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DESIGN AND CHARACTERIZATION OF A BIOFUEL CELL USING A LACCASE ELECTRODE AS CATHODE

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ABSTRACT

The wide and increasing demand of energy of our modern world makes it almost impossible that only one technology satisfies such demand in a sustainable and economic way. For this reason, several researcher groups investigate new technologies in order to obtain technologies that can supply specific ranges of power in a sustainable and economic way. This is the case of biofuel cells that are promising devices for flexible, compact and inexpensive micropower systems operating at "mild" conditions. As a result of a collaboration between the Instituto de Biotecnología (UNAM) and the Instituto de investigaciones Eléctricas, a biofuel cell using a laccase enzyme electrode as cathode was designed and characterized. In this paper, the preparation of laccase electrodes, the performance of such electrodes and the stability in operation of a biofuel cell is presented.

Key words: Laccase, fuel cell, enzymatic electrode, Biofuel cell.

1. INTRODUCTION

Biofuel cells can be defined as devices capable of transforming directly the chemical energy into electric power; through biochemical reactions involved in metabolic routes or specifically of an isolated macromolecule. In this sense, biofuel cells are all systems that use enzymes and/or microorganisms to produce electric power. Biofuel cells are promising devices for flexible, compact and inexpensive micropower source operating at mild conditions. The first enzyme-based biofuel cell was reported 40 years ago [1]. The potential applications of enzymatic biofuel cells include implantable sensors and transmitter systems, pacemakers and nerve stimulators, drug delivery pumps and portable power suppliers.

The biofuel cells consist of two electrodes separated by a semi-permeable membrane, and placed into a solution (like in the conventional fuel cells). The biological catalysts (proteins or microorganisms) must be in solution (or in suspension), in a free way or immobilized inside the anodic compartment (or cathode, depending on the biochemical characteristics of the biological catalyst). The fuel (substratum) that is supplied, is totally or partially oxidized; and the liberated electrons in this biochemical process are transferred to the oxygen reduction to form water. As it was mentioned previously, a biofuel cell using a laccase enzyme electrode as cathode, was designed and characterized. In the following sections of this document, the preparation of laccase electrodes, the performance of such electrodes and the stability in operation of a biofuel cell is presented.

2. EXPERIMENTAL

2.1 Materials and chemicals

Laccase from Coriolopsis gallica UAMH 8260 was obtained and purified as previously described [2]. Carbon Vulcan XC-72R was obtained from Cabot Corporation (Bellerica, MA, USA). Nafion solution 5% was purchased from Fuel Cell Scientific, LLC (Stoneham, Ma, USA). ®SIGRACET graphite laminated sheets (GDL 30BC), used as conducting supports, were obtained from SGL Carbon Group (Wiesbaden, Germany). Nafion® membranes (180 µm) PFSA

117 were purchased from Dupont. The N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochlorine (EDC), 2-(N-morpholine) ethanosulfonic acid (MES), the 4-[2-aminoethyl] benzoic acid hydrochloride (AEBA), and the 2,2´-anzino-bis(3-ethylbensthiazoline-6-sulphonic acid (ABTS) were purchased from Sigma-Aldrich Chemical Corporation (St. Louis, MI, USA). The sodium hydroxide, nitric acid, isopropanol, methanol, and acetone were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and the tetrahydrofuran was from Burdick&Jackson (Muskegon, MI, USA).

2.2 Electrodes preparation

Three different laccase cathode electrodes were prepared.

2.2.1 Laccase-entraped electrodes

Electrodes were prepared with entrapped laccase (non covalent bound) by using a conducting ink. The conducting ink contained 1600 µL of isopropanol, 520 µL of 10mM phosphate buffer, pH 6.0, 52 µL of Nafion solution (5%) and 6.5 mg of carbon Vulcan. The conducting ink was uniformly mixed and maintained in an ultrasonic bath for 1 h at 40 °C. Before deposition on graphite sheets, 50 µL of laccase (75.85 mg/mL) were added and homogenized in an ultrasonic bath for 10 min. The mixture was deposited on a graphite sheet (6.25 cm^2) with a manual system of spraying with x-y-z movements whit an air flow pressure of 50 psi. During the spraying the graphite sheets were maintained at 40 °C. After laccase-ink depositions and drying, the electrode was extensively washed with 10 mM phosphate buffer, pH 6.0 and stored at 4 °C.

2.2.2 Directly covalent bound electrodes (DCB)

Graphite sheets (1cm^2) were carboxylated by a treatment with concentrated nitric acid at 80 °C for 2 h [3]. Then the graphite sheet were extensively washed with distilled water and dried. Laccase was covalently immobilized by submerging the carboxylate graphite laminated sheet into 1 mL of MES buffer containing 50 µL of enzyme solution (75.85 mg/mL) and 2 mg of EDC [4].

The reaction mixture was incubate 4 h at 20 °C and agitated at 80 rpm. Then, the electrodes were extensively washed with phosphate buffer 10 mM.

2.2.3 AEBA-modified electrodes (AEBA)

Graphite sheets were also modified with 4-[2-aminoethyl] benzoic acid (AEBA) according to [5]. AEBA (180 mg) was dissolved into 10 mL of 0.2 M sodium hydroxide. The dehydrochlorinated AEBA was then extracted with 3 mL of toluene, and 3 mL of tetrahydrofuran were added. The graphite sheet (1 cm^2) was submerged into the AEBA solution and heated to 60 °C for 15 h. The AEBA-modified graphite sheets were successively washed with methanol, acetone, tetrahydrofuran and distilled water, and then dried. Due to the AEBA instability, all reagents used in this process were deoxygenated by nitrogen atmosphere. The AEBA-modified graphite sheets were incubated for 4 h in 1 mL of MES buffer 50 mM containing 50 µL of enzyme solution (75.85 mg/mL) and 2 mg of EDC. The modified electrodes were extensively washed with phosphate buffer 10 mM (pH 6.0) and stored at 4 °C until use.

2.3 Construction of a semi-enzymatic fuel cell and characterization

An electrochemical cell was built in acrylic with a zinc anode (22.5 cm^2) and a laccase cathode (1cm^2). A bigger size of anode was used in order to avoid any limiting reaction due to the anode oxidation. Both electrodes were submerged in 50 mM succinate buffer (pH 4.5) and separated by a 180 µm Nafion membrane. The functioning mechanism of this semi-enzymatic fuel cell is described in the reference [6]. Figure 1 shows a view of the constructed cell. The performance of the semi-enzymatic fuel cell was evaluated by potentiodynamic curves using different oxygen flows. On the other hand, the operational stability of the semi-enzymatic fuel cell was evaluated by the chronoamperometry technique.

These electrochemical measurements were carried out on a potentistat (Solartron 1287) coupled to an electrical potential amplifier (Booster).

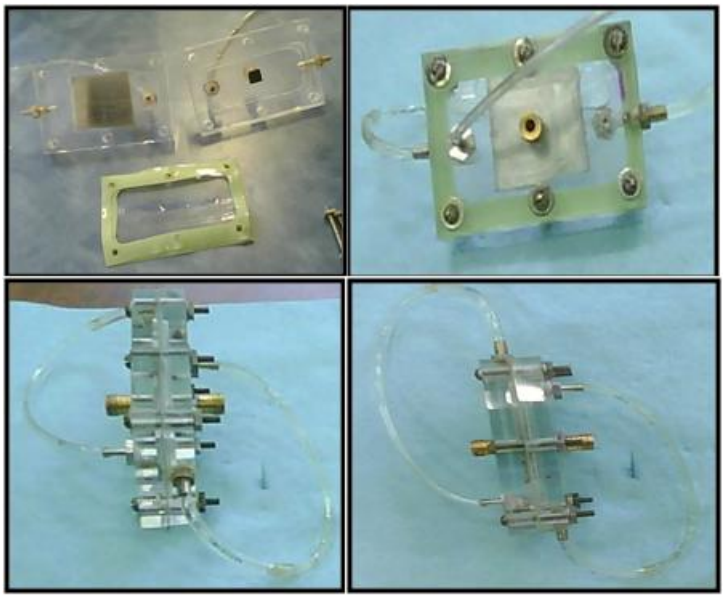

Figure 1. View of the semi-enzymatic fuel cell constructed and its different components.

3. RESULTS AND DISCUTIONS

3.1 Laccase activity in the electrodes

In order to avoid redox mediator compounds in biological fuel cells is imperative to optimize the electron transfer between the active site of the enzyme and the surface of the electrode. The rational of this work was to orientate the coupling of laccase molecule with the electrode surface through the T1 site and thus induce the direct electron transfer between the T1 site and the graphite electrode surface. The oriented laccase molecules leave the T2/T3 site free to interact with molecular oxygen.

The laccase activity of the electrodes was estimated with ABTS as substrate. The enzyme loading was determined by a protein reagent giving the total immobilized protein (see Table 1). Both procedures here used are described detailed in [6].

Table 1. Protein loading and electrode laccase activity.

Electrode	ABTS activity (mU/cm^2)	Loaded enzyme estimated by ABTS activity ($\mu g/cm^2$)[a]	Loaded enzyme by protein assay ($\mu g/cm^2$)[b]	Loaded enzyme oriented in T1 (%)[c]
Entrepped	0.45 (±0.02)[d]	0.04	1414[e]	--
DCB electrode	13.6 (±1.9)	1.35	1.63 (±0.09)	16.94
AEBA electrode	20.2 (±3.5)	2.00	2.74 (±0.18)	26.65

[a] The amount of loaded enzyme was estimated from the activity using the free enzyme specific activity of 10.05 mU/μg.
[b] The amount of loaded enzyme was estimated with the BioRad protein reagent.
c The proportion of the protein molecules oriented was obtained from the values of protein estimated by BioRad reagent and activity, assuming that the oriented molecules are unable to react with the ABTS substrate.
[d] The standard deviations were calculated from three independent replicates.
[e] The total amount of enzyme is retained in the electrode after mixture spraying.

Even if in the entrapped electrode the amount of laccase is significantly higher its activity is very low. It is generally accepted that, among all enzyme molecules immobilized (embedded) onto electrode, only those closely placed onto electrode surface are able to react with the substrate. In addition, the mixture components and the deposition procedure could be affect the enzyme activity. On the other hand, the AEBA electrode showed 68% more total immobilized protein than the DCB electrode, the estimated proportion of oriented laccase molecules in AEBA electrodes was 26.65%, compared with only 16.94% of DBC electrodes. In the case of DBC electrodes, the laccase molecule is randomly oriented and many of T1 active sites could be available for ABTS reaction, showing a lower proportion of oriented molecules.

3.2 Characterization of the semi-enzymatic fuel cell

A semi-enzymatic fuel cell was prepared with a zinc anode and laccase cathodes. The zinc anode was used because the goal was to characterize only the laccase cathode and not the whole enzymatic cell. Due to the fact that the flow and the pressure of oxygen is important to obtain the best performance, different oxygen flows were proved in the semi-enzymatic fuel cell using a zinc anode and the AEBA electrode as cathode. The obtained results are show in Figure 2. As can be see, the best performance of the cell is obtained at the highest flow of oxygen. It is possible to see too, that all curves present the tree typical regions that affect the overall polarization of the cell: activation polarization region, ohmic polarization region and mass transport polarization region. The activation polarization region is related to the energy barrier that must be overcome

to initiate a chemical reaction between reactants. At low current draw, the electron transfer rate is slow and a portion of the electrode voltage is lost in order to compensate for the lack of electro-catalytic activity.

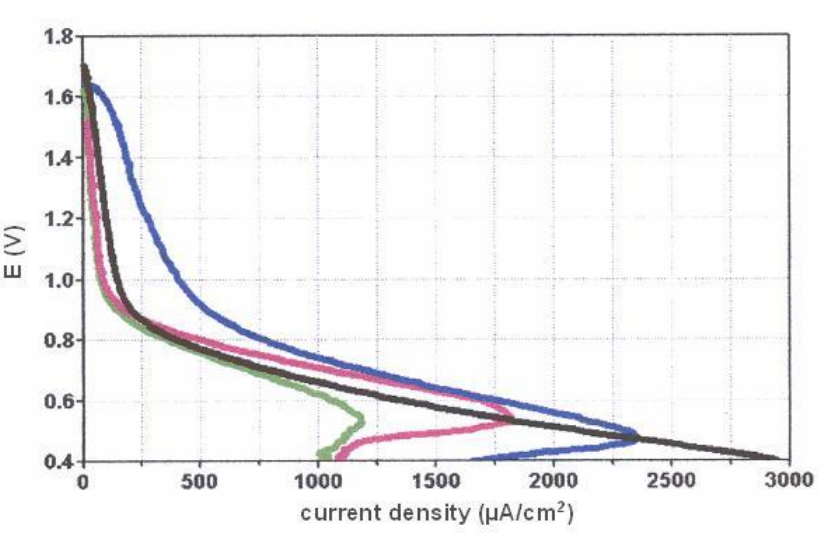

Figure 2. Potentiodynamic curves of the semi-enzymatic fuel cell Zn-AEBA electrode with different oxygen flows. Green line (10-30 mLO_2/min), red line (30-50 mLO_2/min), blue line (50-100 mLO_2/min) and black line (100-110 mLO_2/min).

The ohmic polarization region occurs due to resistive losses in the cell. These resistive losses occur within the electrolyte (ionic), in the electrodes (electronic and ionic), and in the terminal connections in the cell (electronic). The mass transport polarization region results when the electrode reactions are hindered by mass transfer effects. In this region, the reactant (O_2 in this case) becomes consume at greater rates than it can be supplied while the product accumulates at a greater rate than it can be removed. Using an oxygen flow in the range of 100-110 mL/min the problem of the mass transport can be avoided (see Figure 2).

Figure 3 shows the comparison of the potentiodynamic curves when the DCB electrode and the AEBA electrode are used as cathode in the cell. As can be see, the fuel cell constituted by Zn-AEBA electrodes showed a maximum current density of 2977 $\mu A/cm^2$, while the Zn-DCB electrodes showed a maximum current density of 2085 $\mu A/cm^2$. The 42.8% higher current

density of the Zn-AEBA fuel cell could be due to the laccase molecules site-specific orientation, which improve the electron transfer.

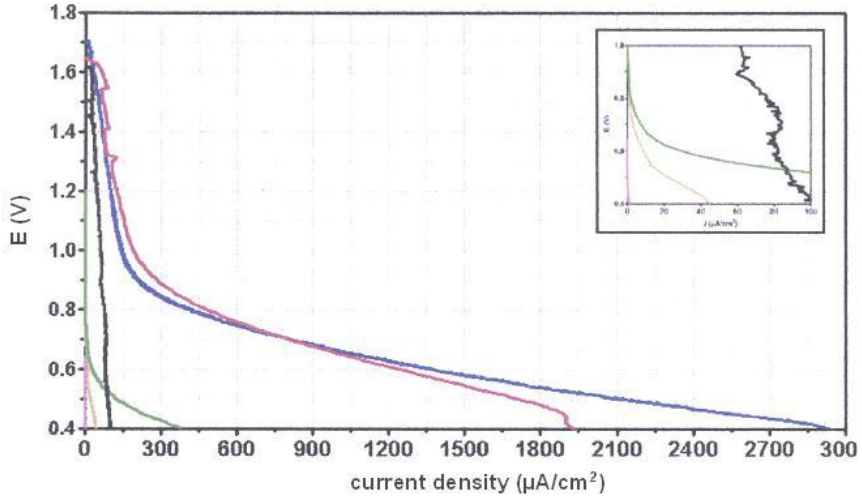

Figure 3. Comparison of the potentiodynamic curves when the DCB electrode and the AEBA electrode are used as cathode in the cell. O_2 or N_2 were feed in the cathode at a flow rate between100-110 mL/min. Red line (Zn-DCB electrode cell, with O_2), blue line (Zn-AEBA electrode cell, with O_2), brown line (Zn-DCB electrode and Zn-AEBA electrode cell, with N_2), green line (Zn-C, without laccase, control cell, with O_2) and pink line (Zn-C, without laccase, control cell, using water as electrolyte).

When the cathode is bubbled with nitrogen instead of oxygen, in both cases, no current is produced. Nevertheless, a control cell constituted with Zn anode and a graphite plate without laccase showed a maximal current density of 763μA/cm^2 in the presence of oxygen. This same cell in which the succinate buffer was substituted by distilled water not showed current. The current density produced in the absence of laccase and in the presence of succinate buffer could be explained by the fact that Nafion is able to catalyze some reactions, such esterification [7]. There are reports of succinic acid esterifications catalyzed by polymers analoges to Nafion [8]. Thus, it seems possible that the Nafion catalyze the succinic acid esterification, originating the electrical current in the cell.

Figure 4 also shows the power densities of the Zn-DCB, Zn-AEBA and Zn-C fuel cells. The Zn-AEBA fuel cell showed a power density of 1190 μW/cm^2 at 0.47 V, the Zn-DCB cell reach only 866 μW/cm^2 at 0.41 V and the Zn-C cell showed a maximum power density of 209 μV/cm^2

at 0.30 V. Eliminating the contribution of the Zn-C cell in the Zn-AEBA and Zn-DCB cells, the maximum power obtained are 982 and 657 $\mu V/cm^2$, respectively.

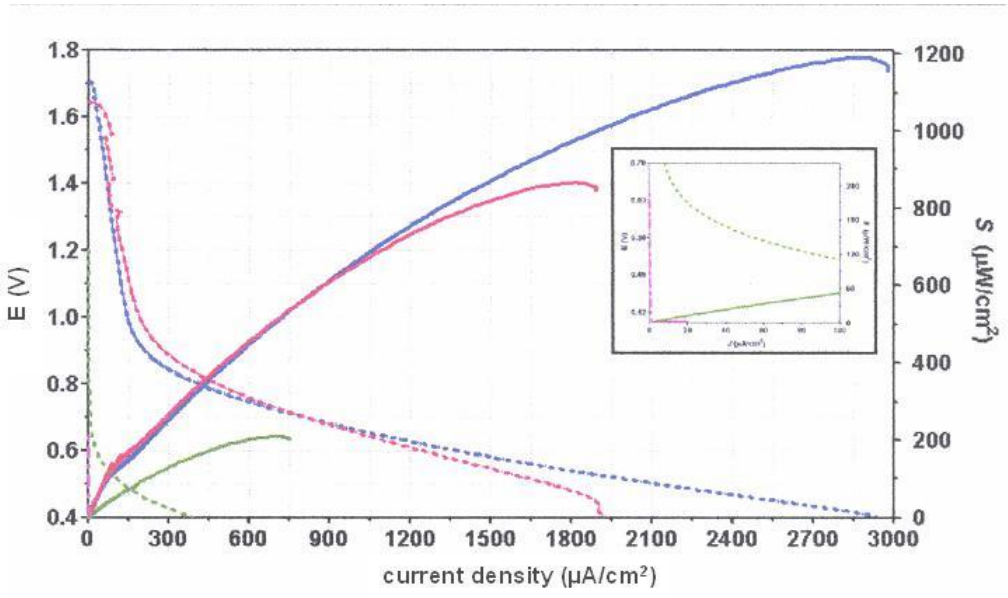

Figure 4. Current density and power density vs. voltage of the Zn-DCB, Zn-AEBA and Zn-C cells. Power densities are presented as continue lines and the current densities are presented as discontinue lines. Red line (Zn-DCB electrode cell, with O_2), blue line (Zn-AEBA electrode cell, with O_2), green line (Zn-C, without laccase, control cell, with O_2) and pink line (Zn-C, without laccase, control cell, using water as electrolyte). In all cases O_2 was feed at (100-110 mL/min).

3.3 Operative stability of the Zn-AEBA laccase fuel cell

In order to evaluate the operative stability of the Zn-AEBA laccase fuel cell a chronoamperometric measurements were carried out. As can be see in Figure 5, after a fast activation of the Zn anode, the current density was stabilized at around 1200 $\mu A/cm^2$ and decreased slightly and uniform during the first 6 h to 950 $\mu A/cm^2$, then decreased faster, possibly due to the Zn anode passivation.

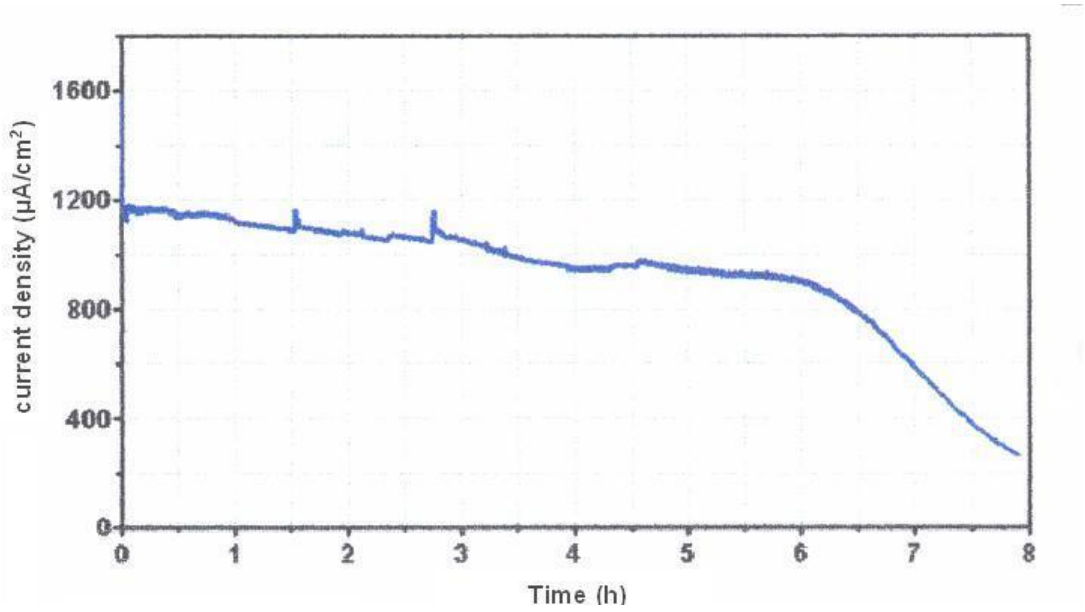

Figure 5. Chronoamperometric behavior of the Zn-AEBA laccase fuel cell. The voltage was fixed at 0.5 V, and the oxygen was feed in the cathode at 100-110 mL/min during this experiment.

4. CONCLUSIONS

- Three different methods to immobilize the laccase were studied in order to fabricate a laccase electrode. It was found that the best method was the AEBA modified, mainly due to the higher proportion of oriented molecules with the T1 site facing towards graphite surface.
- A Zn-AEBA laccase fuel cell was design and constructed. The values of power density obtained (1190 μA/cm^2 at 0.41V) with the oriented laccase electrode (AEBA) are the higher reported so far, even higher than those reported by Kamitaka (8500 μA/cm^2 at 0.41V) [9].
- The operative stability of the Zn-AEBA laccase fuel cell was proven. The current density given for the cell was between 1200 to 950 μA/cm^2 at 0.41V at 0.5 V during a time of 6 h.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1] Yahiro, A.T., Lee S.M., Kimble D.O., *Biochem. Biophys. Acta*, **88**, 375-383 (1964).

[2] Vandertol-Vanier, H.A., Vazquez Duhalt R., Tinoco R., Pickard M.A., *J. Ind. Microbiol. Biotechnol.* **29**, 214-220 (2002).

[3] Lee Y.M., Kwon O.Y., Yoon Y.J., Ryu K., *Biotechnol. Lett.*, 28, 39-43 (2006).

[4] Gilles M.A., Hudson A.Q., Birders C.L., *Anal. Biochem.*, **184**, 244-248, (1990).

[5] Gallardo I., J. Vila N., *J. Phys. Chem. B*, **130**, 19521-19529 (2006).

[6] Javier Martínez, *Tesis de maestria "Diseño y construcción de una celda de combustible Enzimática híbrida Zn-Lacasa"*, (2010).

[7] Beers A.E., Spruijt R.A., Nijhuis T.A., Kapteijn F., Moulijn J.A., *Catal. Today*, **66**, 175-181 (2001).

[8] Benedict D.J., Parulekar S.J., Tsai S., Pari, J. Membr. Sci., **281**, 435-445 (2006).

[9] Kamikata Y., Tsujimura S., Setoyama N., Kajino T., Kano K., *Phys. Chem.*, **9**, 1793-1801 (2007).