained for 2 min. The  
253 temperature of the injector and detector were 200  $^{\circ}\text{C}$  and 230  $^{\circ}\text{C}$ , respectively.  
254 Nitrogen was used as the carrier gas. More information can be found elsewhere  
255 (Fernández-Morales et al., 2010; Infantes et al., 2011). pH values were determined  
256 using a Crison GLP-22 pH probe (Crison Instruments S.A., Barcelona, Spain).

257 Bioelectrochemical calculations were carried out based on the procedures outlined in  
258 the literature (Logan et al., 2006). Potential (V) measurements were recorded with an  
259 auto range digital multimeter (Model 2700, Keithley Instrument, OH, USA). Coulombic

260 efficiency (CE), defined as the ratio of the total coulombs actually transferred to the  
261 anode from the substrate to the maximum possible coulombs if all substrate removal  
262 produces the electrical current (Logan et al., 2006), was calculated by integrating the  
263 current over time and taking into account the total Coulombs associated with the COD  
264 removed in the same period of time.

265

$$266 \quad CE = \frac{M \int_0^t I dt}{F b V \Delta COD}$$

267

268 where M is the molecular weight of oxygen (32), I corresponds to the current intensity  
269 generated, F is Faraday's constant (96.485 C mol<sup>-1</sup> e<sup>-</sup>), b represents the number of  
270 electrons exchanged per mole of COD removed (4), V is the volume of liquid in the  
271 anode compartment (0.7 L), and ΔCOD depicts the change in theoretical COD  
272 concentration over the period of time. The COD concentrations were theoretically  
273 calculated based on the substrate concentration in the bulk liquid. **In this way, it is**  
274 **possible to isolate the contribution of each product in every process. To ratify the**  
275 **accuracy of the theoretical COD calculations, the actual COD concentration of each**  
276 **sample was experimentally determined and compared with the theoretical**  
277 **concentration, with the error in all cases being lower than 8%. The gas composition**  
278 **was analyzed by a multi-component gas analyzer (Rosemount Analytical NGA 2000**  
279 **MLT, Emerson).**

280

### 281 **3. Results and Discussion**

282 Before the study and modeling of the results, the mass balance in all the experiments  
283 performed was verified. Table 3 presents the carbon balance in the glucose-fed MFC  
284 (open and closed circuit) and the ethanol-fed MFC. As seen from Table 3, the carbon  
285 recovery was approximately 90%.

286 [TABLE 3 NEAR HERE]

287

#### 288 3.1 Open circuit MFC

289 To isolate and to study the fermentative processes taking place in the H-type MFC, an  
290 open circuit experiment was performed. Working in this way, the glucose was  
291 fermented but not oxidized by electroactive microorganisms. During the operation, the  
292 liquid bulk of the anodic chamber was analyzed, and the concentrations of the main  
293 compounds were determined. The experimental results obtained are presented in  
294 Figure 3.

295 [FIGURE 3 NEAR HERE]

296 It can be seen in Figure 3 that glucose was fully consumed after approximately 15 d.

297 **Regarding the glucose consumption, it must be noted that a reduction in pH was**  
298 **observed from neutrality to pH values near 5.5, which indicates the existence of the**  
299 **fermentation process.** Several fermentation products appeared as a result of the  
300 fermentation process. The main fermentation products were ethanol and lactic acid,  
301 accounting for more than 95% of the fermentation products generated. In addition,  
302 acetic and formic acids appeared in trace concentrations (data not shown). The

303 maximum concentrations of ethanol and lactic acids reached approximately 6.7 and  
304 3.4 g COD L<sup>-1</sup>, respectively.

305 With the aim of using this information for the description of the processes taking place  
306 in the closed circuit H-type MFC, the model equations corresponding to the glucose  
307 fermentation processes were fitted to the experimental data. From the fitting, the  
308 main kinetics and stoichiometric parameters were determined. The yields of the main  
309 fermentation products, ethanol and lactic acid, were 0.88 and 0.68 g COD per g COD of  
310 glucose consumed, respectively, and the maximum specific uptake rates were 2.3 g  
311 COD (g COD d)<sup>-1</sup> and 1.2 g COD (g COD d)<sup>-1</sup> for ethanol and lactic acid, respectively. The  
312 glucose half-saturation coefficient ( $K_s$ ) was 1.93 g COD L<sup>-1</sup> in both cases. As seen in  
313 Figure 3, the model accurately predicts the substrate and product concentrations along  
314 the fermentation experiment.

315 The equations proposed and the parameter values obtained were subsequently used  
316 to fit the closed circuit experimental data set obtained when glucose was used for the  
317 electricity generation.

318

### 319 3.2 Closed circuit MFC

320 With the aim to studying the fermentative and electrogenic processes simultaneously  
321 occurring in the H-type MFC, a closed circuit experiment with glucose and feedstock  
322 was performed. During the experiment, the patterns of change of the main variables  
323 were determined.

#### 324 3.2.1 Power generation and theoretical COD removal

325 Before the inoculation, the anodic chamber of the H-type MFC was operated with the  
326 sterilized wastewater in the absence of biocatalyst for a period of 2 d. During this

327 period, the electricity generation was negligible. Subsequently, the H-type MFC was  
328 seeded. The H-type MFC was continuously operated in a batch mode for three months.  
329 The experimental data presented in Figure 4 illustrate the voltage generation of the H-  
330 type MFC and the theoretical COD removal rate in the liquid bulk.

331 [FIGURE 4 NEAR HERE]

332 As seen in Figure 4, the electricity generation was proportional to the theoretical COD  
333 removal rate in the system. In this figure, several sections can be identified. Initially,  
334 the system presented an exponential increase of the voltage, which could be explained  
335 because of the high consumption rate of the substrates by the microorganisms in the  
336 anodic chamber. A maximum voltage output of 12.0 mV was observed after 15 d of the  
337 start-up, which corresponds to a CE of approximately 1.5%.

338 After achieving the maximum in the voltage exerted, the voltage gradually dropped to  
339 approximately 6.0 mV. Over more than 40 d, the voltage was maintained at  
340 approximately 6.0 mV, with an average CE of approximately 2.5%. This result could be  
341 explained because of the almost constant COD consumption rate of approximately 0.3  
342 g COD (g COD d)<sup>-1</sup>. The low CE can be explained by a very high glucose concentration  
343 (9.6 g COD L<sup>-1</sup>) in the anodic chamber. This finding could be related to the fact that the  
344 conventional anaerobic organisms outcompete the electrogenic ones when working at  
345 very high glucose concentrations. In the literature (Velasquez-Orta et al., 2011), similar  
346 results were reported for studies working with high glucose concentrations. Finally,  
347 after approximately 70 d of operation, the voltage decreased again to approximately  
348 0.5 mV, which may be related to endogenous electricity generation.

349 *3.2.2 Substrate transformation*



350 Regarding the use of glucose as a fuel, it is remarked that the slight pH reduction  
351 observed (from 7 to approximately 6) and the long-lasting voltage generation over  
352 more than 100 d (even when the glucose was exhausted after only 20 d) indicated that  
353 the glucose added to the MFC was transformed in the anodic chamber. Because of the  
354 importance of the fermentation process in the MFC (Lee et al., 2008), the glucose  
355 concentration and the fermentation product concentrations were monitored (see  
356 Figure 5). In Figure 5, it can be seen that the MFC performed fermentation apart from  
357 power generation.

358 [FIGURE 5 NEAR HERE]

359 During the first 20 d of the operation of the closed circuit MFC, the main  
360 transformations can be explained because of the glucose fermentation to ethanol and  
361 lactic acid, as occurred in the open circuit MFC.

362 As seen in Figure 5, the glucose was consumed in approximately 20 d. During this  
363 period, several fermentation products appeared in the anodic liquid bulk, with ethanol  
364 and lactic acid being the most relevant, but acetic and formic acids also appeared,  
365 similar to the open circuit MFC. Considering the comparison of the production of the  
366 fermentation products in the open and closed circuit MFCs, the existence of a lag  
367 phase in the lactic acid production under closed circuit conditions is remarkable. To  
368 account for this difference, a switch function in the lactic acid production rate (see  
369 process B2 in Table 2) was included. Furthermore, a slightly higher formic acid  
370 production must be noted when working under closed circuit conditions. In the closed  
371 circuit, formic acid was generated at a rate of approximately  $0.03 \text{ g COD (g COD d)}^{-1}$   
372 and with a yield of 0.08 mol COD of formic acid per mol COD of glucose. This higher  
373 production could be explained because of the higher pH in the bulk liquid when

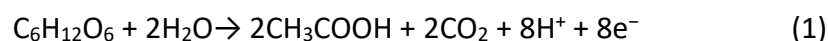
374 operating under closed circuit conditions. In the literature (Temudo et al., 2007), it has  
375 been reported that formic acid production is favored at high pH values. The higher pH  
376 values observed under closed circuit conditions could be explained by the acid  
377 oxidation by electrogenic organisms and proton consumption in the cathodic  
378 compartment of the MFC.

379 In principle, because the fermentation process takes place in the anodic chamber,  
380 some of the electricity generation during the first 20 d of the experiment could be  
381 explained because of the electrogenic fermentation of glucose (Catal et al., 2011). This  
382 event could be possible in an MFC because there are different fermentation pathways  
383 that could be divided into two groups, including the electricity generation processes  
384 (Reactions (1-3)) and the conventional fermentation processes (Fang & Liu, 2002; Fang  
385 et al., 2002). The latter processes do not generate electricity (Reactions (4-8)).

386

***Electrogenic fermentative processes (EFP)***

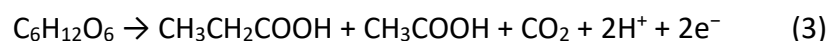
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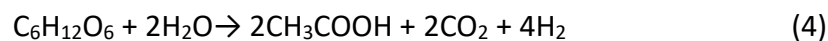


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***Non-electrogenic fermentative processes (NEFP)***

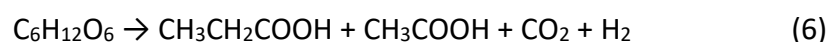
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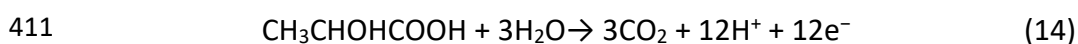
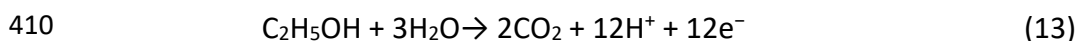
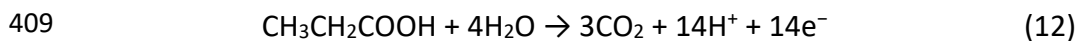
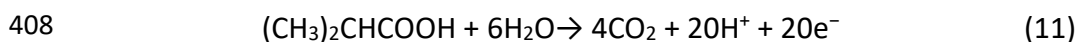
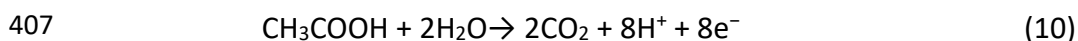


397

398 Additionally, the electricity generation in the MFC could also be due to the direct  
399 conversion of glucose to CO<sub>2</sub> (Reaction (9)) and the conversion of the fermentation  
400 products generated to CO<sub>2</sub> (Reactions (10-14)). The sum of all of these contributions  
401 could explain why the peak in the electricity generation was reached at 15 d after the  
402 start-up of the MFC. After that peak, the glucose was exhausted; therefore, only the  
403 fermentation products could have been used for electricity generation.

404

405 ***Electrogenic oxidative processes (EOP)***



412

413 Taking the above reactions into account, the voltage produced during the first stage  
414 (see Figure 4) could be related to the electrogenic fermentation of glucose, the non-  
415 electrogenic glucose fermentation and the subsequent electrogenic oxidation of the  
416 fermentation products, or it could be due to the electrogenic oxidation of glucose to  
417 CO<sub>2</sub>.

418 In this work, it is important to note that the main fermentation products accumulated  
419 in the liquid bulk of both MFCs (open and closed circuit), corresponding to those not  
420 generated by means of the electricity generation processes (reactions 7-8). There are

421 two possible explanations for this finding, including a negligible contribution of these  
422 electricity generating processes in the closed circuit MFC or a quick consumption of the  
423 fermentation products generated by means of the electrogenic fermentative  
424 processes. In this work, the latter explanation was refused based on the conclusions  
425 reported in the literature when fermenting monosaccharides in MFCs (Catal et al.,  
426 2011).

427 During the second stage (see Figure 4), the main reaction generating electricity was  
428 the oxidation of the fermentation products previously generated, particularly ethanol,  
429 which was the main fermentation product consumed. In this work, lactic acid was not  
430 consumed, which is in accordance with Thurston (1985), who reported the generation  
431 of lactic acid in a MFC without further utilization to generate electricity under  
432 anaerobic conditions (Thurston et al., 1985).

433 Finally, the third stage (see Figure 4), which is characterized by a very low voltage  
434 generation, could be explained due to the oxidation of traces of SCFA presented in the  
435 liquid bulk or to endogenous electricity generation. However, taking the almost  
436 negligible soluble COD removal rate into account, the most probable explanation is  
437 endogenous electricity generation.

438 Because of the simultaneous generation and consumption of ethanol in the closed  
439 circuit MFC, it was considered interesting to uncouple the generation/consumption of  
440 ethanol and determine the kinetics and stoichiometric parameters for ethanol  
441 consumption in a specific experiment. Hence, an additional experiment was performed  
442 in an identical H-type MFC fed with ethanol at 6.0 g COD L<sup>-1</sup>. In this experiment, the  
443 ethanol consumption and the electricity generation were studied, and the results are  
444 presented in Figure 6.



445 [FIGURE 6 NEAR HERE]

446 It can be seen in Figure 6 that the system started to degrade the ethanol and produce  
447 electricity after a short acclimatization period. The ethanol degradation rate was  
448 almost constant over the course of the experiment. Once the electricity generation  
449 started, the voltage generated increased, reaching a maximum voltage generation of  
450 approximately 7 mV. This voltage generation was maintained for approximately 70 d  
451 and then decreased, reaching a new steady state at approximately 1 mV. In the  
452 absence of substrate, this result can only be explained by endogenous electrogenic  
453 generation.

454 With the aim of determining the main kinetics and stoichiometric parameters of the  
455 electrogenic ethanol oxidation, the equations describing the ethanol consumption and  
456 the coupled electricity generation (see process E2 in Table 1) were fitted to the  
457 experimental results, with the ethanol degradation rate being  $0.3 \text{ g COD (g COD d)}^{-1}$   
458 and the half-saturation coefficient being  $0.96 \text{ g COD L}^{-1}$ . The  $K_S$  value obtained is very  
459 similar to that referenced in the literature (Kim et al., 2007). The equations proposed,  
460 as well as the parameter values obtained, were subsequently used for fitting the  
461 closed circuit experimental data obtained when glucose was used for the electricity  
462 generation.

463 Once the kinetics and stoichiometric parameters of the fermentation, as well as those  
464 of the ethanol oxidation and the associated electricity production, were determined,  
465 all of the equations of the MFC model were used to simulate the experimental data set  
466 obtained in the experiment performed with glucose in the closed circuit H-type MFC.  
467 The kinetics and stoichiometric parameters obtained through the fermentation and in  
468 the ethanol electrogenic oxidation tests were used in the model. The obtained results



469 accurately predict the evolution of the substrate, the fermentation product profiles  
470 and the electricity production in the H-type MFC, as seen from the model predictions  
471 and the actual data presented in Figures 5 and 6. The values of the main kinetics and  
472 stoichiometric parameters obtained from the fitting are summarized in Table 4. It was  
473 only necessary to include a small amount of glucose oxidation to accurately fit the  
474 electricity production.

475 From the obtained results, it is interesting to note the almost negligible oxidation rate  
476 of glucose by anodophilic organisms:  $0.03 \text{ g COD (g COD d)}^{-1}$ . A possible explanation is  
477 that the direct anodic oxidation of glucose by pure electrogenic cultures, which has  
478 been previously observed (Chaudhuri & Lovley, 2003), may not be the most important  
479 pathway when working with mixed cultures (Freguia et al., 2008). This phenomenon  
480 could be due to the very low concentration of electrogenic microorganisms in the  
481 mixed culture. Another possible explanation is the lower affinity and lower maximum  
482 consumption rate of the substrate by the electrogenic microorganisms compared with  
483 the conventional ones. This notion was confirmed when comparing the carbon  
484 consumption rate in the fermentation process with the electrogenic ethanol and  
485 glucose oxidation. The carbon consumption rate during the glucose fermentation  
486 process was approximately  $16 \text{ mmol C (L d)}^{-1}$ , whereas the carbon consumption rate  
487 during the electrogenic ethanol and glucose consumption was ten times lower  
488 (approximately  $1.5 \text{ mmol C (L d)}^{-1}$ ).

489 The biomass growth in the system was fitted using an endogenous decay rate of  $0.02$   
490  $\text{d}^{-1}$  and a biomass yield of  $0.07 \text{ g COD (g COD)}^{-1}$  (Romli et al., 1995). According to  
491 theoretical values, the oxidative aerobic biomass growth accounted for approximately  
492 40% of the carbon consumption, and the anaerobic one accounted for approximately

493 5%. The value proposed in this work corresponds to a weighted value between both  
494 processes, i.e., the anaerobic one (fermentative) and the oxidative one (electrogenic).  
495 Similar values for the biomass growth in MFCs have been observed in previous studies  
496 (Kim et al., 2011).

497 In the closed systems fed with glucose or ethanol, the electrons transported through  
498 the circuit represented a small portion, ranging from 1 to 3%. **This finding could be  
499 explained by the loss of MFC electrons by overpotential, ohmic resistance and the  
500 inefficient oxidation of fermentation products and glucose.**

501 [TABLE 4 NEAR HERE]

502

### 503 *3.3 Model validation*

504 Once the calibration procedure was finished, the developed model was compared and  
505 validated using data from a new experiment. During the validation, a satisfactory  
506 agreement was obtained between the measured and predicted values. The goodness  
507 of fit was determined by the Mean Relative Squared Error (MRSE) criterion. The MRSE  
508 values obtained during the calibration and validation stages are presented in Table 5.

509 The obtained results show that the model accuracy was maintained during the  
510 validation stage, with a MRSE value of 12.3%, a value very similar to that obtained  
511 during the calibration of the model.

512 [TABLE 5 NEAR HERE]

513

## 514 **CONCLUSIONS**

515

516 In this work, a model describing the evolution of a MFC fed with high glucose  
517 concentrations was developed. Comparing open and closed circuit operation of the  
518 MFC, similar reaction extensions and product distributions of the fermentation process  
519 were observed. From the obtained results, the importance of the fermentation process  
520 in electricity production was shown by the high ethanol consumption rate by  
521 electrogenic organisms ( $0.3 \text{ g COD (g COD d)}^{-1}$ ) compared with that of the glucose ( $0.03$   
522  $\text{g COD (g COD d)}^{-1}$ ). In terms of electricity generation, ethanol contributed to  
523 approximately 90% the production, whereas glucose accounted for only 10% of the  
524 production.

525

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529

530

531



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Table 1. Petersen matrix, containing the main processes taking place in the microbial fuel cell.

Table 2. Parameters used in the MFC model.

Table 3. Comparison of carbon and electron balances amongst glucose feed (open and closed circuits) after completion of anodic reactions.

Table 4. Main parameter values obtained from the model fitting to the MFC performance.

Table 5. Calibration and validation MRSE values.

Process \ Components	1 Glucose	2 Ethanol	3 Lactic	4 Acetic	5 Butyric	6 Formic	7 H <sub>2</sub>	8 IC	9 Electricity	10 Process rate
<i>Biological conversion</i>										
(B1) Ethanol production	-1	$(1 - Y_b)f_{e,g}$						$-\sum C_i v_{i,B1}$		$k_{G,E} \frac{S_G}{K_{S_G} + S_G} X$
(B2) Lactic production	-1		$(1 - Y_b)f_{l,g}$							$k_{G,L} \frac{S_G}{K_{S_G} + S_G} X \left[ \exp\left(\frac{K_G - S_G}{K_{S_G}} I_X\right) \right]$
(B3) Acetic production	-1			$(1 - Y_b)f_{a,g}$			$(1 - Y_b)f_{H2a,g}$	$-\sum C_i v_{i,B3}$		$k_{G,AC} \frac{S_G}{K_{S_G} + S_G} X$
(B4) Butyric production	-1				$(1 - Y_b)f_{b,g}$		$(1 - Y_b)f_{H2b,g}$	$-\sum C_i v_{i,B4}$		$k_{G,B} \frac{S_G}{K_{S_G} + S_G} X$
(B5) Formic production						$(1 - Y_b)f_{f,IC}$	-1	$-\sum C_i v_{i,B5}$		$K_{IC,E} \frac{S_{IC}}{K_{S_{IC}} + S_{IC}} X$
<i>Electrochemical conversion</i>										
(E1) Electrogenic glucose oxidation	-1							$-\sum C_i v_{i,E1}$	$(1 - Y_b)\gamma_{e^-,COD}$	$k_{G,e^-} \frac{S_G}{K_{S_G} + S_G} \frac{S_{Mox}}{K_{Mox} + S_{Mox}} X$
(E2) Electrogenic ethanol oxidation		-1						$-\sum C_i v_{i,E2}$	$(1 - Y_b)\gamma_{e^-,COD}$	$k_{E,e^-} \frac{S_E}{K_{S_E} + S_E} \frac{S_{Mox}}{K_{Mox} + S_{Mox}} X$



Parameter	Description	Unit
<b>Biological Conversion</b>		
$S_G$	Glucose concentration	g COD · L <sup>-1</sup>
$S_E$	Ethanol concentration	g COD · L <sup>-1</sup>
$S_{Max}$	Oxidised mediator concentration	g COD · L <sup>-1</sup>
$X$	Biomass concentration	g COD · L <sup>-1</sup>
$Y_b$	Biomass yield coefficient	g COD Biomass · (g COD glucose) <sup>-1</sup>
$f_{product, substrate}$	Catabolic yield of product on substrate	g COD product · (g COD substrate) <sup>-1</sup>
$k_{G,E}$	Glucose to Ethanol fermentation rate	g COD Glucose · (g COD biomass·d) <sup>-1</sup>
$k_{G,L}$	Glucose to Lactic acid fermentation rate	g COD Glucose · (g COD biomass·d) <sup>-1</sup>
$k_{G,Ac}$	Glucose to Acetic acid fermentation rate	g COD Glucose · (g COD biomass·d) <sup>-1</sup>
$k_{G,B}$	Glucose to Butyric acid fermentation rate	g COD Glucose · (g COD biomass·d) <sup>-1</sup>
$K_{S_G}$	Monod half-saturation coefficient for glucose	g COD Glucose · L <sup>-1</sup>
$K_{S_E}$	Monod half-saturation coefficient for ethanol	g COD Ethanol · L <sup>-1</sup>
$K_{Max}$	Monod half-saturation coefficient for oxidised mediator	g COD Oxidised mediator · L <sup>-1</sup>
$K_G$	Glucose switching constant	g COD Glucose · L <sup>-1</sup>
$C_i$	Carbon content of component i	mole C · g COD <sup>-1</sup>
$v_i$	Rate coefficient for component I on process j	g COD · m <sup>-3</sup>
$I_X$	Open-closed circuit Boolean switching function	
<b>Electrochemical Conversion</b>		
$k_{G,e^-}$	Electrogenic Glucose oxidation rate	g COD Glucose · (g COD biomass·d) <sup>-1</sup>
$k_{E,e^-}$	Electrogenic Ethanol oxidation rate	g COD Ethanol · (g COD biomass·d) <sup>-1</sup>
$\gamma_{COD}^-$	Electricity generation from COD oxidation	12060 Coulombs · g COD <sup>-1</sup>



	Glucose open circuit		Glucose closed circuit	
	Carbon Balance		Carbon Balance	
	C mmol	Fraction %	C mmol	Fraction %
<b>Amount Added</b>				
Glucose	268.09	100.00	257.25	100.00
<b>Final recovery</b>				
Ethanol	95.20	35.51	0.00	0.00
Lactic acid	69.30	25.85	69.51	27.02
Formic acid	1.40	0.52	4.20	1.63
Acetic acid	0.35	0.13	0.56	0.22
Butyric acid	0.00	0.00	0.03	0.01
Carbon dioxide	45.85	17.10	139.37	54.18
Biomass growth	20.20	7.53	18.34	7.13
<b>Total recovery</b>	<b>232.30</b>	<b>86.65</b>	<b>232.01</b>	<b>90.19</b>



Parameter	Value	Parameter	Value	Parameter	Value
<b>Biological Conversion</b>			<b>Electrochemical Conversion</b>		
$Y_b$	0.07 g COD · (g COD) <sup>-1</sup>	$f_{H2b,g}$	0.17 g COD · (g COD) <sup>-1</sup>	$k_{m,Ee^-}$	0.3 g COD · (g COD·d) <sup>-1</sup>
$f_{e,g}$	0.88 g COD · (g COD) <sup>-1</sup>	$k_{G,E}$	2.67 g COD · (g COD·d) <sup>-1</sup>	$k_{m,Ge^-}$	0.03 g COD · (g COD·d) <sup>-1</sup>
$f_{l,g}$	0.68 g COD · (g COD) <sup>-1</sup>	$k_{G,L}$	1.72 g COD · (g COD·d) <sup>-1</sup>		
$f_{a,g}$	0.50 g COD · (g COD) <sup>-1</sup>	$K_{S_G}$	1.9 g COD Glucose · l <sup>-1</sup>		
$f_{H2a,g}$	0.50 g COD · (g COD) <sup>-1</sup>	$K_{S_E}$	1.0 g COD Ethanol · l <sup>-1</sup>		
$f_{b,g}$	0.83 g COD · (g COD) <sup>-1</sup>	$K_G$	5.8 g COD Glucose · l <sup>-1</sup>		



	Open circuit MFC	Ethanol MFC	Closed circuit MFC
Calibration data	15.1	21.5	10.5
Validation data			12.3

Figure 1. A concept of the bio-electro-chemical model based on the glucose fermentation, supplemented with the glucose and ethanol oxidation with electron-transfer to the electrode.

Figure 2. Schematic view of the H-type MFC set-up.

Figure 3. Performance of the H-type MFC under open circuit conditions (Solid lines correspond to model predictions).

Figure 4. Voltage generation and theoretical COD consumption rate in the H-type MFC fed with glucose (Solid lines correspond to voltage prediction).

Figure 5. Substrate and main products profiles during the closed circuit experiment (Solid lines correspond to model predictions).

Figure 6. Electricity generation and ethanol concentration profile in the MFC feed with ethanol (Solid lines correspond to model predictions).

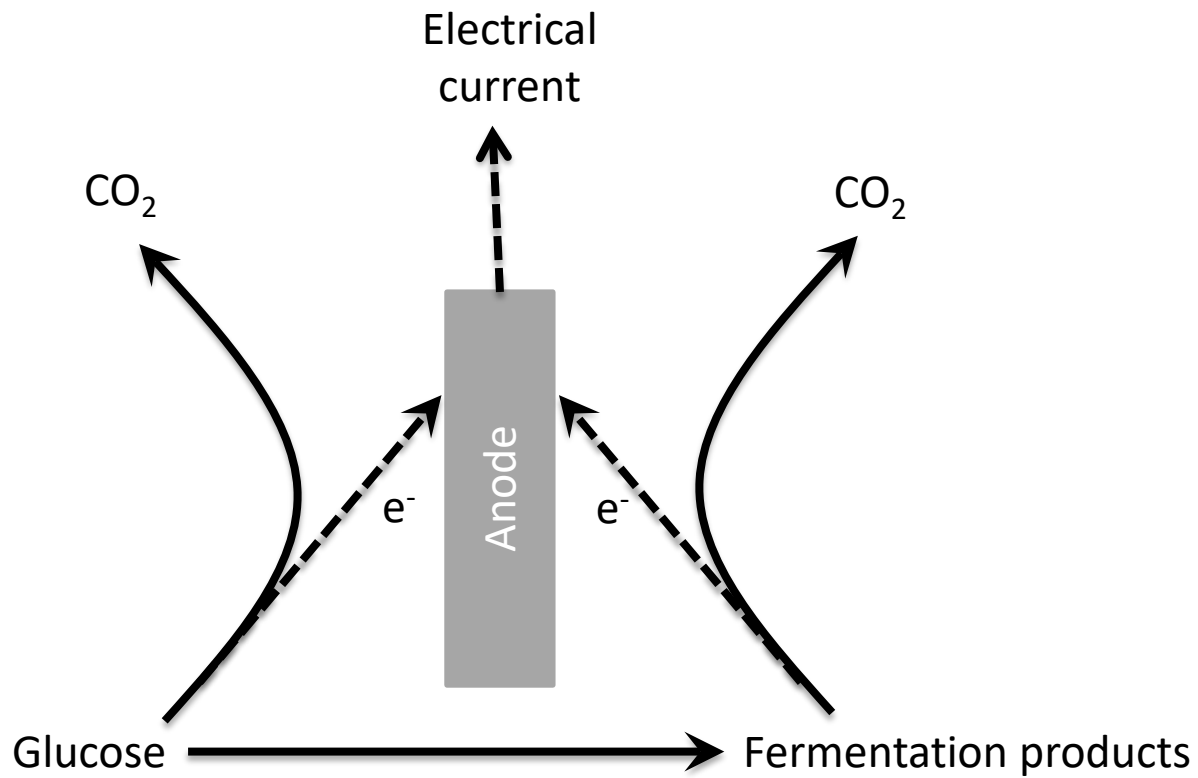


Figure 1. A concept of the bio-electro-chemical model based on the glucose fermentation, supplemented with the glucose and ethanol oxidation with electron-transfer to the electrode.

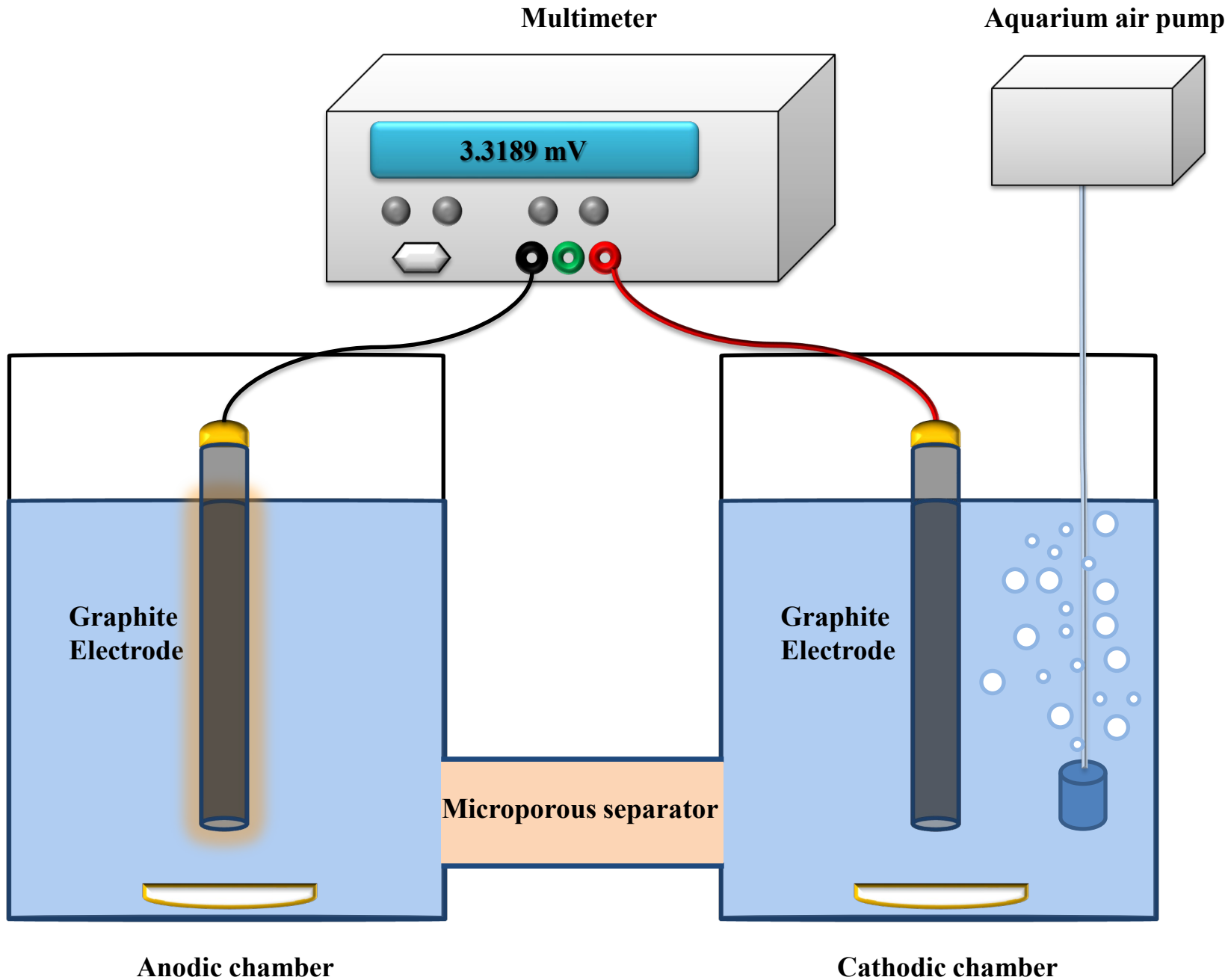


Figure 2. Schematic view of the H-type MFC set-up.



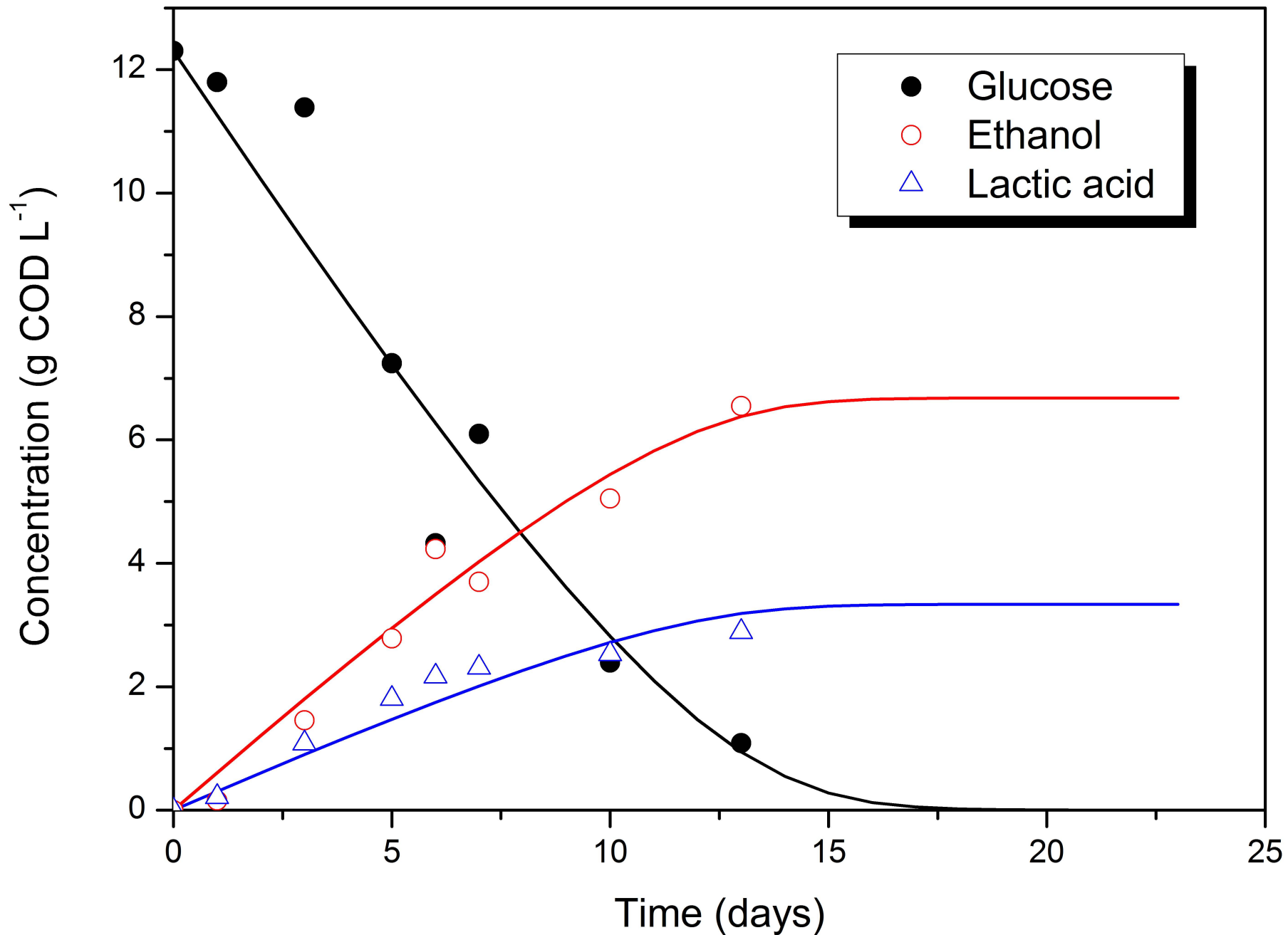


Figure 3. Performance of the H-type MFC under open circuit conditions (Solid lines correspond to model predictions).

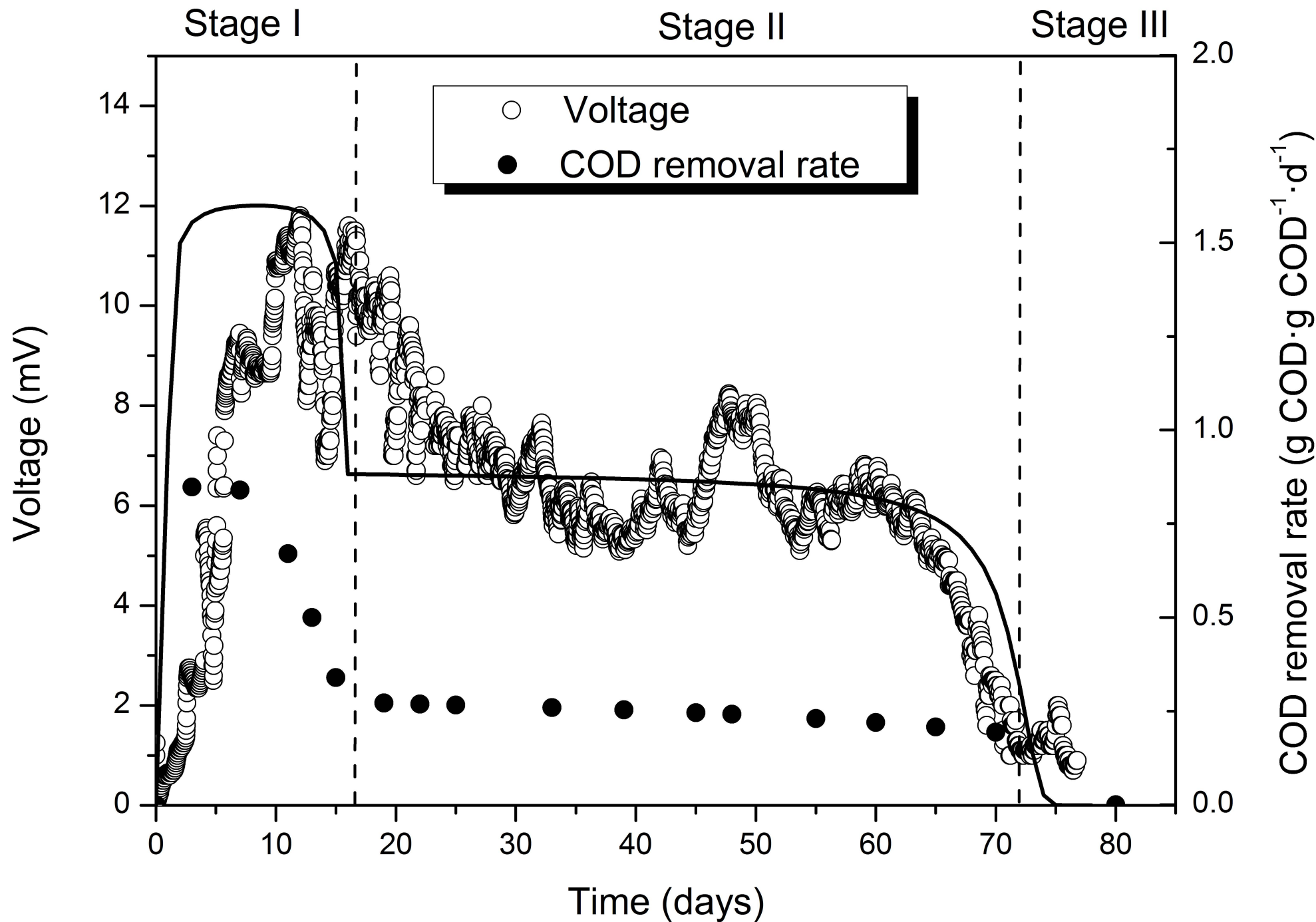


Figure 4. Voltage generation and theoretical COD consumption rate in the H-type MFC fed with glucose (Solid lines correspond to voltage prediction).

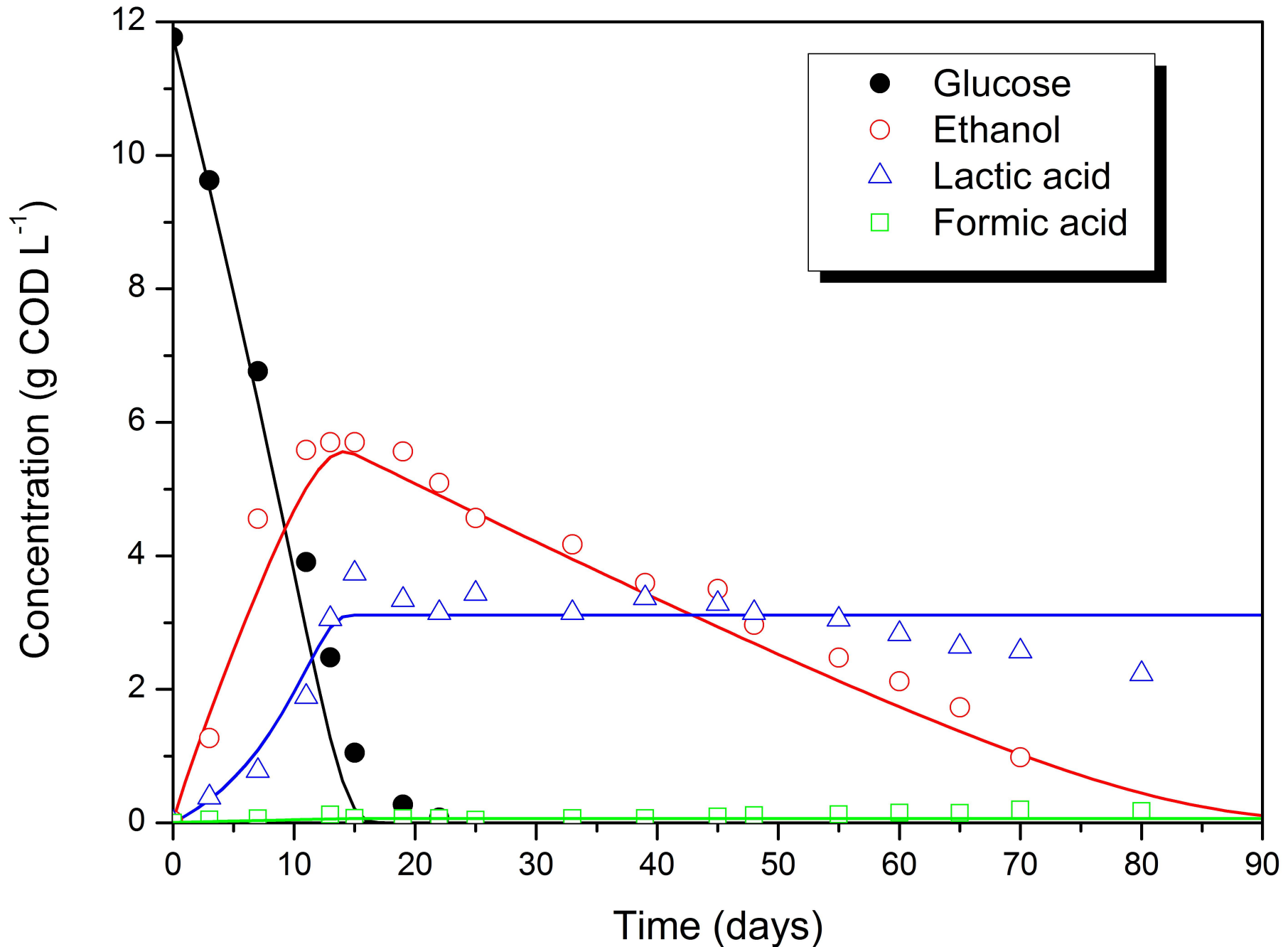


Figure 5. Substrate and main products profiles during the closed circuit experiment (Solid lines correspond to model predictions).