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Electrochemical Evaluation of Ti/TiO_2-polyaniline Anodes for Microbial Fuel Cells using Hypersaline Microbial Consortia for Synthetic-wastewater Treatment

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Abstract: *A Ti/TiO_2-polyaniline (PAni) composite electrode was developed and explored with electrochemical methods. The results of electrochemical impedance spectroscopy (EIS) demonstrate that under abiotic conditions the cathode is the limiting component of the electrochemical cell for the occurrence of the charge-transfer reactions. In contrast, when the Ti/TiO_2-PAni composite is working as anode in the microbial fuel cells (MFCs) studied, the controlling electrochemical processes occur at the anode. The higher power densities, 2317 mW/m², were obtained with a pure culture of Geobacter sulfurreducens, and significant power density was obtained with an uncharacterized consortium, 1137 mW/m². The results showed that the Ti/TiO_2-PAni anode is a suitable material to be optimized for developing high-power MFCs with G. sulfurreducens for wastewater treatment.*

Keywords: microbial fuel cell (MFC), polyaniline (PAni), impedance spectroscopy (EIS), Geobacter sulfurreducens

1. INTRODUCTION

The fossil-fuel based civilization faces numerous problems. On one hand, there is the eventual exhaustion of fossil fuels; and the other, the persistent contamination of water caused by industrial, agricultural and municipal discharges. For the energy problem, several renewable sources have been considered. Bioenergy contributes about 10%–15% of the world primary energy supply—being the most important energy source after fossil fuels. It consists of several resources: (i) solid biomass that can be directly used as fuel, (ii) liquid biofuels such as ethanol, biodiesel, etc., (iii) biogas, (iv) biohydrogen and (v) bioelectrogenesis: direct generation of electricity using biological organisms or their components in fuel cells. Microbial Fuel Cells (MFCs) are devices that generate electricity through the microbial biodegradation of organic compounds, as found in wastewater [1]. In recent years, some investigations have reported power densities of 500 mW/m^2 using domestic wastewater, and 1,500 mW/m^2 to 3,600 mW/m^2 using glucose as carbon source in the anodic compartment of MFCs [2]. The good performance of MFCs depends on their design, the bacteria involved (pure cultures or consortia), the conditions in the anodic compartment (pH, temperature, dissolved oxygen concentration), the type of fuel (a sole carbon source, a complex media or wastewater), and the materials and configuration of the electrodes, among other factors [3,4]. Different electrode materials and electrode modifications have been used in order to improve the performance of these devices. Conductive polymer-based electrodes, such as polyaniline (PAni) over Pt, fluorinated PAni over graphite, and 1,4-naphtoquinone derivatives over carbon cloth, have been evaluated as potential anode materials [5,6,7,8]. PAni is a low-cost and easily synthesized polymer that has shown good electrical conductivity when used in MFCs [9]. In contrast, Ti/TiO_2 has shown to be an insulating material [10]; however, with some modifications, its surface properties can be enhanced, varying from being a highly resistive to a low resistive material [11]. This has allowed Ti/TiO_2-PAni composite substrates to be used as anode in MFCs, using *Escherichia coli* as biocatalyst [9]. This research uses the emeraldine form of polyaniline deposited over Ti/TiO_2 electrodes in membrane-less and mediator-less MFCs. In parallel, most studies on MFCs use pure microbes; *Shewanella putrefaciens*, *Escherichia coli*, *Geobacter sulfurreducens* and

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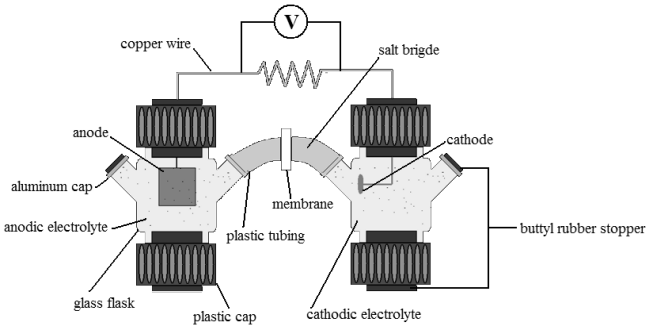

Figure 1. Schematic diagram of the MFC used for this investigation. Electrons are transferred from acetate oxidation to the Ti/TiO_2-PAni composite anodes; oxygen from compressed air is reduced at the cathode.

Rhodoferax ferrireducens are the most commonly used bacteria for this purpose. The two latter are remarkable for the coulombic efficiency (~98%), with which they transfer electrons to the anode from glucose and acetate, although this parameter does not always imply a high energy transfer and it is necessary to know the power density generated. Studies with mixed cultures and microbial consortia have also been investigated; coulombic efficiencies comparable to those of pure cultures (~90%) have been found [12]. Those systems have showed to recover up to 79% of the energy contained in the bonds of the organic molecules that serve as carbon source; however, neither the mixed cultures nor the consortia have shown the energy recovery obtained with *Geobacter sulfurreducens* [12,13]. In consequence, this investigation presents an analysis of the MFCs performance using a stabilized microbial consortia isolated from hypersaline sediments from Yucatan, Mexico, which is compared to a model system that uses *Geobacter sulfurreducens* as sole biocatalyst and it is characterized with electrochemical methods that include Electrochemical Impedance Spectroscopy (EIS). The power output obtained with the anodes and bacteria tested showed good agreement with scientific literature and makes them suitable for optimization for further use in wastewater treatment.

2. EXPERIMENTAL

2.1. Microorganisms and culture conditions

A modified NBAF medium [14] was used with 20 mM acetate as the electron donor and 53 mM fumarate as the electron acceptor, for 96 hours at 37 °C. The media were supplemented with 5g/L HCl cysteine, 0.1% yeast extract and 0.1% of resazurine was added as redox indicator; pH was adjusted to 7.2. The strains were cultured under strict anaerobic conditions, as described in previous works [15]. After the cultures were grown they were used to inoculate the anodic compartment of the MFCs. Ti/TiO_2-PAni coupons (described later in this article) with a surface of 1.5 cm^2 were placed into the culture flasks in order to promote biofilm growth. 10% v/v was used as inoculum. Two different types of microbial cultures were used: A) pure cultures of *Geobacter sulfurreducens* (ATCC 51573), and B) an uncharacterized stable microbial consortia isolated from hypersaline swamp-sediments (~40-50 cm depth) of *Laguna Rosada* in Yucatan, Mexico. 3 g of homogenized sediment sample were placed in the described medium supplemented with 1% of yeast extract, under sterile and strict anaerobic conditions.

2.2. MFCs configuration

The MFCs array is presented in Figure 1. Each compartment of the MFCs was made of a cylindrical flask of 150 mL. The compartments were joined by rubber tubing filled with conductive electrolyte. The rubber tubing had inserted a 0.25 µm pore diameter Millipore membrane for avoiding bacterial contamination of the cathode chamber. No proton-exchange membrane was used. The anodic chamber was completely filled with the inoculated electrolyte; this is the previously described modified NBAF medium without fumarate. The cathodic compartment contained 80 mL of sterile saline solution (0.9% NaCl). Strict anaerobic conditions were maintained in the anodic chamber [15,16]. Avoiding aerobic respiration prevents severe impact in the power output of the MFC. The cathodic compartment was continuously pumped with sterile compressed air, through a 0.22-µM-pore-size Millipore filter. All joints and interstices were sealed with water-impermeable heat-resistant silicone that is non-toxic for bacteria. Both chambers operated in stagnant conditions, since magnetic stirring might affect the performance of the electrochemical monitoring techniques.

2.3. Electrodes

The anodes were made with the emeraldine form of PAni deposited over Ti/TiO_2 electrodes. Ti/TiO_2 electrodes were prepared as follows. ASTM grade 2 titanium (Ti) plates (surface of about 10 cm^2) were thermally oxidized in air at 500°C for 30 min, as described by Ávila-García et al. [17], blue-colored coatings were obtained. The procedure described by Castrellón-Uribe et al. was used [18] for PAni polymerization. Drops of 0.4 mL of aniline monomers (99.7%, J.T. Baker) were added to a 0.2 M HCl solution at room temperature. After dissolution, 60 mL of $(NH_4)_2S_2O_8$ 0.1M were added for initiating the polymerization. Deposition of the PAni formed was carried out by immersion of the Ti/TiO_2 electrodes for 1 h. Green-colored films were obtained over the blue-colored coatings. Subsequently, the electrodes were immersed in aqueous 2M HCL solution and in 4000 ppm aqueous ammonia. Thus, PAni/TiO_2 composite anodes for MFCs were obtained, and this particular method guarantees the formation of emeraldine. The cathodes consisted of mesh electrodes of Pt/Ir (97%:3%) about 5.2 cm^2.

2.4. Electrochemical characterization

The electrochemical interface Voltalab 80 was used for voltage (V, mV)-current (I, mA) analysis and Electrochemical Impedance Spectroscopy (EIS) monitoring. Measurements were carried out at an initial time (0 h) and at 48 h. The Open Circuit Potential (OCP) of the MFCs stabilized before being monitoring during 2 hours; later on it was measured during 30 min, 1 point each 0.2 s. Current was simultaneously measured under the same conditions. Power densities (PD) were calculated in mW/m^2 from the V and I: PD=VI/a, a= area of the anode (m^2). Measurements were carried out at laboratory temperature (25 °C). EIS measurements were taken logarithmically (20 points/decade), with an amplitude of 10 mV, in a frequency range of 100 kHz-1 mHz.

3. RESULTS AND DISCUSSION

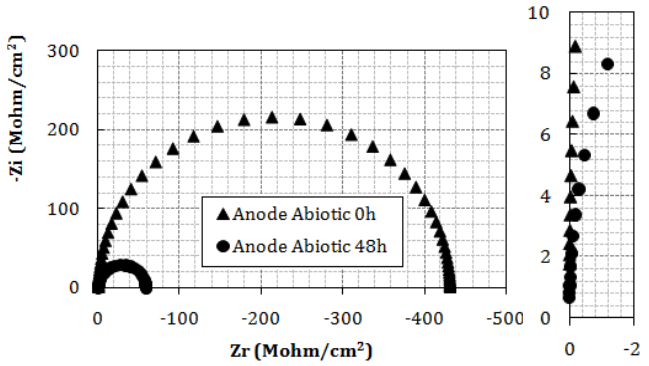

Figure 2. Nyquist plots of Ti/TiO$_2$-PAni anode under abiotic strict anaerobic conditions. The inset on the right illustrates the high-frequency part of the impedance spectra.

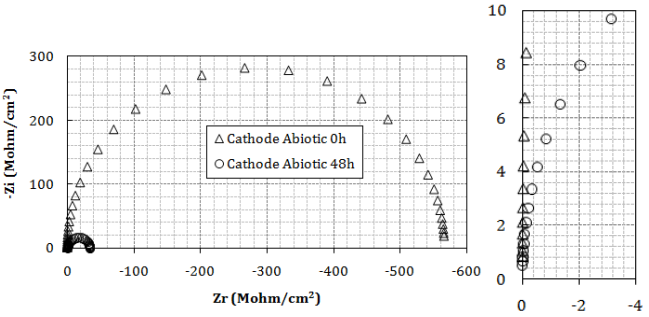

Figure 3. Nyquist plots of Pt/Ir cathode under aerobic environment. The inset on the right illustrates the high-frequency part of the impedance spectra.

3.1. Electrochemical Impedance Spectroscopy

It is convenient to point out that negative real impedances were obtained. This behavior was consistently reproduced in all the systems studied with the Ti/TiO$_2$-PAni anodes. An elucidation of the nature of the negative impedance in detail seems to be rather complicated and requires a special analysis (beyond the scope of this article); thus, this issue limited the analysis of the impedance curves to a qualitative examination on the electron-transfer rates occurring at the electrode-electrolyte interfaces. Negative real impedances are sometimes associated with persistent current reductions as potential increases; this might be characteristic of unstable electrochemical systems e.g. when some of the following processes might take place: a) potential-dependent adsorption/desorption of a catalyst, b) electrostatic effects at low ionic strengths, as in the reduction of anions at a negatively charged surface, or c) when the active surface available for electron-transfer decreases as polarization raises (for example, when the interface shows activity peaks followed by passivity stabilization); in these cases, a part of the polarization curve (or voltammogram if it is the case) has a negative slope—the value of the impedance at zero frequency is related to the slope of the steady-state polarization curve—then the impedance measured in the potential range where this occurs is found with a negative real part [19,20]. A more exhaustive survey on this matter requires information on the kinetics and the mechanisms of the electrochemical processes taking place at the anode surface. Quantitative information is anyways provided taking into account the effective current densities and potentials corrected vs. SCE for calculating the peak power densities.

3.1.1 Abiotic system characterization

First, the modified Ti/TiO$_2$ anodes were characterized with EIS, without the addition of bacteria. Since, only one-time constant was observed in all cases, a simple equivalent circuit (a solution resistance, a constant phase element and a charge transfer resistance) was used for modeling the complex impedances [16]. The frequency response (Nyquist plots) is shown in Figure 2, for the Ti/TiO$_2$-PAni anode operating as working electrode under strict anaerobic conditions, while the cathode was working as counter and pseudo-reference electrode under aerobic atmosphere. A well defined semicircle-like behavior is observed at initial time (0h) and after 48h of exposure to the synthetic media. The charge transfer resistance (R_{ct}) is measured as the absolute value of the intercept of the semicircle with the real axis at low frequencies, minus the electrolyte resistance found in the intercept with the real axis at high frequencies [16]. This parameter is inversely proportional to the exchange current density (i_o) of the reaction that takes place at each electrode [21,22], thus it describes the electron-transfer rate. An important reduction of the charge transfer resistance of the anode under abiotic conditions (R_{ct}^{aa}), in about 4 times on the imaginary axis and about 7 times on the real one, is detected after 48h as a result of a higher activity on the electroactive sites, when a faster activation-controlled process takes place at the anode-electrolyte interface; this means a faster electron-transfer rate to the anode. At about 48h the minimum R_{ct} is detected (Figure 2); this can possibly be attributed to the saturation of the active sites by the electroactive ionic species. At high frequencies there is no significant difference on the electrolyte resistance through time (insert of Figure 2). Subsequently, the Pt/Ir cathodes were characterized with EIS. The frequency response (Nyquist plots) is shown in Figure 3, for the Pt/Ir cathode operating as working electrode under aerobic conditions, while the anode was working as counter and pseudo-reference electrode as described by the Mansfeld group [22]. A well defined semicircle-like behavior is also observed. The charge transfer resistance of the cathode under abiotic conditions (R_{ct}^{ac}) reduction in this case is much bigger than as observed in the anode, 14 times on the real axis and 14 times on the imaginary one (Figure 3). Comparing Figure 2 against Figure 3 it can be observed that under abiotic conditions, at the initial time the R_{ct}^{aa} is about 75% of the R_{ct}^{ac}; this indicates that at 0h the acetate oxidation at the anode is faster than the rate of oxygen reduction at the cathode. The good catalytic performance of the Ti/TiO$_2$-PAni composite-anode is comparable to similar studies [9]. Furthermore, the impedance spectra under abiotic conditions (Figures 2 and 3) demonstrate a higher capacitance for the cathode (C_{ac}) than for the anode (C_{aa}), which is associated to an improved distribution of the active sites in the novel composite here presented [22b]. This implies that the limiting process for the electrochemical reactions takes place at the cathode of the electrochemical system.

3.1.2. MFC characterization

The modified Ti/TiO$_2$ anodes were well characterized with EIS, with the addition of bacteria. A semicircle-like behavior is observed in the anode and cathode impedances of the MFCs (Figures 4 and 5). For the *G. sulfurreducens* MFCs it is observed that the

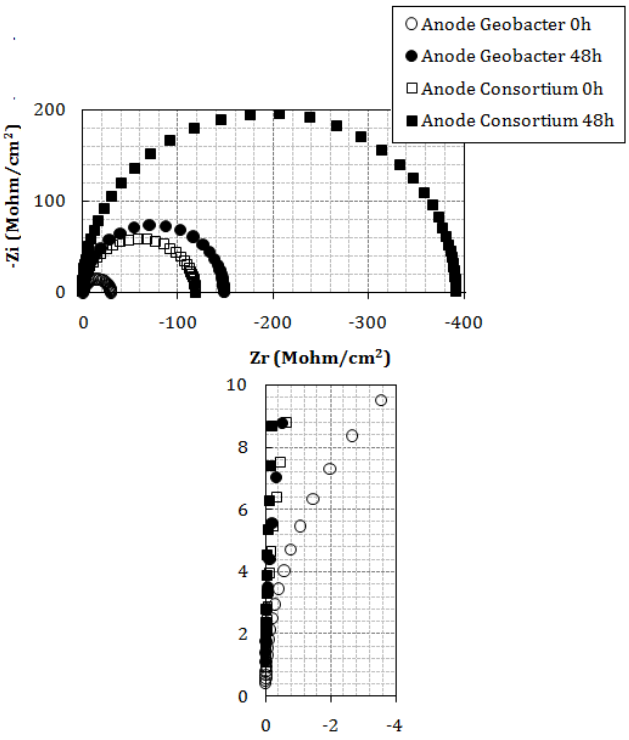

Figure 4. Nyquist plots of Ti/TiO_2-PAni anode operating in MFCs exposed to bacteria: Geobacter sulfurreducens, and an uncharacterized hypersaline consortium. The inset on the right illustrates the high-frequency part of the impedance spectra.

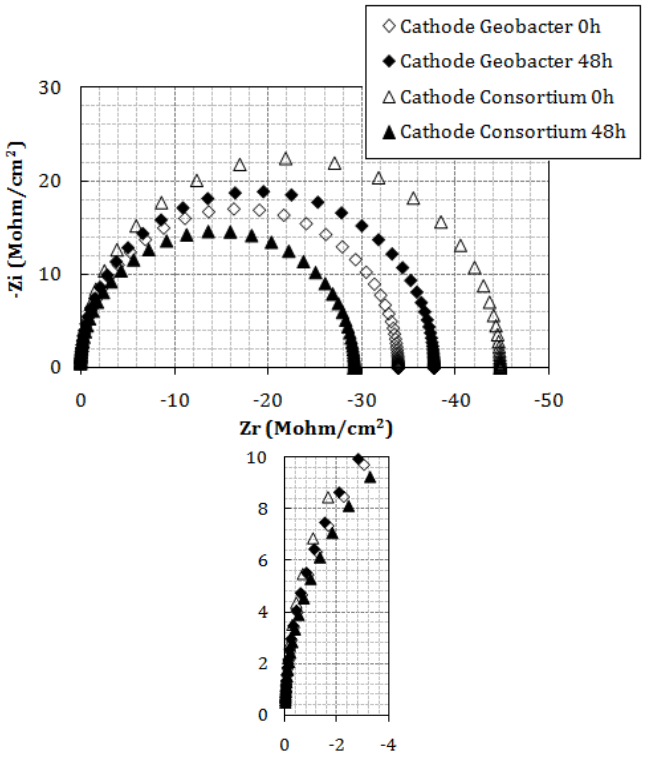

Figure 5. Nyquist plots of Pt/Ir cathode operating in MFCs, connected to the Ti/TiO_2-PAni anode exposed to bacteria. The inset on the right illustrates the high-frequency part of the impedance spectra.

Table 1. Maximim power densities obtained with Geobacter sulfurreducens and bacterial consortium operating in MFCs with Ti/TiO2-PAni anodes.

Cell	Current density [mA/m²]	Potential Difference [mV]	Maximum Power Density [mW/m²]
Geobacter sp.	0.4317	5367	2316.934
Consortium	0.5694	1997	1137.092

charge transfer resistance of the anode (R_{ct}^{ga}) increases in 4 times through time (in 48h) as well as the capacitance of the anode (C_{ga}). The system under the influence of the hypersaline microbial consortium increased through time (48h) its charge transfer resistance (R_{ct}^{ca}) as well 4 times and in 3 times its anode capacitance (C_{ca}), as shown in Figure 4. It is observed that $R_{ct}^{ga} < R_{ct}^{ca}$ in about 3 times. This is an indicative that charge transfer reactions at the anode occur faster in the presence of *G. sulfurreducens* than in the presence of the microbial consortium. However, the larger capacitance of the anode $C_{ca} > C_{ga}$ in the presence of the consortium suggests that the consortium might provide a larger number of active sites for charge transfer reactions to occur. Although the consortium might do a better preconditioning of the surface of the anode for electron transfer, *G. sulfurreducens* is known for its capabilities for producing conductive nanowires (pili) that allow an improved direct electron-transfer to the anode [23]. The direct electron-transfer capabilities of the consortia used are uncharacterized, but it is evident that they do not overcome the ones of the pure *G. sulfurreducens* culture. As for the cathode frequency response under the presence of bacteria, it is shown that R_{ct}^{gc} increases through time (Figure 5), suggesting that as the *G. sulfurreducens* biofilm covers the anodic active sites the electron-transfer reactions in the cathode are reduced as well. The cathode response in the presence of the consortium shows that the presence of this mixture of bacteria favors a faster electron-transfer at the cathode. Comparing the frequency response of the anode and cathode in the MFCs (Figure4 and Figure 5) it is observed that the rate-limiting reactions in the MFC take place at the anode due to the presence of the biofilms formed. Additionally, comparing Figure 2 with 4 and 3 with 5 it is observed that the presence of bacteria increases i_0, associated to power generation.

3.2. Bacterial electrogenesis on TiO_2/PAni anodes in a MFC

Table 1 shows the maximum power densities obtained at about 48h with the different bacterial cultures. *G. sulfurreducens* was

capable of generating 2317 mW/m² while the microbial consortia only reached 1137 mW/m². Both power densities are in good agreement with those that have been obtained with other MFCs. 1495 mW/m² have been obtained in MFCs with nanostructured Ti/TiO_2-PAni anodes and pure cultures of *Escherichia coli* [9]. Other studies using different anode materials and pure cultures of have showed power densities up to 3000 mW/m^2 with *Shewanella oneidensis* (with reticulated vitreous carbon as anode). Studies with pure *G. sulfurreducens* cultures have showed power densities up to 1900 mW/m^2 using an optimized carbon fiber anode. Microbial consortia from anaerobic and aerobic digestor sludge have generated up to 3600 mW/m^2 using solid graphite anodes [13]. In these studies, isolates show lower power densities than the mixed community referenced from the anaerobic and aerobic digestor sludge. In the present study the higher power density was obtained with the pure *Geobacter* MFC, which almost doubled the power generation by the mixed community. This result is very promising because pure cultures are highly reproducible when compared to mixed bacterial populations, so further investigations with the Ti/TiO_2-PAni anodes appear promising for optimization.

4. CONCLUSION

High power densities were obtained in MFCs using Ti/TiO_2-PAni (emeraldine form) anodes. The highest power density, 2317 mW/m², was generated by *Geobacter sulfurreducens*. This proves that the anodes developed are suitable materials for further optimization to be used in wastewater treatment with pure-culture MFCs. Mass transfer limitations were not evident in the impedance response. The negative real impedances obtained will need a more exhaustive survey for elucidating the mechanisms that take place at the Ti/TiO_2-PAni MFCs developed in this study.

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