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Improvement of the Carbon Electrode Treatment to Obtain Bioanodes for Microbial Electrolysis Cell (MEC)

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In this work a process to modify the surface of carbon electrode was studied with the goal of improving the adherence of the bacteria on it. This study was performed through an experimental design to determine the effect of the parameters tested for the biofilm formation. The biofilms were analyzed with cyclic voltammetry technique and the kinetic parameters of alpha and k_{app} were analyzed with a statistical tool called the surface response. The parameters varied in the treatment were: concentration of the substrate, temperature, potential applied and time of the treatment. The results showed differences on the biofilm formed mainly with the concentration of the substrate and the potential applied in the electrode treatment. An alpha of 0.5 obtained suggests an electron transport due to the confined redox compounds within the biofilm and the k_{app} varied from 0.07 s^{-1} to 0.4 s^{-1}. Finally, the biofilms formed were used in a MEC to probe their capability as bio-anodes for hydrogen production and was obtained a production of 0.21 m^3 H_2/m^3 d.

Keywords: Biofilm, bioanode, hydrogen, MEC

1. INTRODUCTION

Hydrogen as a biofuel is a very interesting research area and there are several devices and methods to obtain it. One of the most promising techniques to obtain high purity hydrogen at low cost is by microbial electrolysis cell method (MECs).

Since MEC involves the activity of living microorganisms statistical tools for the experimental designs have been very useful for this research area. For example, the surface response (SR) statistical methodology that allows to obtain a complete information with minimum experiments and in less time [1]. This methodology also allows to experiment with different parameters at the same time with the aim of optimizing the response [2] and even to obtain a mathematical model of the system under study [3, 1].

The methodology of SR has been employed by researchers like Androga et al. 2014 [4] to optimize the hydrogen production by photo-fermentation through the variation in temperature and light intensity. Akman et al 2015 [5] also employed this methodology for a similar process with the variation of the substrate concentration and the light intensity as well.

The methodology of SR was employed in this study since the goal was to improve the process for the biofilm formation to obtain higher hydrogen yields. The parameters studied were temperature, concentration, time and voltage applied during the carbon electrode treatment before the biofilm formation process.

The pre-treatment of the material is required to promote changes on the surface to allow the first bacteria adherence and then the biofilm formation. It is well known that the material of the electrode and its composition affect the bacteria attachment, the electron flow [6, 7], the internal resistance, which is attributed to charge and mass transfer losses [8, 9].

There are just a few procedures reported for the treatments employed before the biofilm formation. The aim of the electrode modification before the biofilm formation is to improve the bioelectrode performance. Some materials employed for the electrode modification are carbon nanotube, stainless steel, conducting polymers, metal oxides and different compositions of electrolytes [7].

Wang et al. 2009, reported a thermal treatment under temperatures of 450 ˚C for 30 minutes and observed changes related to the correlations of O/C (21.8 to 7.4 %) and N/C (2 to 2.9 %) on the surface of the material [10]. The treatment with ammonia gas at high temperatures (5 % at 700 ˚C for an hour) reported by the same group also presented changes with the same chemical species and similar proportions (O/C from 21.8 to 3.8 % and N/C from 2 to 4.6 %) [11]. The presence of these functional groups has been reported as important by several researchers due to the high current densities obtained with the ammonia treatment [11] even when it is applied in different materials like graphite fiber brushes [12, 13] and different treatment temperatures (290 ˚C for 1 h) [14].

Recently, it has been reported other treatments that require the application of considerable temperatures like 450 ˚C and the time of 30 min [15] or the use of acids like HCl (1 M) followed by a process in atmospheric air plasma to improve the electroactivity of the anode. Verea et al 2014, reported a simple treatment method for carbon electrode that consisted of the application of a potential on the electrode during 24 h [16] and this work presents the study of this procedure through an experimental design to obtain the best conditions for carbon electrode treatment. The treatment was followed by the biofilm formation and the hydrogen production as well.

2. MATERIALS AND METHODS

2.1. Electrode treatment

Previous to the biofilm formation the carbon electrode was treated to promote the bacteria colonization on the surface. The treatment consisted of the application of an electric potential in two electrodes immersed in a conductive solution. The electrode of interest was polarized with a negative potential. Both electrodes were made of hE-TEK of 3 x 3 cm^2 without wet proofing and the second electrode was catalyzed with 0.5 mg Pt /cm^2 (20 wt% Pt, E-TEK) and liquid Nafion (5 %), in a ratio of 7 mL Nafion per mg of Pt/C catalyst. The solution was composed of $NaCH_3COO$ and carbonate buffer of pH 9. The concentration of the $NaCH_3COO$ and the potential applied for each treatment was varied as well as the temperature and the time of the treatment. These values are presented in table 1.

Table 1. Parameters tested for the electrode treatment to promote the biofilm formation

Potential applied (V)	2	0.7	0.05	0.7	1.35	2	2.65
Concentration of $NaC_2H_3O_2$ (mM)	30	12	3	12	21	30	39
Temperature (°C)	50	26	14	26	38	50	62
Time of treatment (h)	48	24	12	24	36	48	60

2.2. Biofilm formation

The electrode treated was enriched with bacteria in a sealed electrochemical cell of 100 mL with three electrodes. The treated electrode was used as the working electrode, the electrode of Ag/AgCl 3 M KOH as the reference electrode and the counter electrode was made with the same material (carbon cloth E-TEK without wet proofing) with an area of 10 times the geometric area of the treated electrode. The medium consisted of 70 mL of synthetic wastewater (SWW) with the following composition of 1 g/L NH_4Cl, 1 g/L $NaHCO_3$, 1 g/L Na_2CO_3, 0.2 g/L K_2HPO_4, 10 µL vitamin and 10 µL mineral solutions [17] . The potential applied was -0.42 V vs the reference electrode for 8 h as a selective potential of the exoelectrogenic bacteria from a mixed anaerobic culture as Srikanth et al, 2000 reported [18] and a constant temperature of 37 ˚C [16].

The mixed culture was obtained from the anaerobic sludge and it was previously stabilized as the American Type Culture Collection (ATCC) reports for the *Geobacter Sulfurreduccen's* growth.

2.3. Electrochemical measurements

To characterize the effect of the electrode treatment on the biofilm formation and performance the electrodes enriched with bacteria were analyzed in a Solartron potentiostat with cyclic voltammetry (CV) technique in a three-electrode electrochemical cell. The electrode with the biofilm was connected as the working electrode, the counter electrode was conformed by carbon electrode with an area of 10 times the geometrical area of the working electrode and the Ag/AgCl 3M KOH as the reference electrode.

The CVs were recorded with different scan rates of 10, 20, 40, 80, 100, 150 and 200 mV/s. The potential window was from -0.7 V to 0.4 V vs Ag/AgCl 3M KOH. Here after, all the potentials in this work will be referenced to the Ag/AgCl 3M KOH reference electrode. The voltammograms from the CV were analyzed to calculate the Kinetic parameters like, the apparent electron transfer rate constant (k_{app}), the electron transfer coefficient (α) and the electrons involved in the redox reaction in the biofilm (n). The kinetic parameters were calculated using the equations (1) and (2) from the Laviron method based on the Butler-Volmer approach [19].

The Laviron method is based on the solution of the general equation for the dimensionless current for quasi-reversible and irreversible electron transfer [20]. This method applies when the potential peak difference $\Delta E_p > 200/n$ mV, where $\Delta E_p = E_{pc} - E_{pa}$, E_{pc} is the cathodic peak potential and E_{pa} is the anodic peak potential, n is the number of electrons [21]

$$E_{pc} = E_c^{\circ'} - (RT/\alpha nF) ln \left[\frac{\alpha nF v_c}{RT k_{app}}\right] \qquad (1)$$

$$E_{pa} = E_a^{\circ'} - (RT/(1-\propto)nF) ln \left[\frac{(1-\propto)nF v_c}{RT k_{app}}\right] \quad (2)$$

The R, T, and F have their usual meanings (R=8.314 $Jmol^{-1}K^{-1}$; T=310 °K; F= 96483 $Cmol^{-1}$).
The k_{aap} was calculated from the plot of E_p vs Log *v*, where the term RT/αnF from equation (1) was obtained from the linear part of the cathodic slope and the value of the term RT/(1-α)nF from the linear part of the anodic slope in equation (2).

2.4. Scanning electron microscopy

For the chemical composition of the electrodes treated previous to the biofilm formation, they were analyzed with energy dispersive x-ray spectroscopy (SEM-EDS Hitachi SU 1510). For the qualitative analysis of the biofilm formed on the treated electrode they were analyzed with the Hitachi FESEM S5500 scanning electron microscope. For this analysis the samples were treated with 2.5 % glutaraldehyde for 15 min for adhering the microorganisms. Then they were dried in different alcohol solutions and finally sputtered with gold as Juan Pablo Busalmen et al, reports [22].

2.5. MEC´s Start up and operation

The electrode with the biofilm formed was used as anode in a membrane-free MEC of 100 mL. The electrolyte composition consisted of 80 mL of SWW. The cathode consisted of an electrode made of carbon cloth E-TEK without wet proofing of area 3 x 3 cm^2 catalyzed with 0.5 mg Pt cm^{-2} of commercial Pt/C (20 wt % Pt, E-TEK) and liquid Nafion (5%) in a ratio of 7 mL per mg of Pt/C catalyst.

The electrodes were located 1 cm apart from each other and they were connected to a programmable power supply through an external circuit of titanium wires. The voltage applied (E_{ap}) for the electrolysis was 1 V. The current was recorded using a data acquisition system (2700, Keithley, USA) that was incorporated into the electrical circuit. The electrolysis was conducted in batch mode during 22 h.

2.6. Characterization of MEC development

The gas produced in the MEC was stored and quantified by the displacement method with a graduated probe. The gas collected was sampled and analyzed by the gas chromatograph (Thermo Finnigan) equipped with a thermal conductivity detector (TCD).

The argon gas was used as carrier gas in a TG-Bond Msieve 5AGC. The Injector, column and detector were set at 100 ºC, 90 ºC and 120 ºC respectively. For the gas standard it was used an ultra high purity hydrogen gas. The volume of the hydrogen (V_t) present in the biogas produced was calculated using [23]:

$$V_t = \left(H_s + V_{T,t}\right)G_f \qquad (3)$$

where, $V_{T,t}$ is the total biogas produced and it was composed of H_2, CH_4 and CO_2. G_f is the gas fraction measured by the gas chromatograph, and H_s is the headspace volume of the reactor (mL).

3. RESULTS AND DISCUSSION

3.1 Carbon electrode treatment

The images of the untreated and treated electrodes (figure 1 and figure 2 respectively) were obtained using a SEM-EDS Hitachi SU1510. The electrodes showed differences on the surfaces. The electrode in figure 2 was treated with the conditions of E2 indicated in table 3. It was observed similar particles in different sizes on the entire surface.

The particles deposited on the surface during the pretreatment are very important because they are related to the biofilms performance. Guo et al, 2013 [24] reported that a positively charged and hydrophilic surface is more selective to electroactive microorganisms and therefore the biofilm is conductive. There are some studies where electrodes with hydrophilic chemical species on the surface, like OH, SO_3, $-N(CH_3)^{+3}$, -COOH and nitrates showed major relative abundance of *Geobacter* bacteria

(especially on the surfaces charged positively) than those electrodes treated with hydrophobic species, like -CH_3 [25, 26, 27]. The particles observed on the electrodes were selected to be analyzed with the relative elemental chemical composition technique. The results are shown in table 2.

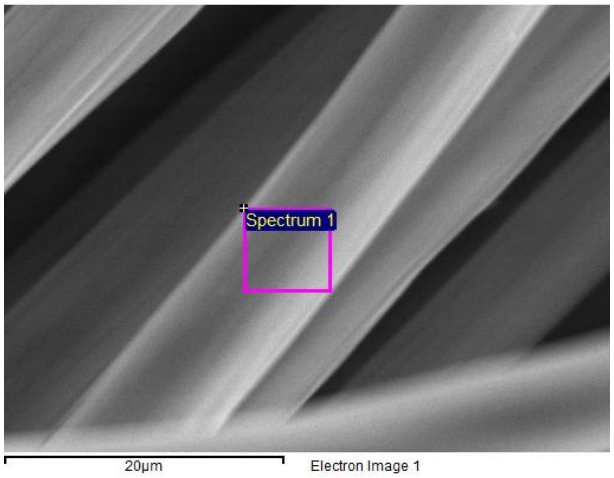

Figure 1. Micrograph obtained with the SEM-EDS Hitachi SU1510. Surface of the carbon electrode before the treatment.

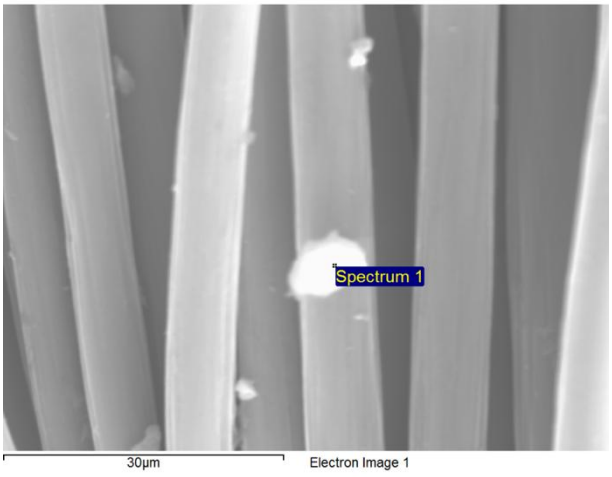

Figure 2. Micrograph obtained with the SEM-EDS Hitachi SU1510. Surface of the carbon electrode treated for 36 h at 38 ºC with a carbon source of 21 mM $NaCH_3COO$ and at an applied potential of 1.35 V (see E2 in table 3).

Table 2. Composition analysis with the SEM-EDS Hitachi SU1510 of the relative elemental chemical composition of the spectrum 1 from figure 1 and figure 2.

Element	Weight %		Atomic %	
	Electrode	Electrode after treatment	Electrode	Electrode after treatment
C	99.00	94.41	99.21	95.87
O	1.01	5.04	0.79	3.84
Na	-	0.45	-	0.24
Al	-	0.09	-	0.04

Table 2 reveals that the content of oxygen in the electrode increased after the electrode treatment and there was a surface deposit of a sodium compound. The chemical species on the electrode treated can be related with metallic oxides or nitrate species formed on the electrode surface.

Table 3. Results of the kinetic parameters of K_{app}, α and n calculated from the voltammograms of the biofilms formed on electrodes treated under different conditions of carbon source concentration, potential applied, temperature and time of treatment.

Experiment	Concentration $NaCH_3COO$	Potential	Temperature	Time	K_{app}	α	n
E	mM	V	ºC	h	(s^{-1})	-	-
1	3	1.35	38	36	0.24	0.33	0.50
2	21	1.35	38	36	0.26	0.31	0.26
3	30	0.7	50	24	0.40	0.44	0.36
4	12	2	26	48	0.20	0.42	0.33
5	12	0.7	26	24	0.20	0.64	0.84
6	21	1.35	38	36	0.26	0.34	0.30
7	30	0.7	26	24	0.24	0.50	0.35
8	30	2	26	48	0.07	0.53	0.33
9	21	1.35	62	36	0.25	0.49	0.15
10	21	2.65	38	36	0.13	0.44	0.17
11	12	2	50	24	0.11	0.51	0.37
12	12	0.7	26	48	0.40	0.58	0.56
13	21	1.35	38	12	0.18	0.56	0.29
14	21	1.35	38	60	0.12	0.41	0.20
15	21	1.35	38	36	0.26	0.38	0.26
16	30	0.7	50	48	0.09	0.44	0.50
17	21	1.35	14	36	0.22	0.62	0.55
18	21	1.35	38	36	0.26	0.32	0.28
19	30	0.7	26	48	0.08	0.66	0.56
20	12	0.7	50	24	0.15	0.79	0.58
21	30	2	26	24	0.09	0.07	0.56

22	12	2	26	24	0.09	0.53	0.33
23	21	0.05	38	36	0.33	0.52	0.24
24	12	2	50	48	0.21	0.47	0.22
25	30	2	50	48	0.13	0.54	0.16
26	12	0.7	50	48	0.24	0.60	0.20
27	39	1.35	38	36	0.08	0.62	0.55
28	30	2	50	24	0.23	0.46	0.17
29	21	1.35	38	36	0.26	0.32	0.27
30	21	1.35	38	36	0.26	0.35	0.22

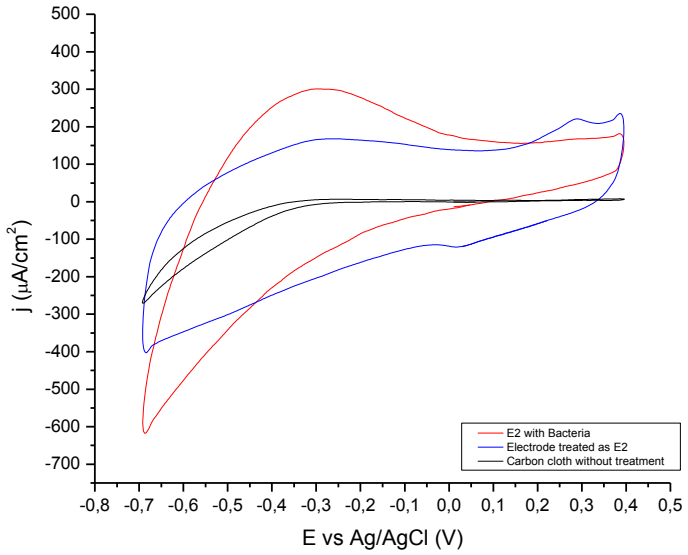

Figure 3. Comparison of the electrode performance. Cyclic Voltammograms at a scan rate of 10 mV/s of the electrode before treatment, after treatment with 21mM $NaCH_3COO$, applied potential of 1.35V at 38ºC during 36h (see E2) and the electrode after biofilm formation.

Since these compounds are good electron acceptors they can promote the electron transfer from the bacteria to the electrode surface and also provide a hydrophilic characteristic [25, 28, 29, 30]. The voltammograms in figure 3 support this fact since the current density of the electrode increased after the treatment under the following conditions: 21mM $NaCH_3COO$, applied potential of 1.35V at 38ºC during 36h. These conditions were the same, which was employed for the analysis of SEM-EDS in table 2.

It has been reported that there are more than one pathway for the electron transfer chain that can be shifted due to the potential applied to develop the biofilm [31]. We observed that the differences on the electrode surfaces due to the treatments provide these differences on the potentials since all the biofilms were formed at -0.42 V vs Ag/AgCl and the kinetic parameters calculated were different (table 3). Then the treatment of the electrodes is related with the type of bacteria in the

biofilm and therefore with the electron chain pathway.

In figure 4 it is observed the different shapes of the biofilm voltammograms obtained from the biofilms formed on electrodes treated with the same applied potential of 2 V and the same concentration of $NaCH_3COO$ of 12 mM but at different temperatures and different time of treatment as E4, E11 and E24 mentioned in table 1. E4 and E24 were treated during 48h at 26 ºC and 50 ºC respectively and it was obtained the current densities of 147 Am^{-2} and 83 Am^{-2} respectively. The current density obtained with E24 was higher (220 Am^{-2}) and the time of the treatment was shorter (24h) as E11. Since the biofilm formed on the electrode treated as E11 showed higher current densities and capacitive response, it shows the need for longer time of treatment to obtain oxidation peaks with better definition.

The changes in the current density may be related to the formation of species with sodium on the electrode surface such as sodium nitrate, for which the main source would be NaCH3COO. The temperatures tested for the electrode treatment can modify the rate of diffusion and deposition on the electrode. Chang et al., 2016 [30] reported the treatment of carbon electrode with the technique of atmospheric pressure plasma jets, where they reported an increase in the current density due to the deposit of the sodium nitrate. This compound, and the carboxyl and the ammonium functional groups can also promote the formation of biofilms and the adhesion of bacteria and therefore the electron transfer from bacteria to the electrode is improved [30]. The composition of the medium for the treatment of the electrode used in this work could promote the formation of the nitrate species to be deposited on the electrode surface.

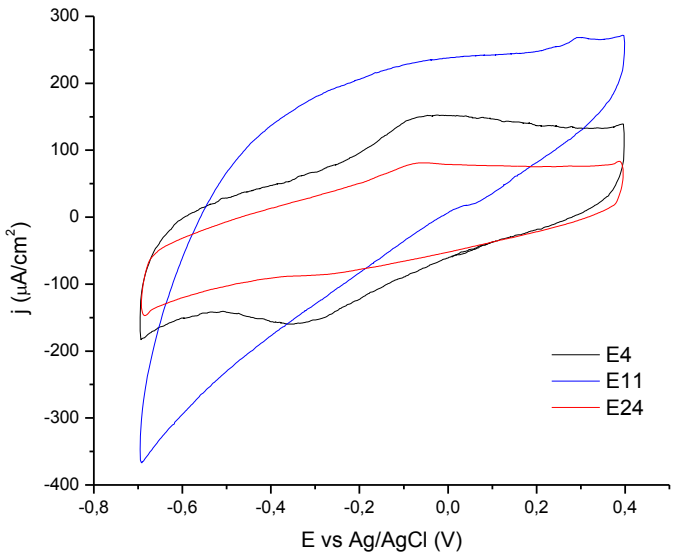

Figure 4. The Cyclic Voltammograms at a scan rate of 10 mV/s for the biofilms formed on the electrodes treated with 12mM $NaCH_3COO$ and applied potential of 2V, at 26ºC during 48h for E4, at 50 ºC during 24h for E11 and at 50 ºC during 48h for E24.

The effect explained before was also observed for the better concentration tested for 30 mM $NaCH_3COO$ and the potential applied for the treatment was 2 V and different temperatures of 26 ºC and 50 ºC for E21 during 26 h and for E25 during 48 h respectively in figure 5. The voltammogram of E25 shows an oxidation peak with higher current density than E21. However, the capacitive response of the voltammograms is probably due to the saturation of the ionic species in the biofilm or to the diversity of the bacteria since it has been reported that different thickness of the same material adsorbed on the electrode resulted in different microbial communities [28].

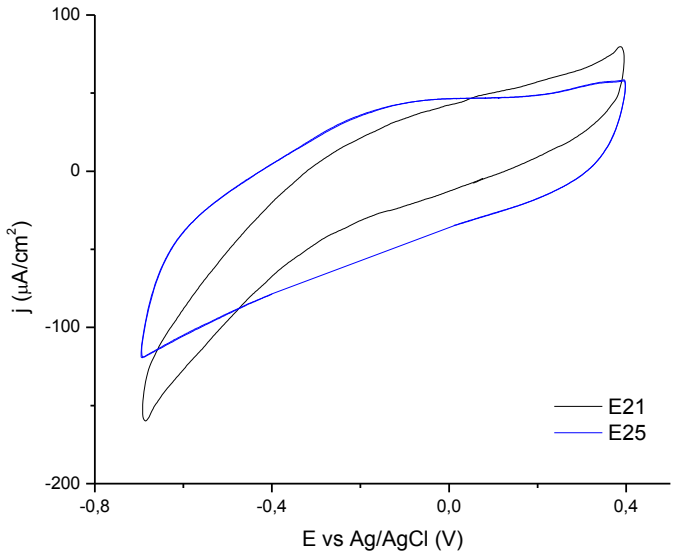

Figure 5. The cyclic voltammograms at a scan rate of 10 mV/s for the biofilms formed on the electrodes treated with 30 mM $NaCH_3COO$ and applied potential of 2 V at 26 ºC during 24 h for E21 and at 50 ºC during 48 h for E25.

The cyclic voltammograms in figure 6 correspond to the biofilms formed on electrodes treated with lower applied potential of 0.7 V. The effect of the concentration of 30 mM of $NaCH_3COO$ was compared during electrode treatments of 24 h and under temperatures of 26 ºC for E5 and E7 and 50 ºC for E20. They showed that the increase in concentration of $NaCH_3COO$ has a major influence on the current density improvement than the effect of the temperature. These conditions compare the species diffusion and promote a biofilm formation, the thickness of layer adsorbed on the electrode must have a limit for the effective electron transfer.

The same effect was observed when higher concentration of $NaCH_3COO$ (39 mM, E27) was compared with 21 mM $NaCH_3COO$ (E2) with an applied potential of 1.35 V at 38 ºC during 36 h. The highest current densities of the oxidation peaks were obtained with the treatment E27, see figure 7. However, the oxidation peaks for E2 and E27 (-0.37 V and -0.04 V respectively) were different.

The figure 7 also compares the highest and the lowest oxidation peaks obtained from the treatments described in table 3. The lowest oxidation peak current density of 74 Am^{-2} was obtained

with E22 when the concentration of the $NaCH_3COO$ was 12 mM and the potential applied was 2 V at 26 ºC for 24 h.

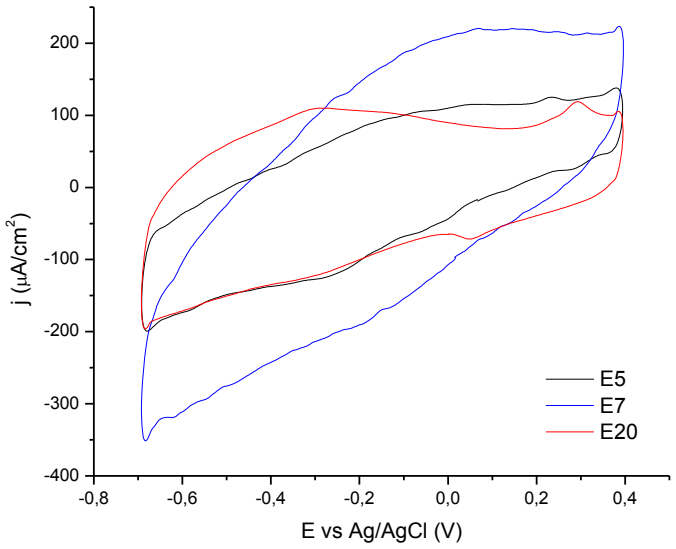

Figure 6. The cyclic voltammograms at a scan rate of 10 mV/s of the biofilms formed on the electrodes treated during 24 h with an applied potential of 0.7 V at 26 ºC and $NaCH_3COO$ concentration of 12 mM for E5, 30 mM for E7, 12 mM and 50 ºC for E20.

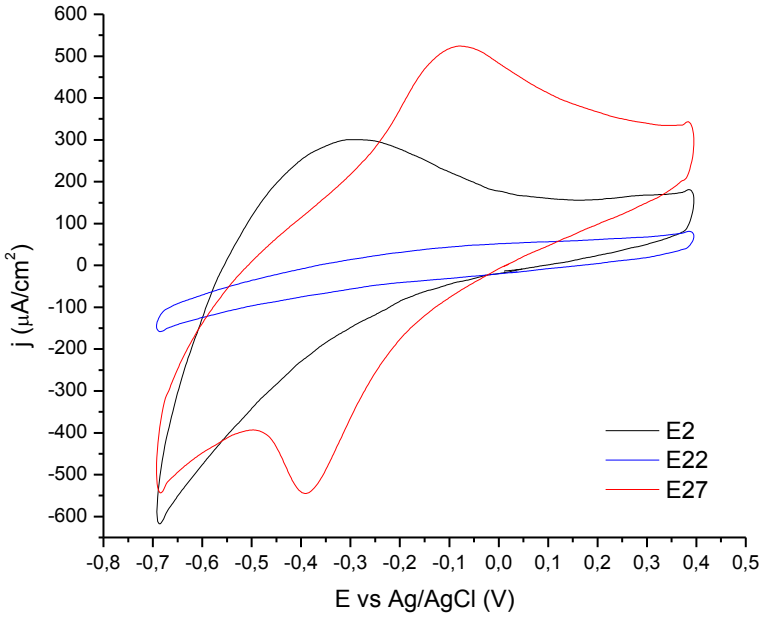

Figure 7. The cyclic voltammograms at a scan rate of 10 mV/s of the biofilms formed on the electrodes treated during 36 h with an applied potential of 1.35 V at 38 ºC and $NaCH_3COO$ concentration of 21 mM for E2, 39 mM for E27 and 12 mM, 2 V, 26 ºC and 24 h for E22.

3.2 Biofilm characterization

All CVs of the biofilm analysis showed a typical response (a linear scan rate dependence) of the species adsorbed on the electrode [32] which proves the relation of the response with the adsorbed bacteria.

The biofilms formed on the electrodes treated under different conditions showed oxidation peaks from -120 mV to 200 mV with current densities of 0.08 $mAcm^{-2}$ to 0.35 $mAcm^{-2}$. These results are comparable with those reported by Martin et al, 2013 [33] for a biofilm formed for 9 days in a MFC operated with similar values of internal and external resistance where an oxidation peak at -170 mV with current density of approximately 0.6 mA/cm^2. This study also suggests that the difference between the resistances allows the development of the different electroactive microorganisms [24]

A recent study also reports the relation of the oxidation peak at -131 mV with the electron transfer through the outer-membrane of c-type cytochromes with an electrode of carbon cloth modified with carbon nanoparticles and at -261 mV with a similar electrode doped with N-doping chemical of pyrrole [34]. Those values of oxidation peaks potentials are close to the ones presented in this study. This is consistent with this study because the conditions employed in the treatments of the electrodes previous to the biofilm formation resulted in different hydrophilic characteristics and internal resistances and hence in the development of different electroactive microorganisms and populations.

The kinetic parameters of α, 1-α and k_{app} calculated from the voltammograms are presented in table 3. A ~ 0.5 was obtained for all cases. This parameter is related to the mechanisms of the electron transport: the first one due to the accumulation of the redox mediator species diffusing from the solution and the second one by the confined redox compounds within the biofilm [35]. To differentiate the mechanism occurring experimentally, Rousseau et al, 2014 have reported to repeat the analysis with CV in a fresh solution [35]. Since the responses of the CVs repeated in fresh solution were the same, the pathway observed experimentally in this study may be due to the confined redox compounds in the biofilm.

Figure 8. Micrograph obtained with the FESEM S5500 Hitachi. Bacteria enrichment during biofilm formation on the surface of the electrode treated during 36 h at 38 ºC with a carbon source of 21 mM $NaCH_3COO$ and an applied potential of 1.35 V (see E2 in table 3).

This is in agreement with the effect observed in the CVs where the current densities decreased for electrodes treated under conditions that favored the diffusion process of the ionic species adsorbed on the electrode surface. This produces a resistive effect and the redox species in the biofilm remain confined showing a capacitive response. This effect is also observed in the kinetic parameters obtained with the biofilms formed on electrodes treated with an applied potential of 1.35 V at 38 ºC for 36 h and concentration of 3 mM $NaCH_3COO$ for E1 and 21 mM $NaCH_3COO$ for E2, E6, E15, E18, E29, E30 were the kinetic parameters were similar except for the number of electrons involved in the redox reaction, which was 0.5 and an average of 0.31 $\pm$0.03 respectively and K_{app} of 0.24 s^{-1} and 0.26 s^{-1} respectively. In both cases this last parameter is higher than the K_{app} of 0.09 s^{-1} obtained from a biofilm formed on carbon paper with conductive nanotube hydrogel electrodeposited in a phosphate buffer (pH7) medium reported by X. W. Liu et al, 2014 [36]. The value of the Kapp < 0.7 s^{-1} has been related with biological electron shuttles such as riboflavin or quinone present on the electrode [37, 36].

However, when concentration of $NaCH_3COO$ was 39 mM in the E27 the number of electrons (n=0.55) was similar to E1 but the apparent electron transfer rate constant was lower (0.08 s^{-1}), which proves that as higher concentration of $NaCH_3COO$ is used in the electrode treatment the current density for the biofilm increases but there is a limit when the electron transfer rate decreases. This effect is in agreement with that reported by Rousseau et al, 2013, [35], where α around unity has no significant effect in the metabolic reaction. This limit was observed when the biofilms compared were formed on electrodes treated with concentrations of 30 mM and 12 mM $NaCH_3COO$ and applied potential of 2 V at 26 ºC for 48 h for E8 and 24 h for E4 and the kinetics parameters were similar.

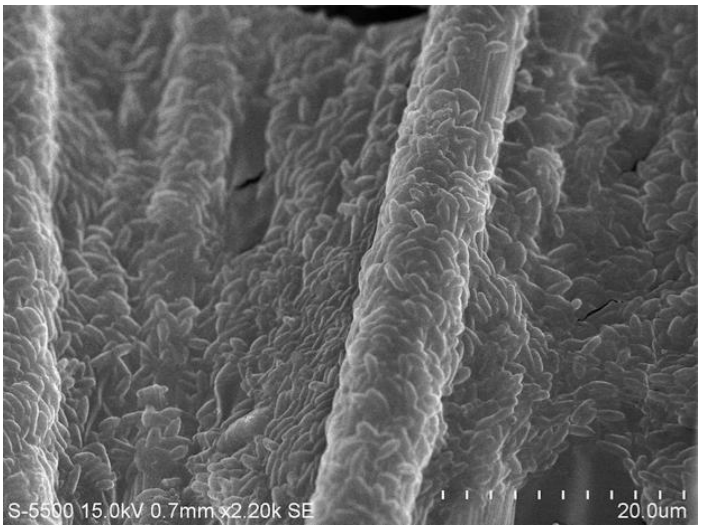

Figure 9. Micrography obtained with the FESEM S5500 Hitachi. Biofilm formed on the surface of the electrode treated during 36 h at 38 ºC with a carbon source of 21 mM $NaCH_3COO$ and an applied potential of 1.35 V (see E2 in table 3).

The comparison of the kinetic parameters for the biofilms formed on electrodes treated with concentration of 12 mM $NaCH_3COO$ and applied potential of 0.7 V at 26 ºC during 24 h for E5 Vs E20 where the temperature was 50 ºC showed different numbers of electrons of 0.84 and 0.58

respectively due to the differences of the temperatures for the electrode treatment. This effect is no longer observable when the time of the treatment is higher (48 h) as in the case of E26 were the number of electrons is 0.2.

Generally, it was observed a relation on the biofilm response due to the conditions of the electrode treatment. The number of electrons calculated from the biofilm increased and the apparent electron transfer rate constant decreased when the concentration of the $NaCH_3COO$ in the electrode treatment increased. However, the effect can be controlled with the adjustment of the potential applied, the temperature and the time of treatment to obtain similar kinetic parameters.

The biofilm formed on the electrode treated (E2) was analyzed with scanning electron microscope (FESEM S5500 Hitachi). The micrograph in figure 8 shows a preference of the bacteria to be attached to the particles formed on the electrode surface during the treatment and the micrograph shows a surface with a population of bacteria with homogeneous morphology and uniform distribution.

3.3 Hydrogen production

The biofilms formed on electrodes treated (E2, E14, E19, E21, E27 and E28) were selected and employed as bioanodes in a membrane-less MEC. The maximum volumetric hydrogen production rate was 0.38 m^3 H_2/m^3 d obtained with the MEC with the electrode treated with 21 mM $NaCH_3COO$, for an applied potential of 1.31 V at 38 ºC for 36 h (E2). There was also hydrogen production with the MEC operated with the bioanode and the electrode treated under the same conditions for longer times (60 h, E14) were the hydrogen production rate was 0.21 m^3 H_2/m^3d. There was no hydrogen production with the other bioanodes tested.

4. CONCLUSIONS

The electrode treatment described in this study is an effective process to promote the biofilm formation with exoelectrogenic bacteria. The kinetics of the bioanode can be controlled and predicted from the parameters employed in the electrode treatment. The parameters of the treatment may provide differences on the surface resistance of the electrode and this suggests that different types of microorganisms can be selected for the biofilm formation. However, it is necessary to identify the microorganisms in the biofilms obtained with different conditions of pretreatment. It was also observed an increase in the kinetic parameters when concentration of $NaCH_3COO$ increased in the electrode treatment and the potential applied for values of 1.35 V and less at ambient temperature. However, when the potential applied was higher and the concentration of the $NaCH_3COO$ increased, the results of the kinetics parameters were lower and have a slightly positive effect with the increase of temperature. In this work it was also proved the capability of the bioanodes to be employed in a MEC for hydrogen production. The information obtained from this work offers different possibilities of treatment that can be useful to obtain bioanodes for scaled-up of MEC.

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