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

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Abstract

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

Keywords: Microbial fuel cell; glucose; fermentation; ethanol; modelling.

1. Introduction

Due to the increasing energy demands and environmental concerns, the interest in the development of renewable energy sources, alternative to fossil fuels, has increased during last years (MacKie et al., 2013). At the same time, more stringent environmental requirements for the wastewater treatments are pressing for the development of more efficient treatment techniques. This is not only an environmental concern, but also an economical issue, because water and wastewater systems are significant energy consumers. For example, an estimated 3-4% of the U.S. electricity consumption is consumed by the water and wastewater industry (Chandrappa & Das, 2014). In such a scenario, a cost reduction of the treatments is compulsory. Currently, the most important operational costs in a conventional wastewater treatment plant (WWTP) is the aeration (Fernández et al., 2011). Because of that, the development of a process allowing to oxidize the pollutants with lower aeration requirements is of crucial importance.

Nowadays, the most adequate technology to solve the combined energetic and environmental problem seems to be the bioelectrochemical technology. Because of that, bioelectrochemical systems, including Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs), have been investigated as alternative energy sources and wastewater polishing systems (Brillas & Martínez-Huitle, 2015; Ding et al., 2015).

The MFCs are electrochemical devices that can directly convert organic and inorganic substrates into electricity by means of a microbial culture. MFCs mimic a biological system, being the only difference that they do not transfer the electrons directly to the electron acceptor. Instead, the MFC anodophilic bacteria transfer the electron to a solid electrode as part of their respiration pathway. Then the electron is externally

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

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

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

The main drawback of the use of glucose as a fuel in the MFC is that glucose rich streams gave rise to a small current when they are used as fuel in the MFC (Kim et al., 2007; Lee et al., 2008). This could be explained because glucose is a complex substrate and can be used as substrate in a widespread number of non-electrogenic anaerobic processes (Freguia et al., 2008).

During the operation of MFCs, multiple/parallel bio-electrochemical reactions take place. One of these simultaneous processes is fermentation. Several MFC studies have shown that fermentation and anode respiration processes are combined very often (Freguia et al., 2008). Fermentative bacteria are able to ferment complex organic substrates to short-chain fatty acids (SCFA), alcohols and other fermentation products. All these products could subsequently be oxidized to produce electricity (Kim et al., 2007; Torres et al., 2007). The feasibility of integrating both processes in the anodic chamber may have a positive influence on the overall wastewater treatment and the electricity generation efficiency. Therefore, the description of the fermentation process at the anodic chamber of MFCs is of great interest.

In this context, the objectives of this study were to evaluate the performance of a MFC fed with glucose, to study the influence of the fermentation process, and the syntrophic oxidation of the fermentation products generated on the performance of the MFC.

2. Experimental

2.1 MFC Model definition

In order to clarify the glucose transformations in the MFC, the fermentation process and the subsequent oxidation of the fermentation products were modelled. The model definition was based on experimental observations when studying the fermentative and subsequent oxidative processes in the H-type MFCs used in this work. The equations involving the soluble compounds participating in the fermentative and oxidative processes were based on ADM1 model (Batstone et al., 2002), these equations are presented in the form of the Petersen matrix in Table 1 and the parameters of the model are presented in Table 2. All the kinetic expressions are based on the classical, commonly used Monod terms (Monod, 1949).

[TABLE 1 NEAR HERE]

[TABLE 2 NEAR HERE]

In the developed model, the reactions proposed were the glucose fermentation to fermentation products, via non electrogenic fermentation processes, and the oxidation of glucose and fermentation products to produce electricity. The fermentation was considered to be non-electrogenic because the main fermentation products accumulated in the liquid bulk of both MFCs, open and closed circuit, correspond to those not generated by means of the electricity generation processes. On the other hand, based on the experimental results, the main electrogenic reactions were the electrogenic oxidation of glucose and the main fermentation product, ethanol, to CO₂. When the anodic and cathodic chambers were connected via the external electrical circuit, closed circuit, environmental conditions for electrogenic organisms metabolisms were made. In contrast, the fermentative processes, rather than

electrogenic ones, might be the predominant processes when working under open circuit conditions. Through the comparison of both (closed and open) circuit MFCs, the fermentation and the electrogenic metabolisms were discriminated.

Taking into account the similarities observed when working with the open and closed circuit MFCs, the fermentation equations proposed in the open circuit MFC were also used for the closed circuit MFC. The only difference was the existence of a lag phase when fermenting glucose to lactic acid under closed circuit conditions but not under open circuit conditions. In order to describe both behaviors, a switching function in the lactic acid production rate was included (see process B2 in Table 2). The different behavior of the closed circuit MFC could be explained because of a switch ability of the microorganisms between the glucose oxidation with electricity production and the lactic acid generation from the glucose fermentation. The switching function was based on an inhibition function proposed in literature (Edwards, 1970). In this function, an additional term called I_x was included. This term is a Boolean function, being its values 0 for open circuit and 1 for closed circuit. The inclusion of this term allowed to describe the differences between the fermentation under open or closed circuit.

Additionally, the glucose and fermentation products generated could be oxidized to carbon dioxide generating electricity. The equations describing these processes in the model are processes E1 and E2 (Table 2). A reaction-scheme of the proposed model is depicted in Figure 1.

FIGURE 1 NEAR HERE

Neither hydrogen production by fermenters nor methane production by methanogens were included in the developed model. This is because neither methane nor hydrogen were detected in the gas phase of the H-type MFC. In the case of methane, this could

be explained because of the acidification of the anodic chamber which inhibits the activity of the methanogenic microorganisms (Fernández-Morales et al., 2010). Regarding to the hydrogen, its absence could be related to the seed used for the inoculation of the MFC. Acetic and formic acids only appeared in trace concentrations, in both open and close circuit experiments, and therefore were not included in the model.

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

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

2.2 MFC Design and configuration

Dual-compartment H-type MFCs were used. The MFCs held 0.7 L in each compartment and the compartments were separated by a tubular central compartment into which the separator was located (see Figure 2). The separator used in this work was a 5 cm thick microporous separator including 16 cm³ of compacted kaolin powder (particle diameter lower than 35 microns). This separator was supported by a glass fiber filter in

order to avoid disintegration due to the action of bulk liquid at both anodic and cathodic sides of the separator. The separator was designed in order to avoid the transport of microorganisms from the anodic to the cathodic chamber. The electrodes used in both the anode and the cathode were porous graphite rods (1 cm OD x 10 cm L) without any surface treatment or catalytic addition. Before the experiments, the electrodes were first soaked in deionized water for a period of 24 h. The submersible external surface of each electrode was 25,9 cm². The electrodes were placed at a distance of 20 cm on either side of the MFC. Cooper wires and a 120-Ohms resistor were used as a contact from electrodes. The cathode was continuously aerated by using an aquarium air pump. During the experiments, the air pump supplied 6 L min⁻¹ of air at 1.5 atmosphere of pressure to the cathodic chamber.

Three identical H-type MFCs were used to study different processes including electricity production from glucose, electricity production from the main fermentation product, ethanol, and the glucose fermentation process. In the latter case, the fermentation process was isolated from the electricity production process by disconnecting the external electrical circuit.

[FIGURE 2 NEAR HERE]

2.3 MFC operation

The anodic chamber of the MFCs were fed with a medium solution containing the organic substrate, 9 g L of glucose or 3.3 g L of ethanol depending on the test, and the following trace minerals (in g L⁻¹): (NH₄)Cl 3.0150; KH₂PO₄ 1.7550; NaCl 0.6570; Na₂SO₄ 0.1290; MgCl₂ 6H₂O 0.2700; EDTA 0.1125; ZnSO₄ 7H₂O 0.0072; FeSO₄ 7H₂O 0.0070; MnCl₂ 4H₂O 0.0056; CuCl 2H₂O 0.0050; CoCl₂ 6H₂O 0.0022; CaCl₂ 0.0014; NiCl₂ 6H₂O

0.0011; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ $0.225 \cdot 10^{-3}$; H_3BO_4 $0.225 \cdot 10^{-3}$. The carbon and mineral solution was sterilized in an autoclave at 110 °C for 20 min.

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

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

2.4 Analytical methods and calculations

Aqueous samples were collected from each MFC and then immediately centrifuged (12000 rpm) and filtered through a membrane filter of 0.45 μm . Once filtered, the samples were analyzed or preserved frozen according to the procedures described in the literature (Eaton et al., 2005).

Glucose concentration was measured by HPLC (Agilent) and refractive index detector (serie 1200). A Zorbax Carbohydrate Column (4.6 x 150 mm 5-micron) was used to separate the components at 35 °C using a mobile phase composed by 75% acetonitrile 25% water v/v and a flow rate of $1.5 \text{ cm}^3 \text{ min}^{-1}$. Furthermore, lactic acid was determined from centrifuged and filtered samples by HPLC (Agilent) equipped with UV-DAD and Zorbax SB-Aq (4.6 x 150 mm 5-micron). A mobile phase was a buffer pH 2

(0.05 M phosphate). SCFA (acetic, propionic and butyric) and ethanol were determined from a centrifuged (12000 rpm) and filtered sample (membrane of 0.45 µm) by gas chromatography (Perkin Elmer) with a flame ionization detector (FID) using a Crossbond Carbowax Column (15 m x 0.32 mm ID, 0.25 mm df). The initial temperature of the oven was 140 °C for 1.5 min and the temperature programmed at 25 °C min⁻¹ until 190 °C where it was kept for 2 min. The temperature of the injector and detector were 200 °C and 230 °C, respectively. Nitrogen was used as carrier gas. More information can be found elsewhere (Fernández-Morales et al., 2010; Infantes et al., 2011). pH values were determined by using a Crison GLP-22 pH probe (Crison Instruments S.A., Barcelona, Spain).

Bio-electrochemical calculations were carried out based on the procedures outlined in literature (Logan et al., 2006). Potential (V) measurements were recorded with an auto range digital multimeter (Model 2700, Keithley Instrument, OH, USA). Coulombic efficiency (CE), defined as the ratio of total coulombs actually transferred to the anode from the substrate, to maximum possible coulombs if all substrate removal produces the electrical current (Logan et al., 2006), was calculated by integrating the current over time, and taking into account the total Coulombs associated to the COD removed in the same period of time.

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

where M is the molecular weight of oxygen (32), I corresponds to the current intensity generated, F is Faraday's constant (96.485 C mol⁻¹ e⁻), b represents the number of

246 electrons exchanged per mole of COD removed (4), V is the volume of liquid in the
247 anode compartment (0.7 L), and ΔCOD depicts change in theoretical COD
248 concentration over the period of time. The COD concentrations were theoretically
249 calculated based on the substrates concentration in the liquid bulk.

3. Results

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

[TABLE 3 NEAR HERE]

3.1 Open circuit MFC

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

[FIGURE 3 NEAR HERE]

It can be seen in Figure 3 that glucose was fully consumed after about 15 d. Regarding to the glucose consumption it must be remarked the pH reduction observed from the neutral pH to pH values near 5.5 which indicates the existence of a fermentation process. Several fermentation products appeared as a result of the fermentation process. The main fermentation products were ethanol and lactic acid, accounting for more than 95% of the fermentation products generated. In addition, acetic and formic acids appeared in trace concentrations (data not shown). The maximum concentrations of ethanol and lactic acids reached approximately 6.7 and 3.4 g COD L⁻¹, respectively.

With the aim to use this information for the description of the processes taking place in the closed circuit H-type MFC, the model equations corresponding to the glucose fermentation processes were fitted to the experimental data. From the fitting, the main kinetic and stoichiometric parameters were determined. The yields of the main fermentation products, ethanol and lactic, were 0.88 and 0.68 g COD per g COD of glucose consumed respectively, being their maximum specific uptake rates 2.3 g COD (g COD d)⁻¹ and 1.2 g COD (g COD d)⁻¹ for ethanol and lactic acid, respectively. Glucose half-saturation coefficient (K_s) was 1.93 g COD L⁻¹ in both cases. As can be seen in Figure 3, the model accurately predicts the substrate and product concentrations along the fermentation experiment.

The equations proposed, as well as the parameter values obtained, were subsequently used for fitting of the closed circuit experimental data set obtained when glucose was used for the electricity generation.

3.2 Closed circuit MFC

With the aim to study the fermentative and electrogenic processes taking place simultaneously in the H-type MFC, a closed circuit experiment was performed feeding glucose. During the experiment, the evolutions of the main variables were determined.

3.2.1 Power generation and theoretical COD Removal

Before the inoculation, the anodic chamber of the H-type MFC was operated with the sterilized wastewater in the absence of biocatalyst for a period of 2 d. During this period, the electricity generation was negligible. Subsequently, the H-type MFC was seeded. The H-type MFC was continuously operated in a batch mode for three months.

The experimental data presented in Figure 4 illustrate the voltage generation of the H-type MFC and the theoretical COD removal rate in the liquid bulk.

[FIGURE 4 NEAR HERE]

As can be seen in Figure 4, the electricity generation was proportional to the theoretical COD removal rate in the system. In this figure, several sections can be identified. Initially, the system presented an exponential increase of the voltage which could be explained because of the high consumption rate of the substrates by the microorganisms in the anodic chamber. A maximum voltage output of 12.0 mV was observed after 15 d of the start-up, which corresponds to a CE of about 1.5%. After achieving the maximum in the voltage exerted, the voltage gradually dropped to about 6.0 mV. During more than 40 days the voltage was maintained in about 6.0 mV, presenting an average CE of about 2.5%. This could be explained because of the almost constant COD consumption rate of about 0.3 g COD (g COD d)⁻¹. The low CE can be explained by a very high glucose concentration (9.6 g COD L⁻¹) in the anodic chamber. This could be related to the fact that the conventional anaerobic organisms outcompete the electrogenic ones when working at very high glucose concentration. In the literature (Velasquez-Orta et al., 2011), similar results have been reported when working with high glucose concentrations. Finally, section III, after about 70 d of the operation, the voltage decreased again to about 0.5 mV, which could be related to the endogenous electricity generation.

3.2.2 Substrate transformation

Regarding to the glucose used as a fuel, it must be remarked that the slight pH reduction observed, from 7 to about 6, and the long-lasting voltage generation, along more than 100 d, even when the glucose was exhausted after only 20 d, indicated that

the glucose added to the MFC was transformed in the anodic chamber. Because of the importance of the fermentation process in the MFC (Lee et al., 2008), the glucose concentration and the fermentation product concentrations were monitored (see Figure 5). In this figure, it can be seen that the MFC performed fermentation apart from power generation.

[FIGURE 5 NEAR HERE]

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

As can be seen in Figure 5, glucose was consumed in approximately 20 d. During this period, several fermentation products appeared in the anodic liquid bulk, being the most relevant ethanol and lactic acid, but also appearing acetic and formic acids as occurred in the open circuit MFC. Comparing production of the fermentation products in the open and the closed circuit MFCs it must be remarked the existence of a lag phase in the lactic acid production under closed circuit conditions. In order to take into account this difference a switch function in the lactic acid production rate (see process B2 in Table 2) was included. Furthermore, a slightly higher formic acid production must be noted when working under closed circuit conditions. In the closed circuit, formic acid was generated at the rate of approximately $0.03 \text{ g COD (g COD d)}^{-1}$ and a yield of $0.08 \text{ mol COD of formic acid per mol COD of glucose}$. This higher production could be explained because of the higher pH in the liquid bulk when operating under closed circuit conditions. In the literature (Temudo et al., 2007), it has been reported that the formic acid production is favored at high pH values. The higher pH values observed under closed circuit conditions could be explained because of the acid oxidation by

electrogenic organisms and proton consumption in the cathodic compartment of the MFC.

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

Electrogenic fermentative processes (EFP)

Non electrogenic fermentative processes (NEFP)

Additionally, the electricity generation in the MFC could also be due to the direct conversion of glucose to CO_2 (Reaction (9)) and conversion of the fermentation products generated to CO_2 (Reactions (10-14)). The sum of all these contributions could

explain the peak in the electricity generation reached 15 d after the start-up of the MFC. After that peak, the glucose was exhausted, and therefore only the fermentation products could be used for electricity generation.

Electrogenic oxidative processes (EOP)

Taking the above reactions into account, the voltage produced during the first stage (see Figure 4), could be related to the electrogenic fermentation of glucose, the non-electrogenic glucose fermentation and the subsequent electrogenic oxidation of the fermentation products or due to the electrogenic oxidation of glucose to CO_2 .

In this work, it is important to remark that the main fermentation products accumulated in the liquid bulk of both MFCs, open and closed circuit, correspond to those not generated by means of the electricity generation processes (reactions (7-8)). This could be explained because of two reasons including a negligible contribution of these electricity generation processes in the closed circuit MFC or due to a quick consumption of the fermentation products generated by means of electrogenic fermentative processes. In this work, the latter statement was refused based on the

conclusions reported in the literature when fermenting monosaccharides in MFCs (Catal et al., 2011).

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

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

Because of the simultaneous generation and consumption of ethanol in the closed circuit MFC, it was considered interesting to uncouple the generation/consumption of ethanol and determine the kinetic and stoichiometric parameters for ethanol consumption in a specific experiment. Hence, an additional experiment was performed in an identical H-type MFC fed with ethanol 6.0 g COD L⁻¹. In this experiment, the ethanol consumption and the electricity generation were studied, and results are presented in Figure 6.

[FIGURE 6 NEAR HERE]

It can be seen in Figure 6 that the system started to degrade the ethanol and to produce electricity after a short acclimatization period. The ethanol degradation rate was almost constant along the experiment. Once started the electricity generation, the

voltage generated increased reaching a maximum voltage generation of approximately 7 mV. This voltage generation was maintained for approximately 70 d and then decreased reaching a new steady state at approximately 1 mV. In the absence of substrate, this can only be explained because of the endogenous electrogenic generation.

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

Once determined the kinetic and stoichiometric parameters of the fermentation as well as the ethanol oxidation and the associated electricity production, all the equations of the MFC model were used to simulate the experimental data set obtained in the experiment performed with glucose on the closed circuit H-type MFC. The kinetic and stoichiometric parameter obtained in the fermentation and in the ethanol electrogenic oxidation tests were used in the model. The results obtained accurately predicts the evolution of the substrate, fermentation products profiles and electricity production in the H-type MFC as can be seen from the model predictions and the actual data presented in Figures 5 and 6. The values of the main kinetic and stoichiometric parameters obtained from the fitting are summarised on Table 4, being

only necessary to include a small glucose oxidation to accurately fit the electricity production.

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

The biomass growth in the system was fitted using an endogenous decay rate of 0.02 d^{-1} and a biomass yield of $0.07 \text{ g COD (g COD)}^{-1}$ (Romli et al., 1995). According to theoretical values, the oxidative aerobic biomass growth account for approximately 40 % of the carbon consumption and the anaerobic one for approximately 5%. The value proposed in this work corresponds to a weighted value between both processes, i.e. the anaerobic one (fermentative) and the oxidative one (electrogenic). Similar values for the biomass growth in MFCs have been observed in previous studies (Kim et al., 2011).

In the closed system fed with glucose or ethanol, the electron transported through the circuit represented a low portion, ranging from 1-3%. This could be explained because of the loss of MFC electrons by overpotential, ohmic resistance and the inefficient oxidation of fermentation products as well as glucose.

[TABLE 4 NEAR HERE]

3.3 Model validation

Once finished the calibration procedure, the developed model was compared and validated using data from a new experiment. During the validation a satisfactory agreement was obtained between measured and predicted values. The goodness of the fit was determined by means of the Mean Relative Squared Error (MRSE) criterion. The MRSE values obtained during the calibration and validation stages are presented in Table 5. The obtained results show that the model accuracy was maintained during the validation stage, presenting a MRSE value of 12.3%, a value very similar to that obtained during the calibration of the model.

[TABLE 5 NEAR HERE]

CONCLUSIONS

- The model developed in this work was capable of predicting the evolution of the fermentation process as well as the electricity generation in the MFC fed with glucose.
- A comparison of the open and the closed circuit operation of the MFC revealed a similar extension and products distributions of the fermentation process, being the main difference the existence of a lag phase in the lactic acid production when working under closed circuit conditions.
- The importance of the fermentation process in the electricity production was shown by the high ethanol consumption rate by electrogenic organisms, 0.3 g COD (g COD d)⁻¹ compared with that of the glucose 0.03 g COD (g COD d)⁻¹.
- In terms of electricity generation, ethanol accounted for about 90% the production whereas glucose only for 10%. Because of that, the possibility of fermenting glucose to fermentation products, such as ethanol, presenting higher consumption rates and therefore electrical current, is considered crucial importance.

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