Study of different carbon materials for their use as bioanodes in microbial fuel cells
Catalina González-Nava, Luis A. Godínez, Abraham U. Chávez, Bibiana Cercado, Luis G. Arriaga and Francisco J. Rodríguez-Valadez

ABSTRACT

Microbial fuel cells (MFCs) are capable of removing the organic matter contained in water while generating a certain amount of electrical power at the same time. One of the most important aspects in the operation of MFCs is the formation of biofilms on the anode. Here, we report the characterization of different carbon electrodes and biofilm using a rapid and easy methodology for the growth of biofilms. The biofilms were developed and generated a voltage in less than 4 days, obtaining a maximum of 0.3 V in the cells. Scanning electron microscopy images revealed that growth of the biofilm was only on the surface of the electrode, and consequently both carbon cloth Electrochem and carbon cloth Roe materials showed a greater quantity of volatile solids on the surface of the anode and power density. The results suggested that the best support was carbon cloth Electrochem because it generated a power density of 13.4 mW/m² and required only a few hours for the formation of the biofilm.

Key words | carbon electrodes, characterization, growth of biofilm, microbial fuel cells, mixed-culture biofilms

INTRODUCTION

Microbial fuel cells (MFCs) represent a new alternative for the treatment of wastewater, since they are capable of removing the organic matter contained in water, while generating a certain amount of electrical power at the same time (Logan 2005). The MFC consists of an anodic compartment where electrochemically active microorganisms grow on the surface of an electrode; the microorganisms oxidize organic matter and the electrons can be transported by an electromotive force (Logan et al. 2006; Logan 2007). These electrons are transferred subsequently through an external circuit to the cathode where the electrons are then released to a terminal electron acceptor, for example, oxygen, nitrate, and sulfate (Logan 2007). One of the most important aspects in the operation of the MFC is the formation of biofilms on the anode, since long time periods are needed for the formation of the biofilm, taking from 4 to more than 40 days (Zhao et al. 2012). The imposition of a potential to the anode, ranging from –0.2 to 0.6 V vs. Ag/AgCl, has also been used for the formation of biofilms and decreases the time required for the biofilm formation and the resistance to electron transfer (Aelterman et al. 2008; Cercado et al. 2013). However, a potentiostat is required to fix the potential on the anode. Biofilms have also been synthesized using pure cultures such as the genera Rhodoferax, Geobacter, Shewanella, Klebsiella, and Clostridium (Dumas et al. 2008). However, the use of pure cultures is complicated and requires the use of sterile conditions, 5 days in incubation, and 5 to 9 days for the formation of the biofilm (Dumas et al. 2008; Richter et al. 2009). In addition, low energy production has been reported (Nevin et al. 2008). On the other hand, the biofilm needs to be supported on a conductive material that allows the growth of the microorganisms. Carbon materials are the most suitable due to their biocompatibility, low cost, good conductivity, and good chemical stability (Logan et al. 2006; Wei et al. 2011). Some configurations have been used in the construction of MFCs such as planar structures (carbon paper, graphite plates, sheets, and carbon cloth), thick porous carbon-based material (reticulated vitrified carbon, carbon felt (CF), and foam), packed structures (granular graphite, graphite rods, granular activated carbon, graphite cubes, or CF) and graphite brush structures (Wei et al. 2011).
order to develop a method which allows the synthesis of biofilms in short periods of time and is easy to implement at low cost, this work evaluated the formation of biofilms on different carbon materials, carbon cloth and felt, using an inoculum of activated sludge from a wastewater treatment plant without the application of an external potential.

**METHODS**

Configuration and operation of the MFC

To perform the experimental tests, MFC acrylic type H with two compartments was used. The volume of the MFC was 375 mL and the compartments were separated by a proton exchange membrane (Nafion 117, DuPont) (Figure 1). The biofilms were grown on electrodes (6 cm length and 3 cm width) using different carbon materials (Figure 2): carbon cloth Electrochem (CC1) supplied by Electrochem, as well as carbon cloth Roe (CC2) and CF, which were supplied by Roe Group. In all cases, CC1 with the same size was used as a cathode to maintain this constant parameter in all cells because this material has been used successfully as a cathode in MFCs in previous works (Zhang et al. 2011; Liu et al. 2012; Martin et al. 2013). The anodic compartment was filled with 150 mL of inoculum, 10 mM of sodium acetate (Richter et al. 2009; Patil et al. 2011; Choi & Chae 2013), and nutrient solution, with a chemical oxygen demand of 933 ± 14.2 mg/L. The inoculum was from sediment formed in an activated sludge reactor and the nutrient solution was 0.4 mL of the following solutions: magnesium sulfate solution: 22.5 g MgSO₄·7H₂O in distilled water and diluted to 1 L; calcium chloride solution: 27.5 g CaCl₂ in distilled water and diluted to 1 L; ferric chloride solution: 0.25 g FeCl₃·6H₂O in distilled water and diluted to 1 L; and phosphate buffer pH 7.2: 8.5 g KH₂PO₄, 21.75 g K₂HPO₄, 33.4 g Na₂HPO₄·7H₂O, and 1.7 g NH₄Cl in about 500 mL of distilled water and diluted to 1 L (American Public Health Association et al. 2012). The anodic compartment was stirred all the time (IKA Color Squid White, USA) and the cathodic compartment was filled with distilled water and remained in aeration using a pump (Elite 800). The cell was maintained at a constant temperature of 25 °C using a temperature bath (Wisd Laboratory instrument) to maintain ambient temperature. All solutions in the anodic chamber were bubbled with nitrogen gas for 15 minutes before use, to exclude air and oxygen in the chamber, and it was sealed to prevent oxygen diffusion into the chamber, as indicated by some authors (Logan 2005; Patil et al. 2011). The voltage (U) of the cells was measured every 60 minutes using an external resistor of 5KΩ and using a data acquisition system connected to a PC. The current (I) was calculated by using Ohm’s law, \( I = \frac{U}{R} \) where \( R = \) resistance, and the power (P) by Joule’s law \( P = UI \) (Choi & Chae 2013). Once exhaustion of the substrate was observed, a new amount of acetate was added to obtain a 10 mM concentration in the solution.

Carbon electrodes’ characterization

In order to characterize the different carbon materials, measurements of contact angle between the surface of each material and a drop of hydrophilic liquid (deionized water) were taken using a microscope (Kruss-DSA30) and the software Drop Shape Analysis DSA4 (version 1.1). Each carbon material was cut into 2×2 cm, the pieces were mounted on the microscope and the drop of water was put over the surface. The volume of deionized water was 5 μL and the measurements were done with the software. Average value for each material was obtained from 10 measurements (He et al. 2012). Raman spectroscopy was achieved with carbon material pieces (2×2 cm), which were mounted on a DXR Raman dispersive microspectrometer (Thermo Scientific), and the measurements were done with 480 nm as the excitation source. The laser
was focused to approximately 1,000 μm in diameter with a power of 10 mW at the sample’s surface in order to prevent thermal degradation of the carbon. The spectra were obtained over the range from 70 to 3,400 cm$^{-1}$ with an average of 100 scans in order to obtain a low signal-to-noise ratio. From the obtained surface spectra intensities, the D and G bands of carbon materials were quantified, and their respective I$_D$/I$_G$ ratio was calculated. The diameter of the fibers of the carbon materials was determined using Raman microscopy. In order to know the surface area of the electrodes, the capacitance ratio method was used (Alves et al. 1998; Wang et al. 2008), performed with cyclic voltammetry, using a three-electrode system in a 0.5 M sulfuric acid solution and a scan rate from 20 to 200 mV/s (Alves et al. 1998). Between each voltammetry, turbulent agitation was used and the area calculations were performed using the following equation:

$$A = \frac{C_{dl}}{C_{st}}$$

where $A$ is surface area (cm$^2$), $C_{dl}$ is double layer capacitance (F), and $C_{st}$ is reference value for the capacitance ($C_{st} = 60 \mu$F/cm$^{-2}$ for rough solid surfaces (Trasatti & Petrii 1991).

**Characterization of biofilm on different carbon electrodes**

The growth of microorganisms on different electrodes was analyzed by scanning electron microscopy (SEM). The biofilm was fixed according to the methodology reported by Zhang et al. (2011) by cutting fragments of electrodes to 2 × 2 cm, which were then immersed for 1 hour in a solution prepared with 0.5 g of collodion (SPI-CHEM) in 40 mL of amyl acetate collodion solution. Subsequently, these biofilms were dehydrated by graded ethanol solutions (30, 50, 70, 80, 90, and 100%) and dried overnight in the dark. Biofilm pieces were collocated on an aluminum plate, fixed with contact adhesive and then the SEM was performed. In addition, the amount of biomass present on the electrodes was determined by measuring the amount of volatile solids (VS). For this measurement, three electrodes of each material (similar surface area), with and without biofilm, were dried at 105°C for 24 hours in an oven (Thermo Scientific) and then weighed. Next, the electrodes were burned at 550°C for 20 minutes in an oven (Wisd Laboratory 800C) and the weight was measured. Finally, the electrodes were dried again at 105°C for 24 hours. The amount of VS was calculated by the difference in weight of each electrode, with and without biomass, after the drying process (Peixoto et al. 2011).

**Electrochemical analysis**

Once the biofilm had grown on the anode, cyclic voltammetry was used to characterize the biofilms. The potential range was from −1.0 to 1.0 V and the sweep rate of potential was 20 mV/s. All experiments were performed using a potentiostat (BASI Epsilon-EC, Bioanalytical Systems), an Ag/AgCl reference electrode (0.209 V vs. normal hydrogen electrode) and CC1 as a cathode in all cases. The polarization curve was obtained by linear sweep voltammetry at a scan rate of 1 mV/s (Liu et al. 2012). The current density and power density were calculated based on measured anodic area. In order to measure the potential of the electrodes, a three-electrode system was set up in the anode and cathode compartments.

**RESULTS AND DISCUSSION**

**Carbon electrodes’ characterization**

Figure 3 shows the Raman spectra obtained with the three carbon materials. As can be seen in Figure 3(a), the spectra have two peaks located at 1,320 cm$^{-1}$ and 1,600 cm$^{-1}$ that are associated with defects of amorphous carbon (D) and graphitized carbon or aromatic rings (G), respectively (Bañuelos et al. 2015). The ratio between amorphous and crystalline material was evaluated by the relationship between the peak intensity ip$_D$/ip$_G$ of each carbon material as shown in Figure 3(b). The ratios were 1.8 for CC1, 0.46 for CC2, and 1.0 for CF, indicating that CC2 contains a lower amount of amorphous material and a larger amount of crystalline material, CC1 has a larger amount of amorphous material, while CF presented equal amounts of both structures. The differences in ip$_D$/ip$_G$ were associated with possible differences in the conditions used for the preparation of the materials. According to Kinoshita (1988), the crystalline material can provide a higher conductivity; thus the conductivity for each carbon material was measured and the results were as follows: 3,533, 3,333, and 613 S/m, for CC1, CC2, and CF, respectively. Using the Raman analysis microscope, the size of the fibers was determined as follows: 6.8 ± 2, 19.5 ± 2, and 11.5 ± 2 μm for CC1, CC2, and CF, respectively.
In regard to the contact angle, the angle \( \theta \) measured in the three carbon materials was 166 ± 4° for CC1, 167 ± 6° for CC2, and 170 ± 4° for CF. These results do not show significant differences between the carbon materials, and the values indicate a high hydrophobicity even greater than that reported by He et al. (2012) for carbon paper with a value of 114°. The large contact angles are related to the high hydrophobicity and roughness of the materials, as can be seen in SEM micrographs. The hydrophobic/hydrophilic properties in carbon material could be beneficial for bacterial attachment, but these results will not contribute to biofilm formation difference.

**Biofilm formation on different carbon electrode materials**

In Figure 4, the generated voltage vs. time of MFC operation is observed. In all cases, the voltage response can be observed before 4 days, and in the case of CC1 and CF appreciable voltage is seen in just a few hours after starting the experiment. The generated voltage is related to the growth of biofilm, because the microorganisms are producing and transferring electrons to the electrode; so the voltage is the result of the rapid formation of the biofilm on the surface of the electrode. In the same figure, it can also be observed that the response to successive feeds of acetate when the substrate had been exhausted reached values in the three cases near 0.3 V, which is a characteristic value obtained in MFCs (Yang et al. 2012).

**SEM micrographs of the structure of the biofilm on felt and carbon cloth**

SEM images in Figure 5 show the surface of different carbon materials without biofilm. This figure shows how the fibers are connected: CC1 and CC2 show an organized similar arrangement, but CC2 shows thick fibers and fabric. CF shows a disorganized arrangement but with micro-cavities that could contribute to the different biofilm formation between carbon cloth and CF. SEM images of the surface of the biofilms grown on the anodes of different carbon materials are shown in Figure 6. As can be seen, the biofilm did not totally cover the electrode surface in all cases and showed extensive voids with marks of roughness and porosity of the electrode material. The uniform structure presented by CC1 is the most suitable since it has been reported that the existence of voids in the biofilm may result in less contact for the transfer of electrons and may decrease the performance of the MFC (Zhang et al. 2011). Images with higher amplification (Figure 6(d)–6(f)) show that the biofilms were only developed on the surface of the electrode and only a few bacteria grew into the porous carbon material’s matrix. Similar phenomena have been reported on glassy carbon (Torres et al. 2009), carbon cloth, and graphite plates, which have been attributed to both the small pore size and the limited amount of substrate supply within the carbon materials (Zhang et al. 2011). The SEM images (Figure 6(d)–6(f)) at higher magnification show an arrangement of similar spherical microorganisms for CC1 and CC2, while on the felt, different
microorganisms are observed. This may be due to the arrangement of the fibers on each of the carbon materials.

Performance evaluation of the biofilm in the MFCs

The measurement of the surface area indicates that CF showed the largest area at 190.6 cm², compared to CC2 and CC1 with 10.2 cm² and 8.2 cm², respectively, as shown in Table 1. By determining the VS in the bioanodes and calculating the VS per unit of surface area, the values of VS were obtained (Table 1). The amount of biofilm formed on the CC2 anode was 8.0 mg/cm², followed by CC1 with 4.8 mg/cm², and 1.6 mg/cm² for the CF anode; so the best support for the growth of the biofilm is the thick carbon cloth. Also CF has the largest surface area. The open circuit voltages determined on the anode just in the stable phase (where the voltage in the cell is near 0.3 V) were very similar for the three different carbon materials, with −0.58, −0.58, and −0.55 V vs. Ag/AgCl being determined in the CC1, CC2, and CF electrodes, respectively (Table 1). These values were comparable to others obtained in earlier studies that reported voltages between −0.30 and −0.42 V vs. Ag/AgCl, as a result of the exoelectrogenic microbial activity on the surface of electrodes (Martin et al. 2013). The voltage on the cathode shows similar values in all cases, where CC1, CC2, and CF generated −0.01, −0.03, and 0.01 V vs. Ag/AgCl. The voltage difference between the anode and cathode assured the operation of the cells, which coincides with a study which reported that a high potential in the cell is obtained when the anode potential is more negative and the cathode is positive (Zhao et al. 2012). As shown in Table 1, the pH in the anodic compartment showed no significant difference because it remained in all cases at values between 8.5 and 9.0 which are suitable for the development of the microbial system. Also, the conductivity in the cells remained at values between 3.0 and 3.3 mS/cm; so, in this case, neither the pH nor conductivity is a factor that determines the response of the biofilm in the MFC.

The polarization curves included in Figure 7 show that the open circuit voltage measured in the stable phase for CC1, CC2, and CF was around 0.6 V in all cases. The initial steeper slope, occasionally observed in polarization curves due to activation losses (Logan et al. 2006), was not observed in the present study (Peixoto et al. 2011). According to these polarization curves, the maximum current density obtained for CC2 was 145 mA/m², while 150 and 5 mA/m² were determined for CC1 and for CF (Table 2).

By determining the maximum power output per unit area from the polarization curves (Figure 7), a value of 13.4 mW/m² was calculated for CC1, 15.6 mW/m² for CC2, and 0.5 mW/m² for CF. Considering that the voltage remains almost constant near 0.6 V in the three electrode materials (Figure 7), the differences in \( P_{\text{max}} \) in the MFC can be associated with the maximum current density \( j \), since CC1 and CC2 showed values around 150 mA/m² while the CF produces only 5 mA/m² (Table 2). At the same time, \( j \) could be determined by the quantity of biomass per unit area (VS) attached to the electrode, which is bigger for CC1 and CC2 (4.8 mg/cm² and 8.0 mg/cm², respectively) compared with the CF (1.6 mg/cm²). According to this, the differences in \( P_{\text{max}} \) are due to differences in the

Figure 4 | Simultaneous monitoring of voltage generated during the growth of the biofilm on different electrode materials: CC1, CC2, and CF; (a) and (b) correspond to test repetitions.

Downloaded from https://iwaponline.com/wst/article-pdf/73/12/2849/363006/wst073122849.pdf by guest on 08 November 2018
amount of biomass on the surface of the electrodes. In addition, cells with CC2 electrodes showed the lowest internal resistance (4.1 KΩ) compared with the CC1 and CF electrodes, which showed internal resistance of 5.9 KΩ (Table 2). The power density in all cases is low because Pt was not used as a catalyst in the cathode, there was no pretreatment of the material of the electrodes, and the distribution of the elements in the cell may not be adequate. The cyclic voltammograms of Figure 8 show oxidation and reduction peaks as a result of the electrochemical activity of the biofilm on the anode surface (Zhao et al. 2012). The signals from the electrodes of CC1 and CC2 are more defined compared with CF, which may be due to the larger area of the material. According to previous reports, the observed peaks in voltammograms can be associated with bioelectrooxidation of acetate due to the appearance of peaks at −0.26 and −0.16 V vs. standard hydrogen electrode (SHE) (Zhang et al. 2011), the mediators produced by microorganisms with peaks at a potential of −0.10 and −0.18 V vs. SHE (Zhang et al. 2011), or the response of
Table 1  | Comparison of different carbon anodes in MFCs

<table>
<thead>
<tr>
<th></th>
<th>CC1</th>
<th>CC2</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area of electrode (cm²)a</td>
<td>8.2</td>
<td>10.2</td>
<td>190.6</td>
</tr>
<tr>
<td>VS on anode (mg/cm²)b</td>
<td>4.8 ± 0.1</td>
<td>8.0 ± 0.9</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>( E_{\text{anode}} ) (V vs. Ag/AgCl)c</td>
<td>−0.58 ± 0.006</td>
<td>−0.58 ± 0.002</td>
<td>−0.55 ± 0.028</td>
</tr>
<tr>
<td>( E_{\text{cathode}} ) (V vs. Ag/AgCl)</td>
<td>−0.01 ± 0.014</td>
<td>−0.03 ± 0.004</td>
<td>−0.01 ± 0.013</td>
</tr>
<tr>
<td>pH</td>
<td>9.0 ± 0.2</td>
<td>8.7 ± 0.04</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>3.3 ± 0.06</td>
<td>3.2 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

*aThe surface area was measured with 2 × 2 cm carbon electrodes.
*bVS on anode (mg/cm²) is related to the calculated surface area.
*c\( E \) = potential.

Table 2  | Comparison of different carbon anodes in MFCs

<table>
<thead>
<tr>
<th></th>
<th>CC1</th>
<th>CC2</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>( j ) (mA/m²)</td>
<td>145</td>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>( R_{\text{int}} ) (KΩ)</td>
<td>5.9 ± 1.9</td>
<td>4.1 ± 0.1</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>( P_{\text{max}} ) (mW/m²)</td>
<td>13.4 ± 1.7</td>
<td>15.6 ± 1.1</td>
<td>0.5 ± 0.03</td>
</tr>
</tbody>
</table>

Figure 7  | Polarization and power output curves obtained for cells with different anode materials; (a) and (b) correspond to test repetitions.

Figure 8  | CV analysis of biofilm on different carbon material anodes, CC1, CC2, and CF; (a) and (b) correspond to test repetitions.
electron transfer sites relating to cytochromes present on the cell membrane of the microorganisms (Patil et al. 2011; Martin et al. 2013).

CONCLUSIONS

A rapid, easy and low-cost methodology for the growth of biofilms in MFCs was developed using CC1, CC2 and CF as a support, and an inoculum obtained from a wastewater treatment plant. In all cases, the response in voltage can be seen before 4 days but, in the case of the CC1 and CF, a signal can be detected as early as 6 hours, obtaining a maximum voltage near 0.3 V in the MFCs. The CF has a greater surface area, but SEM images show that the growth of the biofilm is only on the surface of the electrode; so both cloths CC1 and CC2 show a greater quantity of VS and power density. In accordance with that, the best support is CC1 because it produces a high power density (13.41 mW/m²) and requires only a few hours for the formation of the biofilm. Hydrophobic/hydrophilic properties of the support electrodes were similar; so there is no significant effect from the chemical properties of the material on the growth of the biofilm.

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