Development of mats composed by TiO\textsubscript{2} and carbon dual electrospun nanofibers: A possible anode material in microbial fuel cells

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A new material based on TiO\textsubscript{2}(unite)–C\textsubscript{semi-graphitic}/C\textsubscript{semi-graphitic} dual nanofiber mats is presented, whose composition and synthesis methodology are fundamental factors for the development of exoelectrogenic biofilms on its surface. Therefore, this material shows the required characteristics for possible applications in the bioconversion process of an organic substrate to electricity in a microbial fuel cell. Chronoamperometry, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and electrical conductivity analyses showed excellent electrical performance of the material for the application intended; a resistance as low as 3.149 Ω was able to be measured on this material. Furthermore, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies confirmed the morphology sought on the material for the application intended; dual nanofibres TiO\textsubscript{2}(unite)–C\textsubscript{semi-graphitic}/C\textsubscript{semi-graphitic} with a side by side configuration. The difference in composition of the fibers forming the dual nanofibers was clearly observed and confirmed by energy dispersive X-ray spectroscopy (EDXS), and their crystal structure was evident in the results obtained from selected area electron diffraction (SAED) studies. This nanostructured material presented a high surface area and is biocompatible, given that it can host a dense biofilm of electroactivated Escherichia coli. In this study, the maximum current density obtained in a half microbial fuel cell was 8 A/m\textsuperscript{2} (0.8 mA/cm\textsuperscript{2}).

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1. Introduction

Microbial fuel cells (MFCs) are biochemical-catalyzed electrochemical devices that generate electricity by means of oxidizing biodegradable organic matter (i.e. acetate, glucose, and protein in wastewater) in the presence of microorganisms [1–3]. A MFC is composed of two electrodes (anode and cathode), separated by a proton exchange membrane. In the anode chamber there are electrogenic microorganisms that grow on the anode surfaces and degrade organic compounds to produce electrons, protons and CO\textsubscript{2}. The electrons are transferred to the anode and then flow into the cathode through an external electrical connection and generate current. To preserve the electroneutrality of the system, the transfer of electrons from the anode to the cathode must be accompanied by the transfer of an equal number of protons between the two electrodes through the proton exchange membrane. Finally, the combination of protons with oxygen in the cathode chamber produces water [4]. Even though MFCs are currently far from generating a considerable amount of power, these devices are considered a promising technology, due to MFCs’ most characteristic feature, which is the direct conversion of substrate energy to electricity by catalytic action of electrogenic microorganisms, where this substrate could be basically any organic compound even that found as waste. In addition to the energy problems, with these devices is possible to solve the sustainable waste management [5,6]. The electrochemical characteristics of electrodes greatly affect MFC performance [7–9]. In previous studies, it was found that an enhancement in the MFCs’ performance was associated with the composition and morphology of the anodic material [10], due to the key role that the electrode plays in electricity.

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generation [11]. Thrust by this fact, several investigations have focused on the development of alternative strategies to synthesize new, low-cost anodic materials with high surface areas that can support electroactive biofilms efficiently and with high conductivity for electron transfer, therefore improving the overall performance of MFCs. Carbon-based materials with different morphologies, such as compact graphite plates, rods or granules, fibrous graphite felt, cloth, paper, fibers, foams, and glassy carbon, have been evaluated as anodic materials in MFCs [12]. However, these carbonaceous materials have a small surface area, or a slight amount is available for microorganisms to interact with. Previous investigations suggest that the use of three-dimensional structures [13,14], such as activated carbon nonwoven nanofibers (ACNFN) [15], contributes toward reducing the distance from the free surface to the interior of the structures, hence maximizing the use of the available surface area, which ensures superior performance by decreasing transport limitations. Moreover, additional investigations report the application of nanostructured poly(aniline/TiO2) composite (power density of 1.495 W/m2) [16] and Ti–TiO2 (Current density of 0.48 A/m2) [17] as anodic electrode materials in MFCs, given that a Ti–TiO2 electrode promotes power generation 250 times higher than graphite electrodes for the same purposes due to their morphology and superficial area, allowing microorganisms to have better adhesion to the surface, these improve the charge transfer processes. Modified electrodes have received a lot of attention in recent years; Wu et al. [18] reported an electrode of carbon nanotubes (CNT) modified with Au/TiO2, and this nanocomposite improves the electron transfer rate and promotes the electron exchange at the electrode surface, making it a promising material for MFCs. Composite nanostuctures have been reported to be synthesized previously in the form of nanofibers by a co-electrospinning approach. Duan et al. [19] synthesized hybrid nanofibrous membranes of poly(lactico-glyclic acid) (PLGA) and chitosan with a co-electrospinning approach for the fabrication of substrates for tissue engineering applications. Hu and Yu [20] synthesized chitosan/alginate fibers with a dual-jet system by the same method, for similar applications. Whereas Hyun et al. [21] fabricated layer-by-layer stacks of MnOx–RuO2 nanofiber mats via co-electrospinning, and studied their potential application for electrochemical capacitors. In this work, results on the development of TiO2–carbon/carbon dual nanoﬁber mats, coupled with an electroactive biofilm of Escherichia coli, are presented. Furthermore, this material was assessed as an alternative anodic electrode for MFCs, and its performance is presented.

2. Experimental procedures

2.1. Materials

Polyacrylonitrile, PAN (MW<sub>avg</sub> 150,000); polyaniline emeraldine salt, PANI (MW<sub>avg</sub> 15,000); polyvinylpyrrolidone, PVP (MW<sub>avg</sub> 1,300,000), N,N-dimethylformamide, DMF, Luria Bertani, LB broth, D-(+)-glucose, yeast extract, NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub> salts and methylene blue were purchased from Sigma–Aldrich. Acetic acid and ethanol were purchased from CTR scientific. Titanium (IV) iso-propoxide, Ti(OiPr)<sub>4</sub> was purchased from Fluka. All reagents were used directly without further purification.

2.2. Preparation of electrospray TiO2–C/C dual nanoﬁber mats

Dual nanoﬁber (d-NF) mats were obtained by a bicomponent electrospraying process. The conditions used in this investigation are reported elsewhere [22]. Two distinct precursor solutions (A and B) were ejected simultaneously through a spinneret integrated by two syringes confined in a side-by-side geometry. The precursor solution (A) for TiO2–PVP-PANI fibers was prepared by mixing a polymeric solution that was composed of PVP-PANI at a 1:4 molar ratio, dissolved in 3.6 mL of DMF, and 1 mL of Ti(OiPr)<sub>4</sub> was dissolved in 1 mL of acetic acid and 1 mL of ethanol. Finally, the resulting solution was stirred before electrospinning. The precursor solution (B), for PAN fibers, was created by the dissolution of 8% w/w PAN in 10 mL of DMF [23]. The double jet, solution A and B, was ejected directly to the aluminum collector plate. Afterward, the electrosprun nanofibers were exposed to air at room temperature, under a relative humidity of 40–60%, to promote Ti(OiPr)<sub>4</sub> hydrolysis. The thermal process was carried out in a tube furnace (Mini-Mite<sup>TM</sup>). The nanofibers were heated (3 °C/min) from 20 °C to 280 °C in air atmosphere for 1 h to promote the stabilization of carbon nanoboron fibers [24]; subsequently, they were calcined (5 °C/min) to 425 °C for 1 h in order to obtain the crystalline phase of TiO2. Finally, the nanofibers were carbonized at 1000 °C for 1 h, in 60 cc/min of flowing nitrogen.

2.3. Characterization of TiO2–C/C dual nanoﬁber mats

The morphology and elemental analysis of the nanofibers were investigated using a FEI NovaNanoSEM200 scanning electron microscope (FESEM) that was equipped with an energy dispersive X-ray spectroscopy (EDXS), and EDAX detector. The average diameter and length of the nanofibers were measured from the FESEM images by measuring 50 randomly chosen fibers and using ImageJ analysis software. Alternatively, more detailed studies on the morphology and crystal structure of the d-NF were performed using a Titan G2 80–300 transmission electron microscope (TEM) that was operated at 300 KV. Surface area was determined by N<sub>2</sub> physisorption using the Brunauer–Emmett–Teller method, with a BELSORP Mini II analyzer. Electrochemical properties were studied in a three-electrode system that was coupled with a platinum twisted wire and a silver/silver chloride which served as counter and reference electrode, respectively. Working electrodes for analysis were cut from nanofibers that were cut into squares of 0.5 cm × 0.5 cm and were connected directly to the potentiostat connectors. The electrochemical performance of the electrodes was evaluated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in 0.5 M K<sub>2</sub>SO<sub>4</sub> solutions. The electrical conductivity studies were performed using a two-probe method with a picoammeteric voltage power supply of Keithley 6487; the voltage range applied ranged from 0 V to 200 V at 25 °C of temperature.

2.4. Bioﬁlm growth on TiO2–C/C of dual nanoﬁber mats

E. coli K12 (BioRad, S.A. Mexico) was initially incubated aerobically at 37 °C on a rotatory shaker (1575 Shel-Lab<sup>®</sup>, Oregon, EUA) for 24 h in Luria Bertani, LB broth. The resting cell suspensions were obtained by centrifugation at 3000 rpm for 15 min at 4 °C. Resting cells were resuspended in a standard medium that was composed of anhydrous D-(+)-glucose (10 g/L), yeast extract (5 g/L), NaHCO<sub>3</sub> (10 g/L), and Na<sub>2</sub>HPO<sub>4</sub> (8.5 g/L) for subsequent electroactivation [25]. After the third electroactivation at 20 mV for 10 s, bacterial culture was inoculated in glass vials (WHEATON, United Kingdom), containing dual nanofibers of TiO2<sub>x</sub>(rutile)<sup>–</sup>C<sub>semi-graphitic</sub>/C<sub>semi-graphitic</sub> that were suspended in a fresh standard medium for biofilm growth. The system was incubated at 37 °C under stationary conditions for 30 days; the medium was replenished with a fresh solution every 24 h. This entire process was conducted under sterile conditions. The morphology of the bioﬁlm was characterized using FESEM (FEI Nova NanoSEM200). For this study, the sample was analyzed in a low vacuum mode available in the instrument. The microscope was operated at 5 kV of acceleration voltage and 130 Pa of chamber pressure, to
diminish a possible damage in the cells of biofilm. These conditions of analysis are commonly used for the study of biological samples.

2.5. Electrochemical measurements

The electrochemical measurements were conducted in a conventional three electrode half cell. The electrochemical device was assembled with TiO$_2$ (rutile)–C (semigraphitic)/C (semigraphitic) dual nanofiber mats supporting E. coli electroactived biofilm as work electrode, one silver/silver chloride as reference electrode, and platinum twisted wire as counter electrode. The experiments were carried out in a VMP3 potentiostat/galvanostat/EIS. The anolyte was d-(-)-glucose 11 mM in phosphate buffer solution with methylene blue 23 mM as electron mediator. For the electrode connection, TiO$_2$ (rutile)–C (semigraphitic)/C (semigraphitic) dual nanofibers with E. coli electroactived biofilm were cut into pieces with a size of 1 cm $\times$ 1 cm; the squares were encapsulated between a stainless steel grid (SS 304) that was used as a current collector [26].

3. Results and discussion

FESEM studies were used to reveal the morphology of the calcined d-NF. The micrographs confirmed the presence of interconnected and entangled d-NF that formed a non-woven mat (Fig. 1a). Greater details of FESEM (Fig. 1b) and TEM images (Fig. 1c and d) show that individual nanofibers exhibit a bundle-like structure with two small fibers bound together, suggesting that two parallel jetting solutions traveled together toward the collection plate to form the dual fibers during the electrospinning process. The average diameter of PAN fibers was of 470 $\pm$ 75 nm, whereas for TiO$_2$/PANI/PVP fibers was of 200 $\pm$ 34 nm, however after the stabilization, crystallization and carbonization process (up to 1000 $^\circ$C) the fibers shrunk to an average diameter of 140 $\pm$ 54 nm and a length of 49 $\pm$ 9 $\mu$m. The fibers diameter decreased dramatically and it is associated to the loss of organic components in the as-electrospun fibers after thermal treatment, the particular thermal transformations were reported in a previous work [22]. Furthermore the mat of dual nanofibers remained composed by fibers of TiO$_2$–C and C in a volume ratio about 50/50.
The chemical composition of d-NF was evaluated by energy-dispersive X-ray spectroscopy (EDXS), which confirmed the presence of Ti, O, and C. Fig. 2a shows five peaks with characteristic energies of titanium, oxygen, nitrogen, and carbon, as expected. A line scan analysis was performed to assess the change in the composition of a nanofiber throughout a line running over the transversal section of a dual nanofiber found in the sample. Fig. 2c shows a FESEM image of a dual nanofiber, and the red line indicates the region of the sample studied during the line scan analysis; whereas its corresponding EDXS spectrum is shown in Fig. 2b. The spectrum indicates that one of the nanofibers shows a clear and high Ti and O signal, suggesting a higher presence of TiO2; whereas the other shows a higher C signal, which suggests higher carbon content. More detailed TEM studies on the composition of the dual nanofiber mats are shown in Ref. [22], where the same tendency of the FESEM results was observed.

In order to obtain a more detailed study about the different microstructures observed in a d-NF, transmission electron microscopy (TEM) studies were carried out and the corresponding results are shown in Fig. 3. It could be seen that the nanofibers constituting the dual system, presented two different morphologies i.e. one had a smooth morphology due to carbonaceous structures obtained from polymeric calcined precursors and the other, a polycrystalline material formed by a considerable amount of nanocrystals embedded in a carbonaceous structure. The crystal structure of the material was determined by selected area electron diffraction (SAED) studies. Fig. 3b shows a diffraction pattern that enabled the identification of the interplanar distances related to the families of lattice planes {110}, {200}, {111}, {211}, and {220}. The presence of these lattice planes confirmed the tetragonal crystal structure of the TiO2 rutile phase (JCPDS card # 21-1276). The intimate contact along the length between these two different types of nanofibres, as it was originally intended, has the effect of increasing the contact area between the carbonaceous fibers and the TiO2 fibers; thus increasing the charge transfer at the interface between these two nanostructures, due to the particular alignment of the electronic structure of these two different materials; hence resulting in an improvement in the electrical properties of the composite material, as it was discussed in Ref. [23].

The cyclic voltammograms for dual nanofibers tested at different scan rates are displayed in Fig. 4. It was noticed that when the scan rate increased, it was possible to observe a better resolution of the events occurring within the electrochemical potential window studied. The graphic at 100 mV/s shows a higher increment in the value of cathodic current (−1.45 A/g at −1.0 V vs Ag/AgCl V). The behavior for all graphics is interpreted as an indication that the material favors the catalytic reaction for hydrogen production, along with the generation of an associated anodic peak on the reverse scan, indicating the oxidation of hydrogen adsorbed on the nanofiber surface. These observations could be related to an improved electronic conduction and could be attributed to the presence of the carbon structures, which make available larger amounts of electrons to the nanofiber structure [27], favoring the flow of current through the material and therefore also the faradic cathodic processes taking place, as well as the proton reduction. To gain better insights on these mentioned processes, electrical conductivity experiments were performed on this electrode, and are discussed from here onward.

Fig. 5a shows the Nyquist impedance plot obtained from dual nanofiber mats TiO2(rutile)–C(semi-graphitic)/C(semi-graphitic) tested, and the corresponding electrical equivalent circuit are presented in Table 1. A tendency of forming small semicircles at a high frequency is observed, followed by a transition to linear behavior at lower frequencies. An ideal polarizable capacitance gives rise to a straight line along the imaginary axis. Nevertheless, low frequency lines were maintained to deliver a clear slope, representing the diffusive resistivity of the electrolyte within the surface of the electrodes. The electrochemical behavior for dual nanofiber mats TiO2(rutile)–C(semi-graphitic)/C(semi-graphitic) can be better represented using a $R_0(Q_{eq}(R_{ct}W))Q_{eq}$ equivalent circuit, where $R_0$ is the internal resistance and $R_{ct}$ is the charge-transfer resistance. The constant phase elements, $Q$, are used to relate to the nanofiber
Fig. 3. (a) Bright-field TEM image of TiO$_2$–C/C dual nanofiber (b) SAED pattern of the marked position shown in (a).

Fig. 4. Cyclic voltammetry of TiO$_2$(rutile)–C(semi-graphitic)/C(semi-graphitic) dual nanofiber electrodes at different scan rate.

Fig. 5. Nyquist plot at −0.1 V of nanofiber electrodes. (a) Impedance plot and (b) admittance plot.
high surface area, where $Q_{dl}$ is interpreted as the surface capacitance double layer and $Q_{ps}$ is a pseudocapacitive element. $W$ is a diffusion element represented by the Warburg impedance element to account for possible faradic events due to conductive structures available in the system. To complement the electrochemical information, the Nyquist plots obtained from individual nanofiber mats are also presented (single nanofibers of $C_{\text{semi-graphitic}}$ and TiO$_2$(rutile)$–C_{\text{amorphous}}$), and more information on these systems is presented next.

The equivalent circuit shown in Table 1 was the most adequate to fit the experimental data, showing that the calculated $R_{ct}$ value for TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$ was 3.149 Ω. This value is low if compared with the charge-transfer resistance of individual nanofiber values that are presented in Table 1. A low $R_{ct}$ value promotes a greater diffusion of electroactive species in the interface between the electrolyte and the TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$ dual nanofiber mats. This behavior could also be better appreciated on the Nyquist admittance plot shown in Fig. 5b. It is possible to appreciate that the high-frequency real admittance value is quite large for d-NF TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$/C$_{\text{semi-graphitic}}$ electrode compared to the rest of the mats. The increased ability of these materials to favor the diffusion of electroactive species in individual nanofibers is that the junction between the n-type semiconductor TiO$_2$ and the semigraphitic material can be of the ohmic type [23]. The electrical conductivity data obtained from two tips tests are presented in Table 2, and these also reveal that the net of TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$/C$_{\text{semi-graphitic}}$ dual nanofibers resulted in the highest electrical conductivity values.

The biofilm grown over the new synthetized material was examined by field emission scanning electron microscopy, in a low vacuum mode. Fig. 6 demonstrates that TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$/C$_{\text{semi-graphitic}}$ dual nanofiber mats are able to host a pure culture strain of E. coli K12, previously electroactivated on the electrode surface. The bacterial cells colonized and formed an extensive biofilm that was homogeneously distributed on the surface, with a high cellular density on the host substrate. The support material which was composed of TiO$_2$(rutile)$–C_{\text{semi-graphitic}}$/C$_{\text{semi-graphitic}}$ dual nanofiber mats has a

![Fig. 6. SEM images of electroactivated Escherichia coli K12 biofilm supported in dual nanofiber mats.](image-url)
Fig. 7. Current density vs time curve of dual nanofiber mats anode in a half microbial fuel cell.

high specific surface area above 28 m²/g, which is approximately 60 times larger than that of woven graphite felt (0.47 m²/g), a commonly used anode material [12]. In order to quantify biofilm growth, the amount of biofilm per gram of material was measured for TiO₂(rutile)–C(semi-graphitic)/C(semi-graphitic) anode; the amount of biofilm growth on dual nanofibers represents about 42% weight of the nanofiber-biofilm system. Which suggest that the microorganisms are occupying a considerable area of the nanofibers, this fact is also promoted by the tridimensional morphology of the nanofibers mats, which allows the access of the microorganisms into the interior of the nanofibers arrays.

The current density curve obtained for an electrode tested in a half microbial fuel cell is shown in Fig. 7. The maximum current density obtained was 8 A/m² after an acclimation period of 4.5 h. The current density decreased to a value of 4 A/m²; afterward, it stabilized between 4 A/m² and 5 A/m² after 6 h. This drop in the current density can be attributed to the decrease in the concentration of organic matter available in the reaction media for the catalytic degradation performed by the E. coli.

A comparison between different results for the current density is somewhat complicated, since there are many factors affecting this value, one of them being the microorganisms used. Even though, it can be stated that the current density in the present work was comparable in magnitude to those recently reported by Lanas and Logan [28], who obtained a maximum current density of 4 A/m² using graphite fiber brush as anode in a microbial fuel cell. While Bond and Lovley [29] reported a current density of 1.143 A/m² using a graphite electrode inoculated with Geobacter sulfurreducens in a MFC. An more recently, Hosseini and Ahadzadeh [30] obtained a maximum current density of 1.5 A/m² with graphite anode and an anaerobic activated sludge. The results presented in this work represent the first study applying TiO₂(rutile)–C(semi-graphitic)/C(semi-graphitic) dual nanofiber mats as the anodic electrode in MFCs. Current work in the research group deals with improving the current density obtained by this system.

4. Conclusions

In summary, a new material that was composed of dual nanofiber mats of TiO₂(rutile)–C(semi-graphitic)/C(semi-graphitic) with interesting morphological and electrical properties has been developed; such properties make it suitable to be used as the anodic material in MFCs. TEM and SEM results proved the dual nanofiber mats’ morphology, with one fiber made mostly of Ti, O, and C; whereas the other fiber showed a composition based on C. The electrochemical results of the dual nanofiber mats showed a better performance compared with systems made of single nanofiber. This nanostructured material, with a high surface area, is biocompatible and can host a dense biofilm of electroactivated E. coli bacteria, to generate current, as it was tested in a half microbial fuel cell. Future work should focus on improving the performance of the MFCs using this material as their anode material.

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