

## Article

# Use of Cyclic Voltammetry to Describe the Electrochemical Behavior of a Dual-Chamber Microbial Fuel Cell

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**Abstract:** Cyclic voltammetry (CV) was used in this work to describe the electrochemical behavior of a dual-chamber microbial fuel cell (MFC). The system performance was evaluated under vacuum and non-pressurized conditions, different reaction times, two sweep potentials, 25 and 50 mVs<sup>−1</sup> and under different analyte solutions, such as distilled water and domestic wastewater. CV experiments were conducted by using a potentiostat with three different configurations to collect the measurements. A dual-chamber MFC system was equipped with a Dupont<sup>TM</sup> Nafion<sup>®</sup> 117 proton exchange membrane (PEM), graphite electrodes (8.0 cm × 2.5 cm × 0.2 cm) and an external electric circuit with a 100-Ω resistor. An electrolyte (0.1 M HCl, pH ≈ 1.8) was used in the cathode chamber. It was found that the proton exchange membrane plays a major role on the electrochemical behavior of the MFC when CV measurements allow observing the conductivity performance in the MFC in the absence of a reference electrode; under this potentiostat setting, less current density values are obtained on the scanned window potentials. Therefore, potentiostat setting is essential to obtain information in complex electrochemical processes present in biological systems, such as it is the case in the MFCs. Results of the study showed that wastewater constituents and the biomass suspended or attached (biofilm) over the electrode limited the electron charge transfer through the interface electrode-biofilm-liquor. This limitation can be overcome by: (i) Enhancing the conductivity of the liquor, which is a reduction of the ohmic drop, (ii) reducing the activation losses by a better catalysis, and (iii) by limiting the diffusional gradients in the bulk liquor, for instance, by forced convection. The use of the electrolyte (0.1 M HCl, pH ≈ 1.8) and its diffusion from the cathode to the anode chamber reduces the resistance to the flow of ions through the PEM and the flow of electrons through the anodic and cathodic electrolytes. Also reduces the activation losses during the electron transfer from the substrate to the electrode surface due to the electrode catalysis improvement. On the other hand, vacuum also demonstrated that it enhances the electrochemical performance of the dual-chamber MFC due to the fact that higher current densities in the system are favored.

**Keywords:** dual-chamber MFC; cyclic voltammetry; electrolyte; electrochemical behavior; potentiostat settings; vacuum and non-pressurized conditions

## 1. Introduction

A microbial fuel cell (MFC) is a device employed to recover energy from wastewater. It is made of anode and cathode compartments separated by a proton-selective membrane. The anode and cathode are electrically connected to a resistor or a power sink which consumes the energy generated by the reactions. In the anode, bacteria degrade the organic matter and catalyze the electron transfer to the anode electrode. In the cathode, the reduction of the terminal electron acceptor takes place [1].

Microbial Fuel Cells (MFCs) have importantly drawn the attention of researchers around the world due to the potential to treat wastewater and produce bioenergy simultaneously [2]. In this respect, important advances have been recently achieved in electrode materials, ion-selective membranes, bioreactor configurations, applications on different types of wastewater and MFC modeling, that that scientific community is excited with the idea of scaling up this technology to the “real-world”. The practical applications of MFCs have recently been reported in electronic/robotics with prototypes that demonstrated the potential of MFCs as a power source [3]. Power sensors by using benthic (seafloor) and marine MFCs is the case of another field trial [4–6]. In these field trials, electronics was used to match the MFCs’ lower but constant energy generation to the higher and intermittent energy needs. More recently, other applications such as large pilot-scale MFCs treating real wastewater and urine in a continuous flow mode, MFC in constructed wetlands, prototypes integrated to wastewater treatment plants, and floating MFCs combined with plants to power sensors installed in natural water bodies have been evaluated [6,7]. Despite these achievements, power generation is still limited for municipal wastewater systems ( $26 \text{ mW m}^{-2}$ – $146 \text{ mW m}^{-2}$ ) and remains one of the main challenges to be overcome in the coming years for the full-scale and massive application of MFCs. In this regard, López Zavala et al. [8] have enhanced the performance of dual-chamber MFCs for municipal wastewater, achieving chemical oxygen demand (COD) reductions of up to 95%, voltage peaks of 0.954 V, maximum power densities on the order of  $2.1 \text{ W m}^{-2}$  and  $36.9 \text{ W m}^{-3}$ , and energy generation peaks of  $99.4 \text{ J mg}^{-1}$  COD removed. These values are greater than those reported in the MFCs’ literature for municipal wastewater.

Geometry and other operation conditions can be optimized to produce more power in the MFC; however, it is very complicated to determine the main reasons for an increase in power generation in this MFC [9]. Through an electrochemical characterization of the MFC, it is possible to obtain useful information to reduce the ohmic drops, the activation over-potential, and the concentration over-potential on the MFCs. Furthermore, electrochemical kinetics (electro-catalysis) and the mass transport on the MFCs under different setup conditions and geometries can be described. Cyclic voltammetry is an electrochemical technique useful to elucidate the electrochemical behavior of the system and has been commonly used to describe the electron transfer interactions in the interface electrode-biomass/biofilm-liquor in the anode chamber of MFC [10,11]. By performing CV at different sweep potentials on the MFCs, it is possible to compare quantitatively the electrochemical kinetics, the electrodes performance over time, and the effectiveness of the electron transfer between the biofilms and the electrodes [12]. Additionally, CV can provide information of the peak potential on the scan rate by using the Laviron Theory [12–14], can monitor the bacteria activity in the anode of the MFC, and can explore the formal potentials of the redox species involved in the system if peaks are observed; in addition, CV can describe whether or not the electrochemical reactions follow an electrochemical reversible kinetics according to Nernstian behavior; CV also provides insights of a possible complex mass transfer process occurring at the electrode interface. Furthermore, CV of different electrode materials allows the comparison of different catalysis, giving an overall description of the MFC performance under different operation conditions [15]. On the other hand, due to the complexity of wastewater composition and the processes involved during degradation, it is critical to use in the MFC different electrode configurations in the CV measurements in order to visualize individually the electrochemical behavior on each electrode; as a result, this protocol allows us to understand for instance the electron transfer interactions in the interface electrode-biomass/biofilm-liquor in the anode chamber of MFC, as well as identify if a particular electrochemical reaction can be detected with the

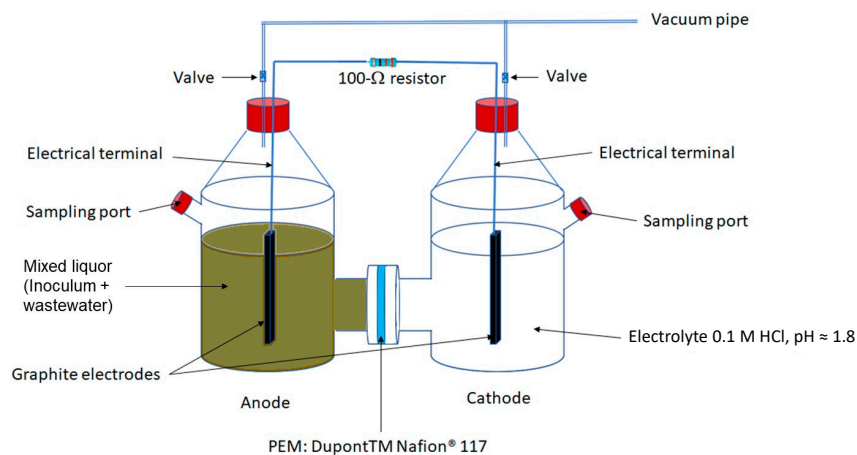
presence of peaks associated with a standard potential, using a reference electrode [10]. Furthermore, with the use of the CV it is possible to obtain information of the conductivity of the studied system such, as it is the case of the electrochemical storage systems where the measurements are collected in the absence of a reference electrode; as a result the measurements are recorded on a working electrode and a counter electrode [16].

Thus, in this paper, cyclic voltammetry regarding three potentiostat settings was used to describe the electrochemical behavior of a dual-chamber MFC operated under different inner pressure conditions, reaction times and sweep potentials. The electrochemical technique of CV allowed understanding the electron transfer interactions in the anode liquor [wastewater constituents and the biomass suspended or attached (biofilm) over the electrode]. The liquor presented low current conductivity that limited the electron charge transfer through the interface electrode-biofilm-liquor. CV assessment also allowed elucidating the reasons of the power generation increase in the dual-chamber MFC when it is operated under vacuum conditions, and the conductivity of the anode liquor is enhanced by the use of an electrolyte in the cathode chamber that diffuses towards the anode.

## 2. Materials and Methods

### 2.1. Experimental Device

The experiment devised and used in this study was that reported by López Zavala et al. [9] that consisted in a dual-chamber microbial fuel cell (MFC) system (Adams & Chittenden Scientific Glass, Berkeley, CA, USA) equipped with a Dupont™ Nafion® 117 proton exchange membrane (PEM) of 3.85 cm diameter. Graphite electrodes (8.0 cm × 2.5 cm × 0.2 cm) placed in each chamber were connected to an external circuit with a 100  $\Omega$  resistor (Figure 1). In the cathode chamber, an electrolyte based on a hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8) was used. Additionally, the device was prepared to apply a vacuum in both chambers by means of a vacuum pump.



**Figure 1.** Schematic representation of the dual-chamber microbial fuel cell.

### 2.2. Operation of the Dual-Chamber MFC

First, inoculum was prepared using mixed liquor of a municipal wastewater treatment plant bioreactor. The mixed liquor was acclimated during 10 days under anaerobic conditions and a temperature of  $25 \pm 1$  °C. Simultaneously, the anode chamber electrode was kept submerged into the mixed liquor to facilitate the attachment and biomass growth over the electrode surface. Second, with the conditioned electrode and inoculum prepared, domestic wastewater was degraded in the anode chamber during 160 h under anaerobic conditions (vacuum: 77.3 kPa), temperature of  $25 \pm 1$  °C, and the initial conditions shown in Table 1. The system was operated on batch mode. Third, in parallel another MFC system was also operated on batch mode, but distilled water replaced the wastewater in the anode chamber (Table 1). Sampling in both chambers was conducted every 12 h. Chemical oxygen

demand (COD) and pH were determined based on the protocols described in the Standard Methods for the Examination of Water and Wastewater [17].

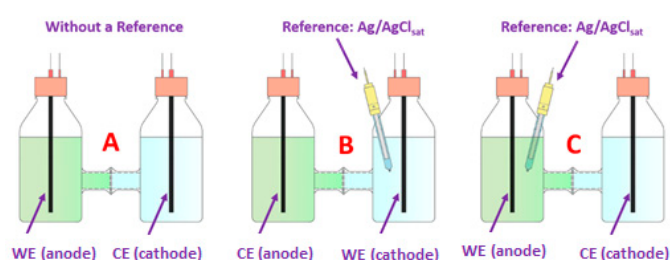
**Table 1.** Initial operating conditions of the microbial fuel cell (MFC).

Chamber	Constituent		Unit	MFC 1	MFC 2
Anode	Inoculum (MLVSS)	Vol.	mL	33.0	33.0
		Mass	mg	112.4	
	Wastewater/distilled water	Vol. Total COD	mL mg L <sup>-1</sup>	250.0 906.7	250.0
Cathode	Electrolyte	Vol.	mL	283.0	283.0

MFC 1: Wastewater was degraded. MFC 2: Distilled water was used instead of wastewater in the anode chamber. MLVSS: Mixed Liquor Volatile Suspended Solids.

### 2.3. Electrochemical Characterization

The electrochemical characterization of the MFC was done by cyclic voltammetry using a potentiostat model CHI700E (CH Instruments, Austin, TX, USA). Cyclic voltammetry (CV) measurements were carried out using three different potentiostat connecting configurations and five different experimental scenarios. In the CV experiments, the first three cycles were recorded by scanning the potential under cathodic direction in order to observe the evolution of the electrochemical behavior between cycles that can indicate an evolution on the electrochemical kinetics by degradation and/or formation of the species. Variation on potentiostat settings was conducted to evaluate the electrochemical response of the MFC system and to understand the main reason of the power increase in the dual-chamber MFC; meanwhile, different experimental conditions were set up to evaluate the effect of the electrolyte, the pressure and the scan rates over the electrochemical behavior of the MFC. Figure 2 shows the three potentiostat settings used in the study. In setting A, the reference electrode (RE) was absent and the anode acted as working electrode (WE), while the cathode of the MFC system worked as a counter electrode (CE). In configuration B, the RE was set in the cathode chamber and the cathode acted as WE, while the anode of the MFC system worked as a CE. For the setting C, the RE was set in the anode chamber and the anode acted as working electrode WE, while the cathode of the MFC system worked as a CE. On the other hand, the experimental conditions were carried out as follows:



**Figure 2.** Potentiostat settings for cyclic voltammetry (CV) measurements.

Scenario 1 (S 1). In this scenario, both the effect of the electrolyte and the pressure on the electrochemical behavior of the MFC System were assessed. The anode chamber was filled up with distilled water and the cathode chamber with a hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8) as an electrolyte. CV measurements were done at scan rates of 25 and 50 mV/s and under vacuum (77.3 kPa) and non-pressurized conditions in both chambers.

Scenario 2 (S 2). In this case, the electrochemical behavior of the MFC was assessed when domestic wastewater was degraded under vacuum conditions (77.3 kPa). Degradation of wastewater was done as described in Section 2.2. In parallel, another system was also operated, but distilled water replaced the wastewater in the anode chamber. The electrolyte (0.1 M HCl, pH  $\approx$  1.8) was used in the cathode chamber. The CV measurements were conducted at a scan rate of 50 mV/s.

Scenario 3 (S 3). In this scenario, the electrochemical behavior of the MFC was analyzed at different reaction times and under vacuum (77.3 kPa) conditions. CV measurements were done at scan rates of 25 and 50 mV/s. Distilled water was used in the anode chamber and the electrolyte (0.1 M HCl, pH  $\approx$  1.8) in the cathode chamber.

Scenario 4 (S 4). In this experiment, the electrochemical behavior of the MFC was analyzed at different reaction times and under non-pressurized conditions. CV measurements were done at scan rates of 25 and 50 mV/s. The anode chamber was filled up with distilled water and the cathode chamber with the hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8).

Scenario 5 (S 5). In this scenario, the electrochemical behavior of the MFC was analyzed at different reaction times when domestic wastewater was degraded in the anode chamber under vacuum (77.3 kPa) conditions and the electrolyte (0.1 M HCl, pH  $\approx$  1.8) was used in the cathode chamber. Degradation of wastewater was conducted as described in Section 2.2.

The CV measurements were run at 50 mV/s. All potentiostat settings and experimental conditions evaluated in this study are summarized in Table 2.

**Table 2.** Potentiostat settings in each scenario.

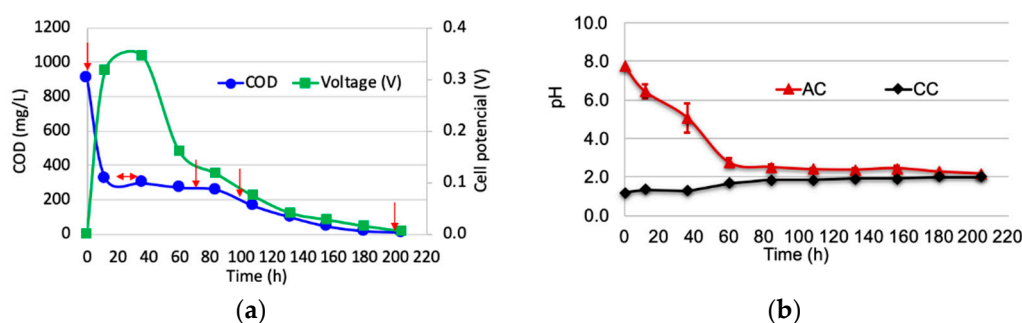
Scenario	Potentiostat Settings	Experimental Conditions	Sweep Potential, Cathode Direction (mV/s)
S 1	A: Without a Reference; B: CE in Anode Chamber; WE & RE in Cathode Chamber C: CE in Cathode Camber; WE & RE in Anode Chamber	Electrochemical behavior of the MFC under vacuum and non-pressurized conditions. Distilled water was used in the anode chamber	25 & 50
S 2	A: Without a Reference B: CE in Anode Chamber; WE & RE in Cathode Chamber C: CE in Cathode Camber; WE & RE in Anode Chamber	Electrochemical behavior of MFC under vacuum condition with two different anodic solutions, domestic wastewater and distilled water	50
S 3	A: Without a Reference B: CE in Anode Chamber; WE & RE in Cathode Chamber C: CE in Cathode Camber; WE & RE in Anode Chamber	Electrochemical behavior of the MFC at different reaction times and under vacuum conditions. Anode chamber filled up with distilled water	25 & 50
S 4	A: Without a Reference B: CE in Anode Chamber; WE & RE in Cathode Chamber C: CE in Cathode Camber; WE & RE in Anode Chamber	Electrochemical behavior of the MFC at different reaction times and under non-pressurized conditions. Distilled water was used in the anode chamber	25 & 50
S 5	A: Without a Reference C: CE in Cathode Camber; WE & RE in Anode Chamber	Electrochemical behavior of the MFC during degradation of domestic wastewater at different reaction times and under vacuum conditions	50

### 3. Results and Discussion

#### 3.1. Biodegradation of Wastewater and Generation of Electricity in the MFC

Figure 3 shows the evolution of COD reduction, voltage and pH during the biodegradation of domestic wastewater in the dual-chamber. The reaction times at which the CV measurements were conducted is indicated with red arrows. These reaction times were strategically selected, trying to explore important stages of the wastewater biodegradation (COD reduction) and electricity generation (voltage evolution) in the MFC. Such stages were the beginning of the degradation process, the peak of the electricity generation, the beginning of the steady decline of the voltage profile, and the end of the biodegradation process. The electrochemical behavior of the MFC might be different at each stage because the substrate availability, biomass activity and electricity generation were different. Detailed analysis of the electrochemical behavior of the MFC is conducted in the following sections.





**Figure 3.** Performance of the MFC during wastewater degradation. (a) Evolution of chemical oxygen demand (COD) reduction and voltage; (b) Evolution of pH in the anode chamber (AC) and cathode chamber (CC).

### 3.2. Electrochemical Behavior of the MFC under Vacuum and Non-Pressurized Conditions (S 1)

Figure 4 shows the CV results for the first scenario. All CV measurements were performed under cathode direction (negative direction of the window of potential explored). The sweep potential of Figure 4a–c cases was 25 mV/s, while for the Figure 4d–f cases the scan rate was 50 mV/s. In cases Figure 4a,d, less magnitude of current density was observed on the window of the potentials scanned due to the presence of the proton exchange membrane, which played a major role on CV measurements, because of the absence of an ideal polarizable reference electrode. On the other hand, in cases shown in Figure 4b,c,e,f, the higher magnitude of current density was due to the presence of an ideal polarizable electrode as a reference (Ag/AgCl). Thus, current density data were linked to the oxidation and reduction reactions that occurred on the working electrode. The highest cathode potential corresponded to the lowest values of negative current density, which were related to the reduction of water and the generation of hydrogen gas (0 vs. SHE =  $-0.197\text{ V Ag/AgCl}$ ). On the other hand, the highest anode potential was linked to the greatest values of current density, which correspond to the oxidation of water to form oxygen gas (1.23 vs. SHE =  $1.033\text{ V Ag/AgCl}$ ).

As seen in Figure 4, the magnitude of the current density of the system under vacuum was greater than that under non-pressurized conditions, except in the case (Figure 4d), where the magnitude of current density was slightly greater under non-pressurized conditions. However, important noise was observed on the measurements, which can be associated with the higher scan rate used that caused a perturbation and fluctuation on the CV measurements. Thus, vacuum conditions improved the electrochemical behavior of the MFC; therefore, this result validates one of the strategies implemented by López Zavala et al. [8] to enhance the power generation in the dual-chamber MFC. Indeed, vacuum enhances the electron charge transfer to the anode surface because the activity of oxygen as an electron acceptor is limited.

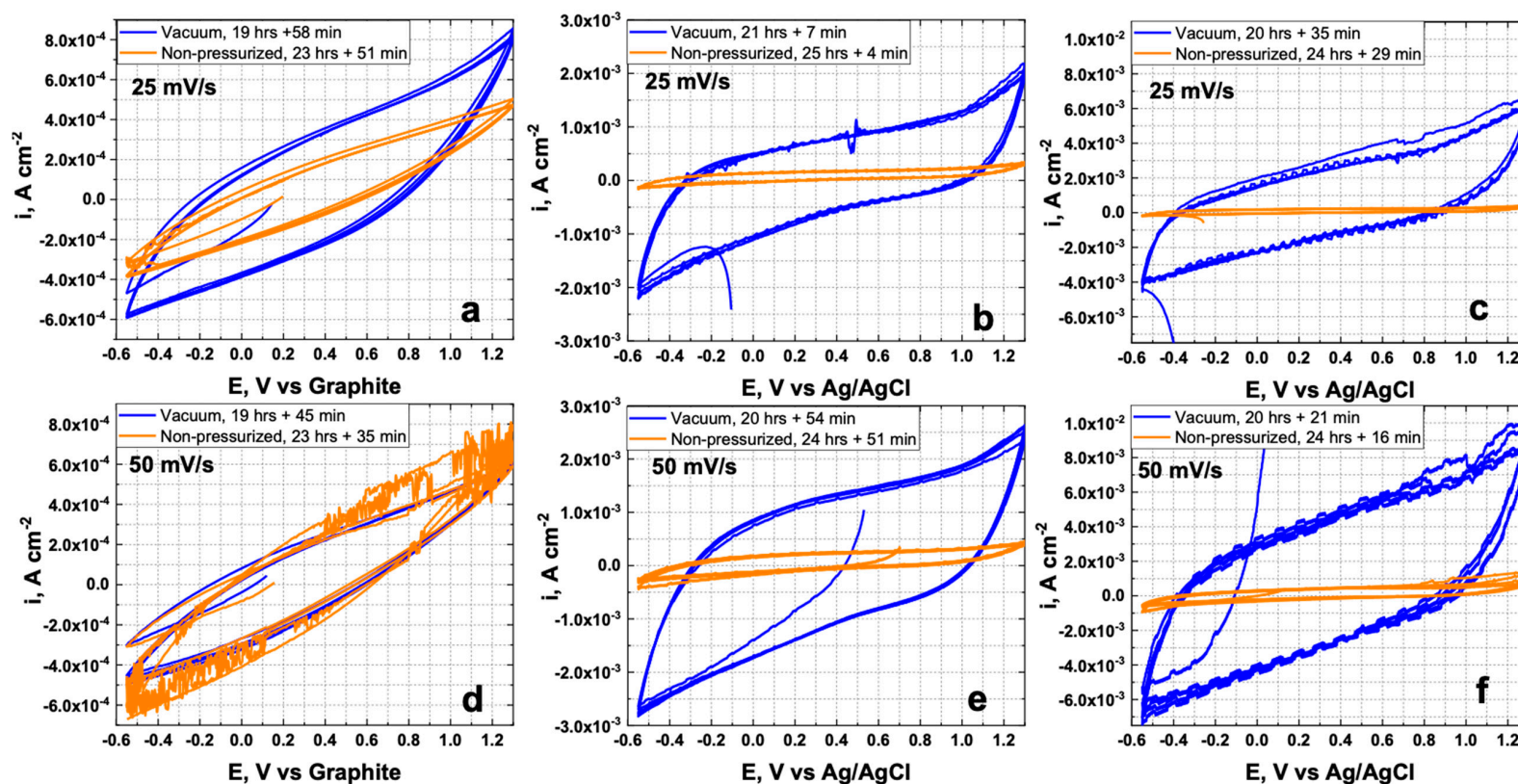
### 3.3. Electrochemical Behavior of the MFC during Degradation of Domestic Wastewater (S 2)

In Figure 5, results of the second scenario are shown. CV measurements were conducted at the reaction times where the highest biodegradation rates and electricity generation occurred (Figure 3). In the case of Figure 5a, less magnitude of current density on the window of potentials scanned was observed in comparison with cases Figure 5b,c. The lower magnitude of the current density was associated with the potentiostat settings, as it was explained in Section 3.1. Moreover, the highest cathode potential corresponded to the lowest values of negative current density, which are related to the reduction of water to generate hydrogen gas. On the other hand, the highest anode potential was linked to the greatest values of current density, which correspond to the oxidation of water to form oxygen gas. As seen, the magnitude of current density was much greater when the anode chamber was filled up with distilled water. This means that the conductivity of current in the anode liquor (wastewater + inoculum) was limited by the wastewater constituents and the biomass suspended or attached (biofilm) over the electrode; i.e., electron charge transfer through the interface electrode-biofilm-solution was

less effective than the interface electrode-distilled water. In fact, the limited electron charge transfer in the MFC systems is one of several factors that affect the power generation rates. This bottleneck can be overcome by enhancing the conductivity of the liquor, by reducing the activation losses, and by limiting the diffusional gradients in the bulk liquor by, for example, forced convection [8]. The electrolyte (0.1 M HCl,  $\text{pH} \approx 1.8$ ) diffuses through the PEM from the cathode to the anode chamber, thus enhancing the conductivity of the liquor and reducing the resistance to the flow of ions and electrons through the PEM, and the anodic and cathodic electrolytes, respectively. The diffusion of the electrolyte to the anode chamber also reduced the activation losses during the electron transfer from the substrate to the electrode surface due to the electrode catalysis improvement. These effects are not visible in Figure 5 because at 20–30 h reaction times, the electrolyte diffusion to the anode chamber was still limited. However, at longer reaction times, like those discussed in the Section 3.6 (scenario S 5), the conductivity improvement was clearly observed. Finally, mixing limits the diffusional gradients and enhances the flux of substrate to the suspended biomass and biofilm attached to the anode electrode, thus reducing the bacterial metabolic losses. However, in this study, mixing was not conducted, but it is important to take it into consideration for improving the electron charge transfer and the power generation in MFCs [8].

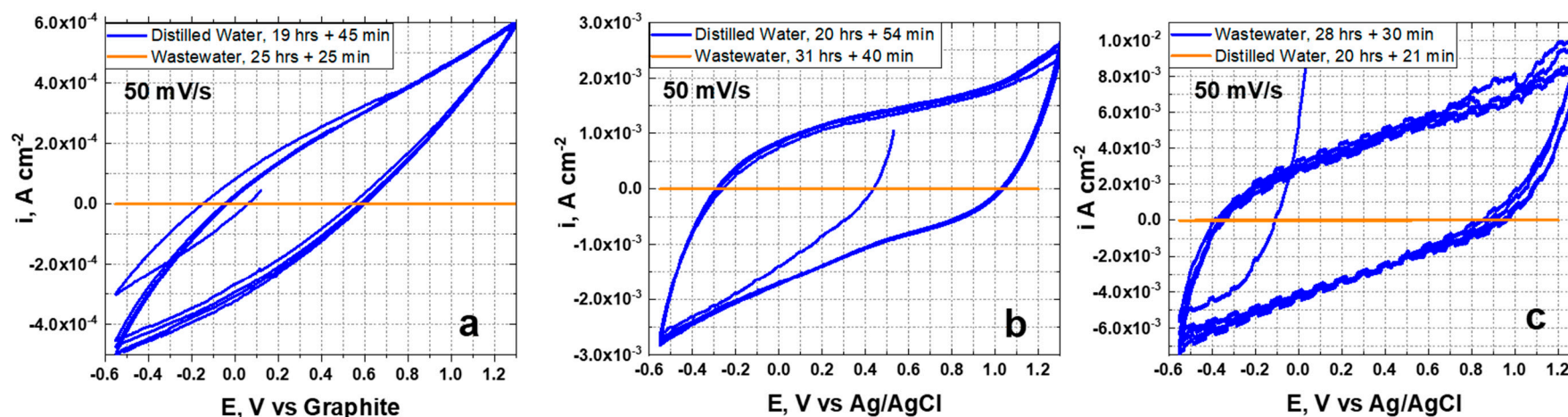
#### 3.4. Electrochemical Behavior of the MFC at Different Reaction Times and under Vacuum Conditions (S 3)

Figure 6 shows the results of the scenario S 3. All CV curves showed less current density at shorter reaction times; indeed, at the first hours of the experiment, less activity was observed. Approximately at 68 to 70 h reaction time, current density showed more noise due to the abrupt change of pH that could cause fluctuations of CV measurements. Evolution of pH during degradation of domestic wastewater is shown in Figure 3. A similar evolution pattern was observed when distilled water replaced the wastewater in the anode chamber. In the case of Figure 6c,f, a peak reduction in the first cycle of CV (69 h) was observed at the potential interval  $-0.2$  to  $-0.5$  V vs. Ag/AgCl; however, this peak might be associated to noise that is due to abruptly disappear in the second cycle. As discussed in Section 3.3, electrolyte diffusion to the anode chamber enhanced the electron charge transfer due to the reduction of the resistance to the flow of ions and electrons through the PEM, and the anodic and cathodic electrolytes, respectively; therefore, as diffusion progressed with the time, the pH in the anode chamber decreased, thus enhancing the conductivity of the liquor, and consequently, the electrochemical performance of the system.

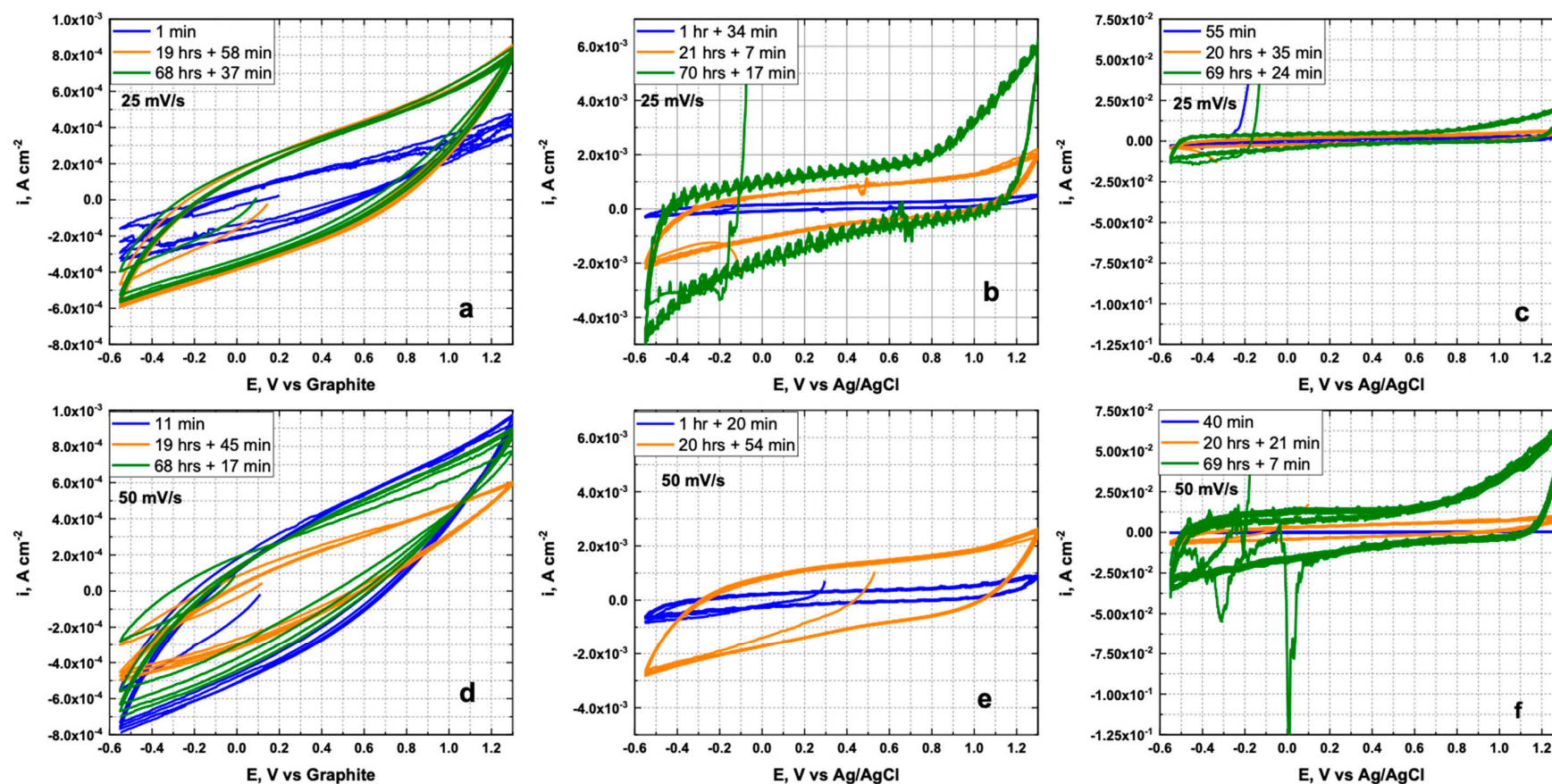


**Figure 4.** S 1: Electrochemical behavior of the MFC at different sweep potentials on cathode direction and under vacuum and non-pressurized conditions. The anode chamber was filled up with distilled water and the cathode chamber with a hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8). It is also seen that (a) and (d) (b), and (e) and (c) and (f) were obtained with the potentiostat settings A, B and C, respectively (Figure 2).





**Figure 5.** S 2: Electrochemical behavior of the MFC during degradation of domestic wastewater under vacuum conditions at 50 mV/s under cathode direction. Domestic wastewater was degraded in the anode chamber. In a parallel system, distilled water replaced wastewater in the anode chamber. In the cathode chamber of both systems the electrolyte (0.1 M HCl, pH  $\approx$  1.8) was used. Voltammograms (a), (b) and (c), correspond to the potentiostat settings A, B and C, respectively.



**Figure 6.** S 3: Electrochemical behavior of the MFC under vacuum conditions, at different reaction times and sweep potentials under cathode direction. The anode chamber was filled up with distilled water and the cathode chamber with a hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8). (a) and (d), (b) and (e), and (c) and (f) correspond to the potentiostat settings A, B and C, respectively.

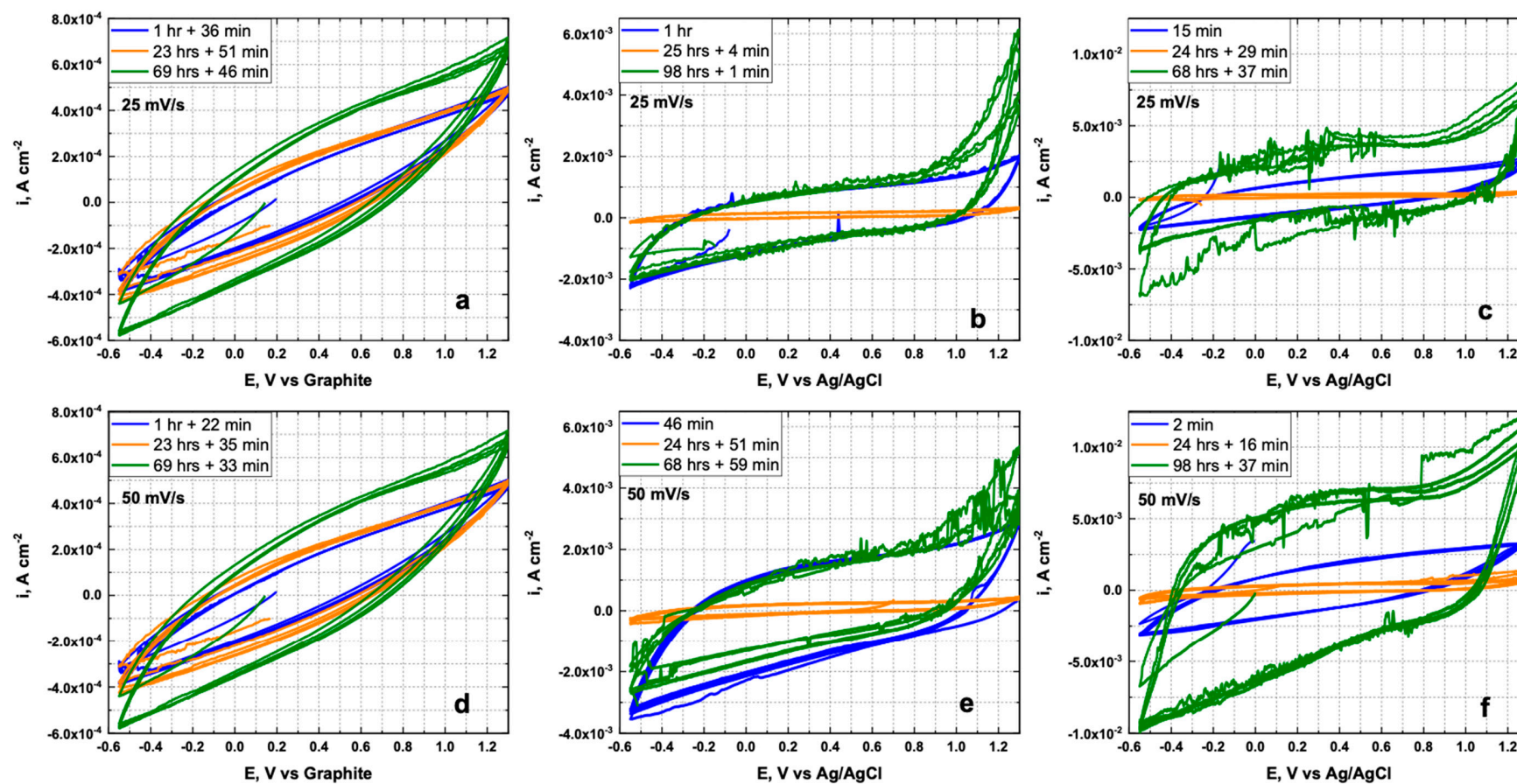
### 3.5. Electrochemical Behavior of the MFC at Different Reaction Times and under Non-Pressurized Conditions (S4)

Figure 7 presents the results of the scenario S 4. In general, the CV curves present more noise at longer reaction times, and this behavior can be associated with the reduction of pH in the anode chamber; it is also observed that the current density measurements present more noise under non-pressurized than under vacuum conditions. As seen, at reaction times lower than 1.5 h, the current density was greater than that measured at reaction times about 25 h. However, at reaction times longer than 69 h, the magnitude of current density increased, getting the highest values of the experiments. This trend can be associated with the fact that at the beginning the pH of the anode chamber was nearly neutral, and consequently, possessing of less electrochemical activity. At longer reaction times, conditions in the anode chamber became more acidic, due to the electrolyte diffusion from the cathode chamber. Furthermore, non-pressurized conditions caused the decreased of the system efficiency. In cases with respect to Figure 7a,d, the CV measurements were done without an RE; in this case there was observed less magnitude of current density on the window of the potentials scanned, mainly due to the presence of the proton exchange membrane, as explained in Section 3.2. Moreover, at larger overpotential values of the CV curves, the dissociation of water occurred, and the hydrogen and oxygen evolution reactions happened at the highest cathode and highest anode potentials, respectively. The results of this scenario confirm that which was stated in Section 3.2, in the sense that vacuum conditions improved the electrochemical behavior of the MFC; therefore, MFC should be operated under these conditions. Also, as reaction times increased, electrolyte diffusion to the anode chamber enhanced the electrochemical performance of the MFC, as discussed in Section 3.4.

### 3.6. Electrochemical Behavior of the MFC during Degradation of Domestic Wastewater at Different Reaction Times and under Vacuum Conditions (S 5)

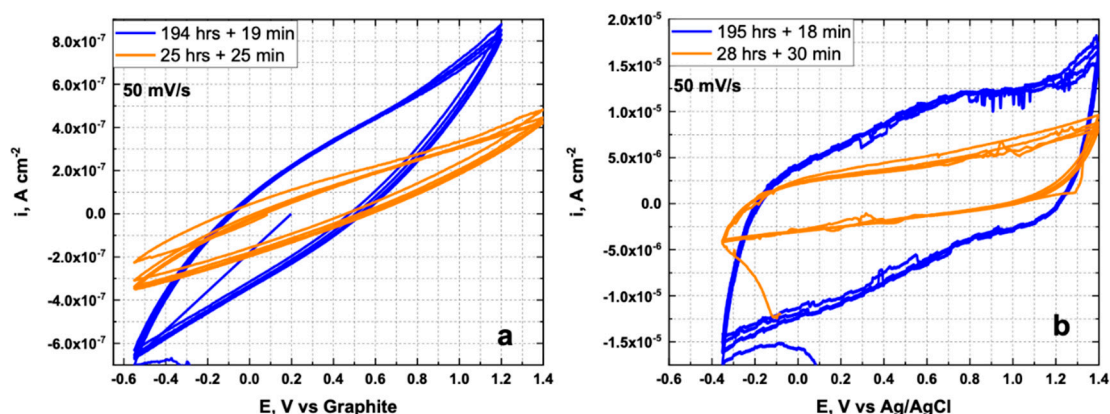
Figure 8 shows the results of the scenario S 5. In the case of Figure 8a, a reduced magnitude of current density on the window of potentials scanned was observed, mainly due to the presence of the proton exchange membrane. Moreover, at the extreme potential values of the CV curves, the dissociation of water occurred, and the hydrogen and oxygen evolution reactions happened at the highest cathode and highest anode potentials, respectively. The CV curves present an increase of current density as reaction times increase, i.e., when the pH in the anode chamber becomes more acidic due to the  $H^+$  diffusion from the cathode chamber. In addition, the increase of conductivity is also associated with the acidogenic wastewater degradation, since the electrochemical behavior denotes an activity variation throughout the operation of the MFC [18]. Furthermore, in the case concerning Figure 8b, the magnitude of current density is greater at longer reaction times, and it can be associated to some phenomena implicit on the system; for instance, there is more capacitance at the working electrode since the hysteresis on the CV increased. The increase of the capacitance on the working electrode occurred due to  $H^+$  concentration increases at the surface of the interface electrode-biofilm-solution. Additionally, the increase of the current density throughout the operation of the system can also be related to the oxidation of the organic matter. On the other hand, the trend of the CV curves is similar to that observed in the CV study reported by Jadhav and Ghangrekar, when in the MFC there predominates large amount of organic matter [19]. The results of this scenario clearly confirm that the electrochemical performance of the MFC is improved as the conductivity of the liquor is enhanced by the electrolyte diffusion from the cathode to the anode chamber, being more evident at longer reaction times. Thus, the use of the hydrochloric acid solution as an electrolyte is recommendable to enhance the power generation in a dual-chamber MFC.





**Figure 7.** S 4: Electrochemical behavior of the MFC under non-pressurized conditions, at different reaction times and sweep potentials under cathode direction. The anode chamber was filled up with distilled water and the cathode chamber with a hydrochloric acid solution (0.1 M HCl,  $pH \approx 1.8$ ). (a) and (d), (b) and (e) and (c) and (f) correspond to the potentiostat settings A, B and C, respectively.

Even though wastewater was degraded in the dual-chamber MFC (Figure 3), peaks of the oxidation and redox reactions were not clearly detected in cyclic volt-ammo-grams presented in Figures 5 and 8, as those observed in the volt-ammo-grams reported by Fricke et al. [11] and Peixoto et al. [20]. The reasons for these differences on the shape and pattern of the cyclic volt-ammo-grams might be the different electrode materials, sweep potentials, organic substrate and the conductivity of the anode liquor used in this work in comparison with those used in the previous mentioned studies. Indeed, the volt-ammo-grams' shape and pattern of this study agree with those reported by the literature when wastewater was degraded and similar graphite electrodes were used in the MFC. In all cases, peaks in the volt-ammo-grams were not clearly observed [14,21–26].



**Figure 8.** S 5: Electrochemical behavior of the MFC at different reaction times and under vacuum conditions at 50 mV/s under cathode direction. Wastewater was degraded in the anode chamber and a hydrochloric acid solution (0.1 M HCl, pH  $\approx$  1.8) was used as an electrolyte in the cathode chamber. Figures (a) and (b) correspond to the potentiostat setting A and C, respectively.

#### 4. Conclusions

Cyclic voltammetry, an electrochemical technique, was used in this study to describe the electrochemical behavior of a dual-chamber MFC. The effect of the electrolyte, pressure, sweep potentials, reaction time and potentiostat settings on the electrochemical behavior of the MFC, were all evaluated. It was found that the proton exchange membrane plays a major role on the electrochemical behavior of the MFC when CV measurements are conducted in the absence of a reference electrode. Under this potentiostat setting, lower current density values are obtained on the scanned window potentials. Therefore, the potentiostat setting is essential to describe properly the electrochemical behavior of the MFC. It is important to mention that low scan rates ( $<25$  mV/s) are desirable in these kinds of studies; however, such low scan rates cause noise in the CV measurements due to the big over potentials applied that makes difficult its interpretation; in addition, by recording CV experiments at lower scan rates, it will be impossible to analyze if there is a potential presence of an electrochemical kinetics reversibility according to Nernstian behavior, because the MFC systems can evolve roughly every 20 minutes.

Results of the study showed that wastewater constituents and the biomass suspended or attached (biofilm) over the electrode limited the electron charge transfer through the interface electrode-biofilm-liquor. The CV curves present an increase of current density when the pH in the anode chamber becomes more acid (of lower values) due to the  $H^+$  diffusion from the cathode chamber. Thus, the use of the electrolyte (0.1 M HCl, pH  $\approx$  1.8) and its diffusion from the cathode to the anode chamber reduces the resistance to the flow of ions through the PEM and the flow of electrons through the anodic and cathodic electrolytes. This also reduces the activation losses during the electron transfer from the substrate to the electrode surface due to the electrode catalysis improvement. Therefore, the limitation of the electron charge transfer can be overcome by: (i) Enhancing the conductivity of the liquor, (ii) reducing the activation losses, and (iii) by limiting the diffusional gradients in the bulk liquor



by forced convection (not evaluated in this study). On the other hand, vacuum also demonstrated that it enhances the electrochemical performance of the dual-chamber MFC due to the fact that higher current densities in the system are favored.

**Author Contributions:** M.Á.L.Z. obtained the funds of this research, established the methodology for the preparation of the inoculum, conditioning of the anode and the biodegradation of wastewater under anaerobic conditions, performed figure one, analyzed and discussed the results of the biodegradation, then revised and edited the final manuscript. O.I.G.P. established the methodology for the electrochemical experiments, performed the CV experiments, constructed the CV graphs, performed figure two, analyzed and discussed the results, and wrote the manuscript. H.C.R. and C.D.M. prepared the inoculum, conditioned the anode, characterized the biodegradation of wastewater, and helped in the CV experiments. M.G. provided a revision of the manuscript.

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