Long-term effects of the transient COD concentration on the performance of microbial fuel cells

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Abstract

In this work, the long-term effects of transient Chemical Oxygen Demands (COD) concentrations over the performance of a microbial fuel cell were studied. From the obtained results, it was observed that the repetitive change in the COD loading rate during 12 hours conditioned the behavior of the system during periods of up to 7 days. The main modifications were the enhancement of the COD consumption rate and the exerted current. These enhancements yielded increasing Coulombic Efficiencies (CEs) when working with COD concentrations of 300 mg/L, but constant CEs when working with COD concentrations from 900 to 1800 mg/L. This effect could be explained by the higher affinity for the substrate of *Geobacter* than that of the non-electrogenic organisms such as *Clostridia*.

Keywords: Microbial Fuel Cell; COD; long-term; electricity.

1. Introduction

The world population has experienced continuous growth during the last centuries. Current projections show a continuous increase of population up to approximately 9 billion inhabitants by the year 2050 [1]. This population should be supplied with two basic commodities: water and energy. Energy demands for conventional water and wastewater processes are a significant part of the problem in supplying these commodities; e.g., in the US, approximately 5% of the electricity consumption is due to the water treatment processes [2]. Renewable energy sources represent some of the most appropriate options to reach the sustainable energy production objective [3, 4]. In addition, the development of processes that can use microorganisms to produce electricity from wastes represents an interesting approach for bioenergy production [5]. On the one hand, microorganisms are not as selective and sensitive as the chemical catalysts, and hence, the spectrum and quality of the potential fuels to be used can be expanded with the use of microorganisms, relative to chemical catalysts. In addition, microorganisms are self-replicating, and consequently, the catalyst for the substrate oxidation is self-sustaining. On the other hand, wastewaters contains a significant amount of organic pollutants [6]. Unfortunately, the large number of constituents and dilute nature makes it very difficult to extract the chemical energy contained in waste [7]; hence, biotechnological processes that tend to function well under dilute circumstances represent an option for its energetic valorization.

Microbial Fuel Cells (MFCs) are electrochemical devices able to extract the chemical energy contained in a fuel by means of microbial metabolisms [8]. However, in spite of the importance of the influent COD concentration on the microbial metabolisms, little information can be found in the literature related to its influence on MFC performance. The COD concentration effects are of great importance in the practical application of MFCs in both the short term and

64 the long term, because the COD concentration profiles of the domestic wastewaters change
65 due to daily and seasonal changes [9, 10]. Domestic wastewaters are one of the main fuels used
66 in MFC because of its widespread availability and its COD content [11].
67 In the long term, the importance of the COD concentration profiles is related to the
68 composition of the microbial community. These changes are very important because full-scale
69 MFCs operate with mixed cultures, combining electrogenic microbes such as *Geobacter* and
70 *Shewanella* with non electrogenic microbes such as *Bacteroidia*, *Clostridia*, *Bacilli*,
71 *Lactobacillales*, etc. [12]. The use of mixed cultures is justified by the results of studies in which
72 mixed cultures were shown to have power densities that were similar to those observed in
73 pure culture experiments [13]. Moreover, the use of mixed cultures reduces the operating costs
74 and allows the system to use low-quality fuels, such as wastewaters [14].
75 Regarding the COD influence over the microbial population, on the one hand, the increase of
76 the influent COD concentration reduces the competition for the substrate, allowing for the
77 development of a wide spectrum of microorganisms. On the other hand, low COD
78 concentrations lead to a competition between microorganisms for the substrate, with only the
79 most adapted to the environmental conditions prevailing [15]. In this sense, the low COD
80 concentrations are more selective for the microbial population than the high COD
81 concentrations [12, 14]. In general, electrogenic organisms present lower growth rates. In the
82 particular case of *Geobacter*, it has been reported[16] that a maximum growth rate of 2.4 d^{-1} can
83 occur whereas non electrogenic microorganisms such as *Clostridia* presents maximum
84 specific growth rate of 3.3 d^{-1} [17, 18]. However, under substrate limitations *Geobacter* growth
85 was faster due to its higher substrate affinity [19].
86 These changes in the microbial populations in the MFC could affect not only the COD
87 removal rates but also the electrochemical performance of the MFC [20]. In the particular case

of the MFC, reporting the CE is sufficient to evaluate its performance as an electrochemical power system based on wastes as fuel [2].

The use of mixed cultures reduces the operational costs and enhances the adaptive capacity due to microbial diversity. These characteristics lead to an improved capacity to degrade mixed substrates and the possibility of continuous processing, which is quite interesting when operating with an MFC.

In this context, the goal of this work was to assess the long-term influence of a transient change of the influent organic concentration on the performance of MFC. To accomplish this goal, a MFC seeded with a mixed culture was used. Once started-up and acclimated, the MFC was subjected to transient COD concentrations, and then, the long-term effects on COD removal and current exerted were monitored. The results are discussed in light of the present knowledge of the technology, and they are of great interest because the operation of MFCs in actual application would be under these changing conditions.

2. Experimental Procedure

2.1 Experimental set-up

The experimental micro-scale set-up used in this work consisted of a two-chambered micro-scale MFC, as described elsewhere [21]. The anodic and cathodic electrodes were based on Toray carbon papers TGPH-120 (E-TEK, USA): the anodic electrode had a Teflon content of 20 %, and the cathodic one had a Teflon content of 10 %. In the cathode, a catalytic layer with 0.5 mg Pt/cm^2 loading was deposited onto a microporous layer. The anodic and cathodic chambers were built on a graphite plate. Both chambers were separated by a Sterion® membrane. The active area of the anodic chamber was 4.65 cm^2, and its volume was 0.95 cm^3. The active area of the cathodic chamber was 2.85 cm^2, and its volume was 0.50 cm^3. The membrane-electrode assembly was performed according to the literature [22]. An air breathing

cathode was used. Air-breathing systems use free convection airflow to supply oxygen to the cathode. This air breathing cathode is a very robust cathode, as reported in the literature [21]. A schematic view of the set-up is shown in Figure 1.

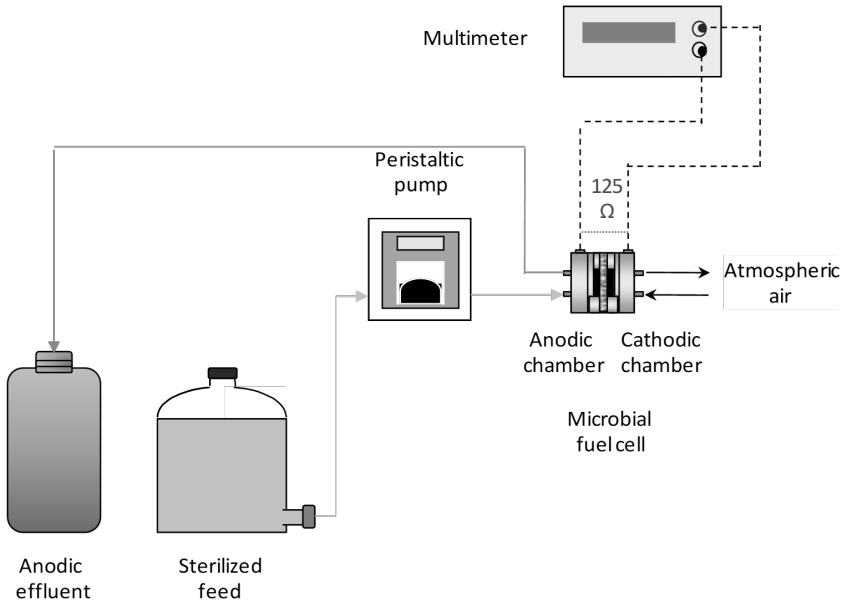


Figure 1. Schematic view of the set-up.

The MFC was operated in continuous mode. The anodic compartment of the MFC was fed from a wastewater reservoir 800 mL in capacity. A peristaltic pump was used for feeding the wastewater from the reservoir to the anodic chamber of the MFC at a flow rate of 0.5 mL/min. To avoid the degradation of the wastewater during its storage, the wastewater was sterilised for 20 min at 120 ºC. The anodic chamber of the MFCs was seeded two days after the start-up of the MFCs with a *Geobacter* enriched mixed culture taken from the effluent of a working MFC [23]. In this way, the absence of electricity generation before the seeding of the MFC was verified, with these data serving as abiotic control data.

The composition of the synthetic wastewater used in the experiments is presented in Table 1. The main organic substrate was glucose because it is often used as a model substrate [24, 25]. The trace minerals concentration was always maintained constant, whereas the organic COD concentration was increased at the values of 300 mg/L, 900 mg/L, and 1800 mg/L, corresponding to n, 3n, and 6n COD concentrations, respectively.

Table 1. Synthetic wastewater composition.

Compound	Concentration n	Concentration 3n	Concentration 6n
Glucose (mg/l)	300	900	1800
$(NH_4)_2SO_4$ (mg/l)	74.2	74.2	74.2
KH_2PO_4 (mg/l)	44.5	44.5	44.5
$NaHCO_3$ (mg/l)	115	115	115
$MgSO_4 \cdot 7H_2O$ (mg/l)	50	50	50
$CaCl_2$ (mg/l)	30	30	30
$(NH_4)_2\,Fe(SO_4)_2$	3	3	3

The primary reactions occurring in the MFC were the following ones:

Anodic glucose oxidation: $C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 24\,H^+ + + 24\,e^-$

Cathodic oxygen reduction: $O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O$

Cell reaction: $C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$

The standard potential value, E_{emf}^0, of the glucose oxidation is 0.104 V vs. Normal Hydrogen Electrode (NHE) and 1.229 V vs. NHE for the oxygen reduction reaction[26]. These values can be modified when changing the operational conditions. As a result, the actual potentials can be determined according to the Nernst Equation.

$$E_{emf} = E_{emf}^0 - \frac{R \cdot T}{n \cdot F} \ln(\Pi) \quad (1)$$

where

E_{emf} is the actual potential

R is the universal gas constant

T is the absolute temperature

n is the number of moles of electrons transferred in the cell reaction or half-reaction

F is the Faraday constant

Π is the reaction quotient.

In the Nernst equation, it can be observed that the higher the reactant concentration, the higher the exerted potential. The whole cell potential can be calculated as the difference between the cathodic and anodic half cell potentials.

$$E_{emf} = E_{cat} - E_{an} \quad (2)$$

However, the cell potential not only depends on electrolyte concentration but also on over potentials and Ohmic losses[27]. Thus, the actual cell potential can be determined using eq. 3:

$$E_{cell} = E_{emf} - (\Sigma \eta a + |\Sigma \eta c| + IR\Omega) \quad (3)$$

where

Σηa and |Σηc| are the cathodic and anodic overpotentials, respectively

IRΩ is the sum of the Ohmic losses, which are proportional to the generated current (I) and Ohmic resistance of the system (RΩ).

During normal operation, the anode and the cathode were connected by means of copper wires and a resistor (125 Ω). The potentials between the edges of this resistor were continuously monitored. These potentials are directly related to the current flowing between the electrodes by Ohms law (eq. 4).

$$I_{MFC} = \frac{E_{cat} - E_{an} - (\Sigma \eta a + |\Sigma \eta c| + IR\Omega)}{125} \quad (4)$$

where I_{MFC} is the intensity of the electrical current exerted by the MFC.

In the set-up used in this work, electrical current was generated due to organic matter oxidation. This oxidation was monitored by measuring the parameter of the COD removal rate (r_{COD}). This parameter can be calculated, once steady state is achieved, according to eq. 5, where Q is the influent flow rate to the anodic chamber, and ΔCOD is the COD removed from this stream.

$$r_{COD} = Q \cdot \Delta COD \quad (5)$$

The relationship between the COD removal rate and the theoretical current intensity can be determined by taking into account the stoichiometry of the oxidation of the COD, for which every mmol of COD corresponds to 4 mmol of e^-, as represented in the following equation with (n), as well as the Faraday constant (96485 C mol^{-1} e^-), the molar mass of oxygen (M) and the COD removal rate (r_{COD}). This relationship is presented in eq. 6.

$$I(mA) = \frac{n\ (4\ mmol\ e^- \cdot mmol^{-1}\ O_2) \cdot F\ (964854\ mC \cdot mmol\ e^-) \cdot r_{COD}(mg\ O_2 \cdot d^{-1})}{M\ (mg\ O_2 \cdot mmol^{-1}\ O_2) \cdot 86400(s \cdot d^{-1})} \quad (6)$$

Therefore, the CE is defined as the ratio of total quantity of Coulombs actually transferred from the substrate to the anode, to the maximum possible Coulombs transferred if all the substrate removal generated current. High CE values are desirable for increasing energy recovery. The CE can be calculated integrating over time the Coulombs actually transferred divided by the stoichiometric theoretical value (eq. 7).

$$CE = \frac{M \cdot \int_0^t I \cdot dt}{n \cdot F \cdot V_{anodic} \cdot r_{COD}} \quad (7)$$

2.2 Methodology

To determine the long-term effect of transient COD concentrations, the influent COD was step-wise modified from 300 mg/l to 1800 mg/L. This step-wise modification was repeated by changing the time gaps (see Figure 2). The effects were studied in a time period ranging from 1 to 7 days. The perturbation intervals were selected based on the perturbations experienced in a conventional wastewater treatment plant [10]. These plants are subjected to daily and weekly changes, which could affect the performance of the facility [10, 28]. As it is well known, one of the main applications of the microbial fuel cell is wastewater treatment combined with clean energy generation. As a result, we were interested in evaluating the influence of the perturbations for periods ranging from 1 to 7 days on the performance of the microbial fuel

cell. A description of the procedure is presented in Figure 2. During the transient COD changes and afterwards, the main operational parameters were determined.

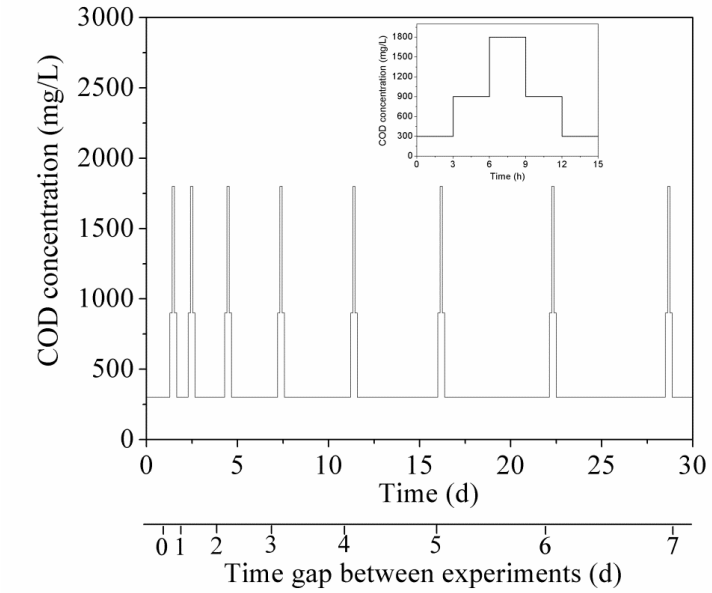


Figure 2. Schematic view of the experimental procedure. Inset: close-up of the step-wise transient COD modifications.

2.3 Characterization techniques

The quantities of the total suspended solid and the COD were measured according to processes reported in the literature [29]. The conductivity, dissolved oxygen, and pH were measured by means of a LF538 WTW conductivity-meter, an Oxi538 WTW oxy-meter, and a GLP22 Crison pH-meter, respectively. A digital multi-meter was connected to the system to continuously monitor the value of the cell potential. Polarization curves were recorded using an Autolab PGSTAT 30 potentiostat/galvanostat (Ecochemie, the Netherlands). Polarization curves provide interesting information about the operating conditions of the MFC, particularly about the actual capabilities of the MFC. These curves allow us to discern three important parameters: the open circuit voltage (OCV) or the maximum allowable MFC

voltage (for a nil current), the maximum intensity reachable (for a nil potential) and the maximum feasible power density. In addition, the shape of the curve provides information about the limiting stage, which controls the performance of the cell.

Typically, the shape of the polarization curve can be divided into three zones, which can be explained by the three major sources of loss of the fuel cells: the activation losses, voltage loss due to Ohmic loss, and voltage loss due to concentration loss.

In MFCs, linear polarization curves are most often obtained [2, 30] because, in most MFC devices, the operation mainly occurs in the Ohmic region of the curve [2].

The presence of the *Geobacter* and *Clostridia* genus were detected by MALDI-TOFF fingerprints. The matrix solutions were prepared by saturation of cyano-4-hydroxycinnamic acid in 1: 48:2 acetonitrile: water: trifluoroacetic acid matrix solution. The microorganisms were sterilized with ethanol at 75%. The solution was centrifuged (1000 rpm) for ten minutes and the supernatant removed. The microorganism was then extracted from the precipitate using 20 µL of acetonitrile/formic acid/water (50:35:15).

3. Results and discussion

The system was continuously operated for more than two months in order to ensure operation under steady-state conditions. Once the steady state was achieved, the culture was characterized being the main communities *Geobacter* and *Clostridia*, and then the transient COD tests were performed. These tests consisted of the modification of the influent organic COD concentration fed to the MFC. The transient modifications were maintained for 12 hours, and the effects were evaluated for up to 7 days. The experiments were performed at constant flow rates by modifying the influent COD but not the hydraulic retention time. During these tests, the remaining operational conditions were kept constant. The COD concentrations tested were the following: the COD concentration feed to the system under

steady state (used as the control value and named "n"), three times the control concentration (named as 3n) and six times the control concentration (named as 6n). To study the possible hysteresis of the system, when modifying the influent COD concentration, the forward scan was complemented with a reverse scan.

It is important to remark that similar trends, but with different values, were observed for the main parameters in each transient COD test.

3.1. Long-term effect on the COD removal

Regarding the COD removal, it must be noted that the values obtained in the forward and reverse scans were found to be different. The values obtained in the reverse scans are represented by the corresponding symbols for the forward scans but with open characters. During the step-wise procedure, the highest concentration, 6n, was studied once, and therefore, no reverse scan is presented.

In Figure 3, the COD removal rates versus the time break between experiments are presented. This figure shows two different behaviors. In the first stage, with a time gap of 3 or lower than 3 days, the system showed increasing COD degradation rates when it was subjected to transient modifications in the influent COD concentration. The increase in the degradation rate presented a gentle slope when operating at the low COD concentrations of the n series, but a very stepped slope when the system was operated at the highest COD concentrations of the 6n series. In the case of the highest influent COD concentrations, the enhancement of the COD degradation rate was higher than 20% and could be caused by the increase of the biomass concentration on the surface of the anodic electrode due to the transient exposition to high COD concentrations [23]. To corroborate the biomass growth, the biomass concentration in the effluent wastewater was determined. The values obtained are presented in Fig. 3. The trend was very similar to that obtained for the COD removal rate, indicating a direct relationship between them. As shown in Fig 3, when several transient modifications occurred

in a short period of time the enhancement was more evident, suggesting an accumulative effect. The highest response was obtained when the time gap between transient COD tests was 3 days. When operating with longer time gaps ranging between 4 and 7 days, the behavior of the systems was different, decreasing the COD removal rate when increasing the time gap. This reduction of the COD removal rate could be explained by the decay of the excess of biomass grown when the MFC was exposed to high COD concentrations. According to literature this reduction presents a linear trend [31], this trend can also be observed in the biomass concentration data presented on Figure 3. These results verify that the MFCs behave in different ways, depending on the events that occurred a few days prior. For longer time gaps between the experiments (higher than 7 days), the system reached a similar response to that obtained before the tests, indicating that after 7 days, the system was no longer influenced by the previous events and behaves as usual.

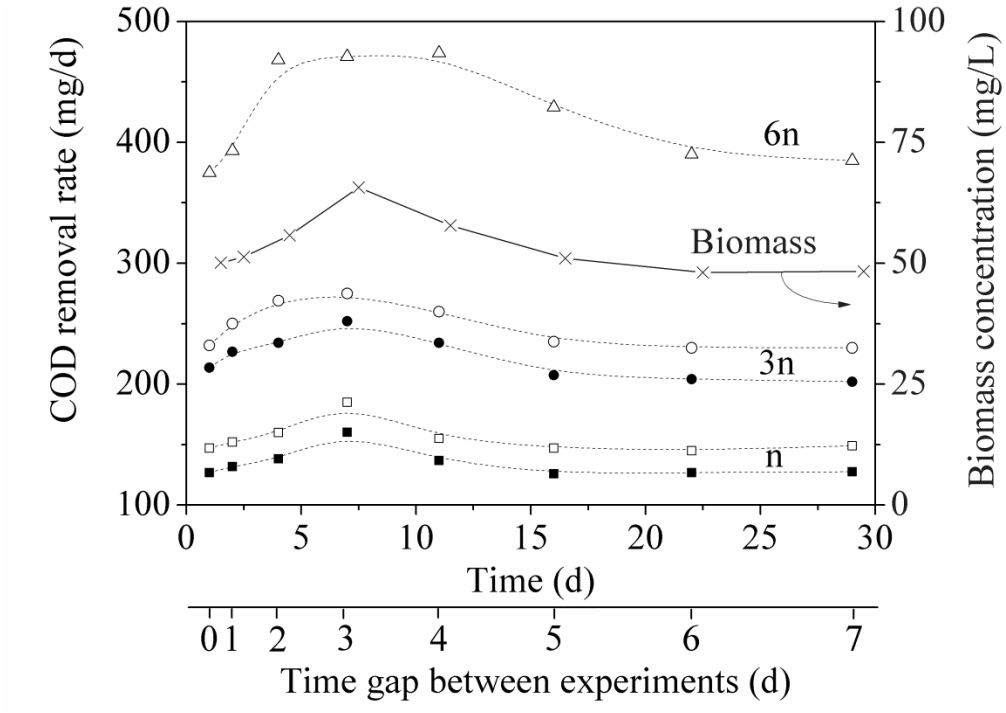


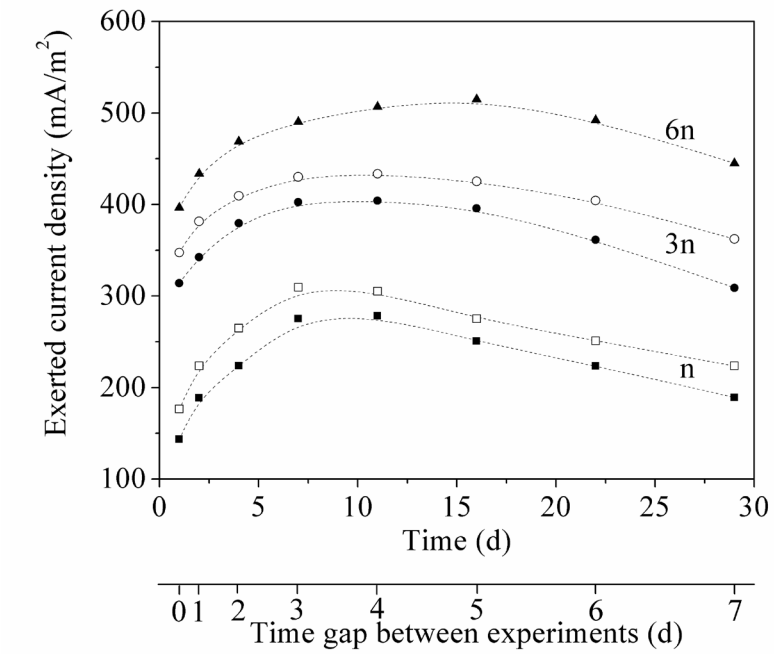
Figure 3. COD removal rates versus time and versus the time gap between experiments. Full and open symbols correspond to the forward and reverse scans, respectively. Crosses correspond to the effluent biomass concentration.

3.2. Long-term effect on the current density generation and the CE

Regarding the exerted current density, the obtained results are shown in Figure 4, where the values obtained in the reverse scans are represented by the corresponding symbol for the forward scan, but with open characters.

In this figure, two stages are also shown. However, the exerted current densities presented an opposite trend to that obtained when studying the COD removal rate. In the case of the exerted current density, the trend was more stepped when low COD concentrations were studied, presenting a maximum between 3 and 4 days of time gap between experiments, depending on the COD concentration. The control experiment, n, and three times the COD concentration, 3n, reached the maximum after 3 days, whereas the experiment with six times the COD concentration, 6n, reached the maximum after 4 days.

Subsequently, the current density generations of the 3n and 6n tests decay, reaching a value similar to that obtained at the beginning of the tests when the time gap between experiments was 7 days. However, in the case of the n test, the exerted current density remained higher than that obtained at the beginning of the tests, even after 7 days, presenting an increase in the exerted current density of approximately 15%. This phenomenon could be related to the enhancement of the electrogenic population, with this enhancement being maintained even when the influent COD concentration was reduced. This behavior could be explained by a higher affinity of *Geobacter* microorganisms for the substrate than that of the non-electrogenic organisms [16, 32]. Therefore, when the influent COD concentration is reduced, the *Geobacter* outcompete the non-electrogenic ones because of the lower affinity for the substrates of the latter microbial group.



303

304 Figure 4. Exerted current density versus the time gap between experiments. Full and open
305 symbols correspond to the forward and reverse scans, respectively.

306

307 Because of the different trends in the COD removal rate and the current density generation,
308 changes in the CE with time were expected. The determined CE values are shown in Figure 5,
309 where the error bars correspond to measurement replicates. This figure shows that the trend
310 of the CE highly depended on the COD concentration tested. In the case of the highest
311 concentrations, i.e., 3n and 6n, the CE was almost independent of the time gap between
312 experiments. This behavior could be related to a compensated development of both
313 electrogenic and not-electrogenic microorganisms. In these cases, the COD concentration is
314 very high and allows the development of both types of microbial populations, electrogenic
315 and not-electrogenic, without limitations. The compensated growth when no limitations occur
316 indicated that the growth rate of both groups were very similar. However, in the case of the
317 lowest COD concentration, n, the CE was clearly influenced, ranging between 35 and 65%.
318 This behavior could be explained because of the combined effect of the higher substrate
319 affinity of *Geobacter* for the substrate and because of the biomass growth rate of electrogenic

and non-electrogenic microorganisms. In this case, when the COD concentration is scarce, the *Geobacter* microorganisms prevailed, and the MFC culture was enriched in electrogenic microorganisms, leading to a better electrochemical performance. In the case of the lower COD concentration, the enhancement of the CE was maintained up to 4 days.

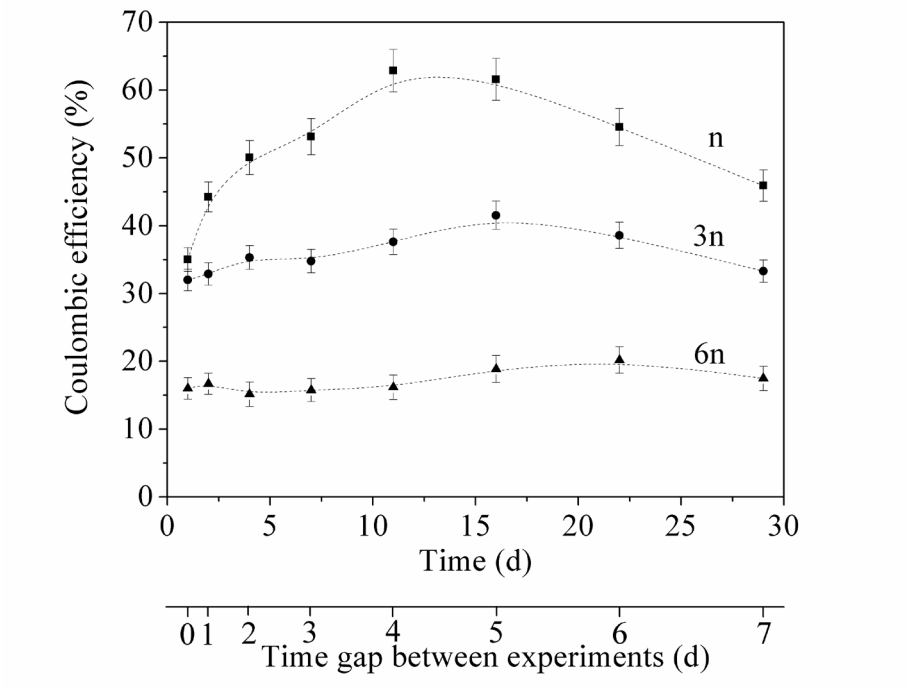


Figure 5. CE versus the time and time gap between experiments.

3.4. Short and long-term effect comparison

To compare and evaluate the differences in the exerted current density in the short and long-term, both results are presented in Figure 6. Data for the short-term effects were taken from the literature [23]. In Figure 6, the current density exerted during the steady state as well as the values reached after the transient COD modification in the short-term, after 6 h, and long-term, after 3 d, are presented.

In this figure, it can be seen that the exerted current obtained when operating at low COD loading rates presented an increasing trend in the short term and in the long term. The

enhancement in the long term could be explained because of the enrichment in *Geobacter* microorganisms in the culture after the repetitive transient COD modifications. However, when working at the highest loads, COD concentrations 3n and 6n, the system showed a higher enhancement at the short term than at the long term. This behavior could be explained because of the enrichment of the non-electrogenic organisms in the microbial culture when working with high COD concentrations. Under these conditions, a significant amount of acetate could be produced from the glucose fermentation by *Clostridia*. Then, some of the acetate could be consumed by *Geobacter* [33]. These results are in accordance with the higher affinity of the *Geobacter* microorganisms for the substrate[16], which leads to better electrogenic performance when operating at low COD concentrations.

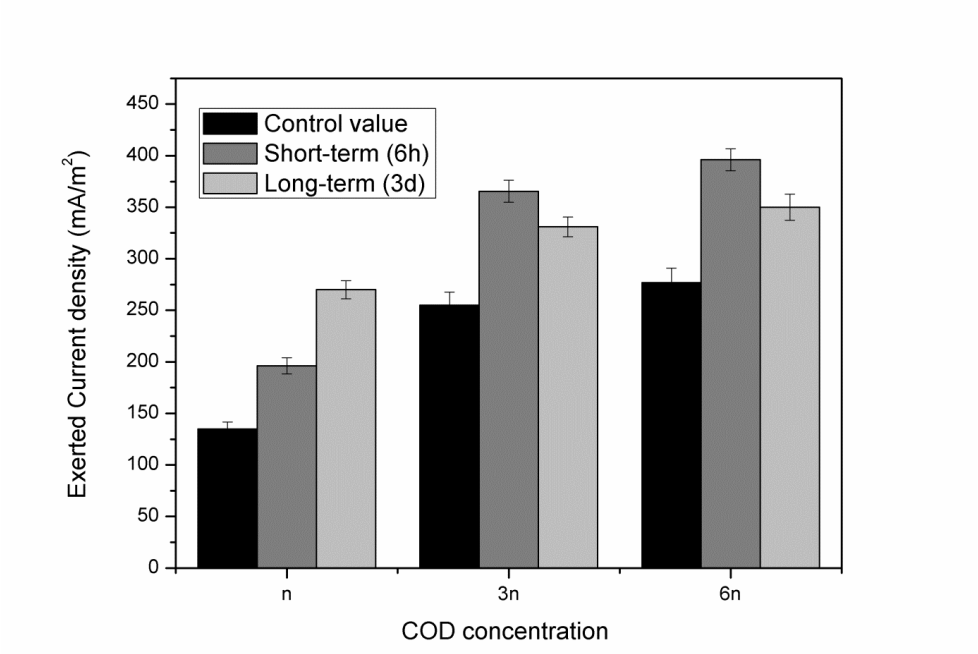


Figure 6. Exerted current density versus COD concentration in the short-term and the long-term.

Finally, to characterize the electrochemical abilities of the system the polarization curves were performed. From the obtained results, the Open Circuit Voltages (OCVs) and the Ohmic resistance values were determined. The white area of Fig. 7 shows the OCV values obtained in different stress tests performed on the system.

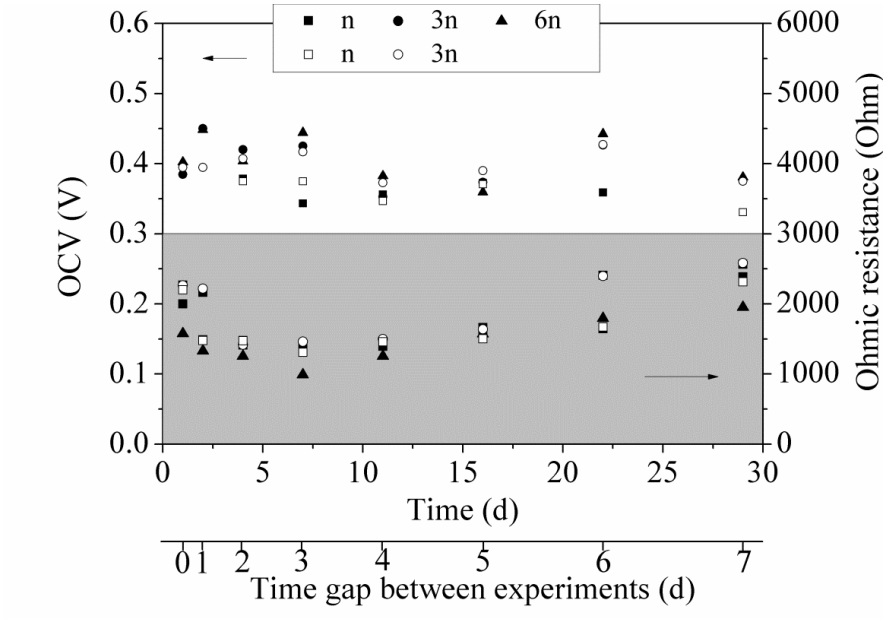


Figure 7. Open circuit voltage and Ohmic resistance obtained during the COD stress tests. Full and open symbols correspond to the forward and reverse scans, respectively.

It can be seen that the response was kept constant, in spite of the COD concentration. This behavior can be explained by the fact that the OCV only depends on the potential difference between the electrodes. As is known, the potential of an electrode is a function of the oxidation and reduction reactions , but not of the extension of the reaction [34]. The stability of the chemical reactions, and therefore of the OCV, indicates that the electrogenic microbial population located at the anodic compartment did not change significantly during the experiments [23] being the most important group *Geobacter*. Regarding the Ohmic resistance, its values are presented in the grey area of Fig. 7. This figure shows that the values initially presented a descending trend. This behavior could be explained by the development of a more active electrogenic biofilm at the surface of the electrode [3]. In this case, the higher activity reduced the resistance to the electrical current. However, when the time between transient modifications was increased, the Ohmic resistance also increased, which could be explained because of the decay of the

371 *Geobacter* microorganisms that had previously grown. The average value of the sum of the
372 overpotentials experienced was approximately 0.07 V.
373
374 **4. Conclusions**
375 From the results obtained in this paper, the following conclusions were made.
376 The time dependence experienced in the electrogenic behavior of the MFC makes these devices
377 sensitive to transient COD concentrations. This time dependence can be explained by changes in
378 the microbial population and the metabolisms while the electrochemical performance remains
379 almost constant. This behavior is very interesting for the potential application of MFCs as
380 bioelectrochemical sensors. Modifications in the COD loading rate maintained during 12 hours
381 caused long-term modifications in the microbiological behavior of the cell, increasing the ability
382 of the microorganisms to degrade the COD. The main effects were observed during the first 4
383 days, although the influence was identified up to 7 days.
384 Note that the electrochemical behavior of the cell was also affected. In the long term, the
385 Coulombic efficiency decreased when the system was exposed to higher COD loading. In this
386 case, the effects were significant for at least 7 days.
387 Finally, it was also observed that the *Geobacter* microorganisms presented higher affinity for the
388 substrate than the non-electrogenic organisms. This higher affinity leads to better results when
389 working with low COD concentrations, which is a very important finding for enhancement of the
390 population of electrogenic microorganisms in a mixed culture MFC.
391
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395

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