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Short communication

Enzymatic Based Biocathode in a Polymer Electrolyte Membrane Fuel Cell.

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In this communication results of a fuel cell operating with a conventional platinum anode and a peroxidase based biochatode are presented. The biocathode was prepared with peroxidase extracted from vegetal tissue of brazilian zucchini, *Cucurbirta pepo*, via four different methods. The biomaterial was used either immobilized or adsorbed on carbon cloth and on carbon powder. The tests were carried out at 25 ^{o}C in a proton exchange membrane fuel cell, in which cathode and anode were fed with oxygen and hydrogen, respectively. The crude extract from brazilian zucchini (*Cucurbirta pepo*) was used as peroxidase source in the cathode of the biochemical fuel cell. In spite of the poor stability, evidence of the cathode activity towards oxygen reduction is presented and a maximum power density in the range of 80 to 120 μW cm^{-2} was observed.

Keywords: biochemical fuel cell, enzymes, biocatalysts, oxygen reduction reaction.

1. INTRODUCTION

The proton exchange membrane fuel cell, PEMFC, converts chemical energy into electrical one with high efficiency and low emission of pollutants. These systems can be used in portable equipment, as a power source for vehicles and in stationary applications [1]. The PEMFC is composed of two electrodes (electronic conductors) and between them an electrolyte (ionic conductor). At the anode takes place the oxidation of hydrogen or low molecular weight alcohols and at the cathode the reduction of oxygen. The PEMFC uses gas diffusion electrodes and platinum, supported on high surface area carbon, is commonly used as catalyst in both the anode and the cathode.

An alternative to the conventional platinum based catalysts, is the use of biocatalysts like enzymes. In this context, a biochemical fuel cell is a system that uses biocatalysts [2,3] and can be used in low power applications such as implanted pacemarkers, and can also find applications in biosensors [4]. To be used as catalysts, enzymes must be conveniently immobilized in a conductive substrate. The objective to immobilize an enzyme is to get a biocatalyst with activity and stability that are kept unaffected during the operation, when compared to its free form. The procedure of immobilization of a protein can be achieved for instance via cross-linking, van der Waals' interactions, and hydrogen bonding, among others. Ideally, an immobilized enzyme must show a superior catalytic activity, keeping at the same time its structural properties and the active sites integrity [5]. There are many methods to immobilize enzymes, including physical and chemical procedures, like adsorption or inclusion [6]. Aiming at the attainment of more resistant enzymes to the aggressions of the environment and keeping a high enzymatic activity, many studies on enzyme immobilization have been carried out. For instance, laccase [7,8] and peroxidase [9] have been immobilized on different substrates like polianiline (PANI) [10].

In general, much less attention has been given to enzymes for the reduction reaction at the cathode than to the enzymes for the anodic reaction [2]. Given its ability to reduce oxygen, immobilized laccase has been used as a biocathode [11]. This enzyme is classified as an oxidoreductase that uses diphenols and other substances as electron donnors. Studies have shown that there are no significant differences in the electrochemical reduction of oxygen when using laccase itself when compared to the immobilized form [11]. In contrast to laccase, it has been reported that peroxidase uses as electron donator the Fe^{3+} through a covalent link with the porphyrin heme [9]. Because of the active redox center ($Fe^{3+/}Fe^{2+}$), peroxidase can in principle be successfully immobilized, keeping its bioelectroactivity [12]. Indeed, Dong and co-workers [13,14] have reported the imobilization of horseradisch peroxidase in a composite using an ionic liquid and carbon. The modified electrode presented electroactivity towards oxygen and hydrogen peroxide reduction in a phosphate buffer (pH =7) [13,14].

The main goal of the present communication is to compare the activities of peroxidase based biocathodes prepared by four different methods. The biomaterial was extracted from vegetal tissue of brazilian zucchini, *Cucurbirta pepo*, and the tests where carried out in a PEMFC composed of the biocathode and a conventional platinum based anode, fed with oxygen and hydrogen, respectively.

2. EXPERIMENTS

2.1 Preparation of the crude peroxidase extract

Crude extract from brazilian zucchini (*Cucurbirta pepo*) was used as peroxidase source. The procedure used to extract the enzyme was previously suggested by Fatibello-Filho *et al.* [15]. First, 40 g of *C. pepo* was washed, dried, peeled and homogenized in water in a mixer resulting in about 400 mL of suspension. After filtration, 1 % (m/v) of active carbon was added. This solution was centrifuged at 2000 rpm, and the top part was separated, frozen in liquid nitrogen and lyophilized. This crude enzyme extract was used in four different forms for the cathode preparation. The carbon cloth (PWB 3,

Stackpole) used as support of the electrode was subjected to a treatment first in nitric acid and then in water in order to break the hydrophobicity as previously described [16,17].

2.2 Preparation of the cathode

Four types of cathodes were prepared from the crude enzyme extract. In the first two cases, the electrodes were prepared using as substrate a carbon cloth (PWB-3, Stackpole), where the enzyme was either adsorbed or immobilized. A piece of carbon cloth was immersed in a 15.0 mg mL^{-1} aqueous solution of the crude enzymatic extract and then lyophilized, resulting in the adsorbed biomaterial on carbon cloth, hereafter referred to as the CC_{ads} cathode. For immobilization, the dried carbon cloth with adsorbed crude extract as obtained in the previous case was then immersed in a 1 mmol L^{-1} diciclohexilcarbodiimida (Aldrich) solution in chloroform (Merk). Finally, the CC_{im} cathode was dried in an air flow. For the other two cases, active carbon powder (Riedel-of Raën) was used. The adopted procedure was quite similar to that just described, the main difference being that carbon powder (which was characterized and found to adsorb 18 mg of methylene blue per gram of active material) was added to the crude extract of peroxidase for adsorption. For immobilization it was used a 1 mmol L^{-1} solution of diciclohexilcarbodiimida (Aldrich) in chloroform (Merk). The load of the electrode was approximately 2 mg cm^{-2} of crude extract with an activity of approximately 126 units mg^{-1}, determined by the same methodology adopted by Fatibelo-Fillho *et al.* [15].

2.3 Preparation of the anode

As anode it was used a commercial Pt/C catalyst supplied by E-TEK. This electrode is prepared by distributing uniformly the Pt catalyst on a carbon cloth containing carbon powder (Vulcan XC-72R) and Teflon® (DuPont) [16]. The Pt load was 0.4 mg of Pt cm^{-2}.

2.4 Membrane Electrode Assembly (MEA) preparation

A Nafion® 117 membrane (DuPont) was used as electrolyte. To prepare the membrane electrolyte assembly (MEA), the anode was hot pressed on one side of the membrane. The enzymatic cathode was just placed in contact with the membrane, in order to avoid damaging the biomaterial, since enzymes do not tolerate high pressure and temperature as used in the conventional preparation of the MEA [5]. Because all experiments were carried out with the same Pt anode and membrane, the differences observed in cell performance can be basically attributed to the enzymatic cathode.

2.5 Fuel Cell Experiments

The experiments were carried out under galvanostatic control. With a constant current source it was controlled the current density through the system and with multimeters it was measured the potential between the cathode and the anode of the biochemical fuel cell. The cathode was fed with O_2 humidified and the anode was fed with H_2 also humidified, both at ambient temperature. All the experiments were carried out at 25 ºC ± 1 ºC. The geometric area of electrodes were 1.13 cm^2.

3. RESULTS AND DISCUSSION

Figure 1 shows current density vs. cell potential curves obtained for the four types of enzymatic cathodes studied. Overall, the following activity sequence was observed: $CP_{ads} > CC_{im} > CP_{im} > CC_{ads}$. Differences of about 20% in the cell voltage were observed when the enzymatic cathode prepared with the biomaterial adsorbed on carbon powder was employed (CP_{ads}).

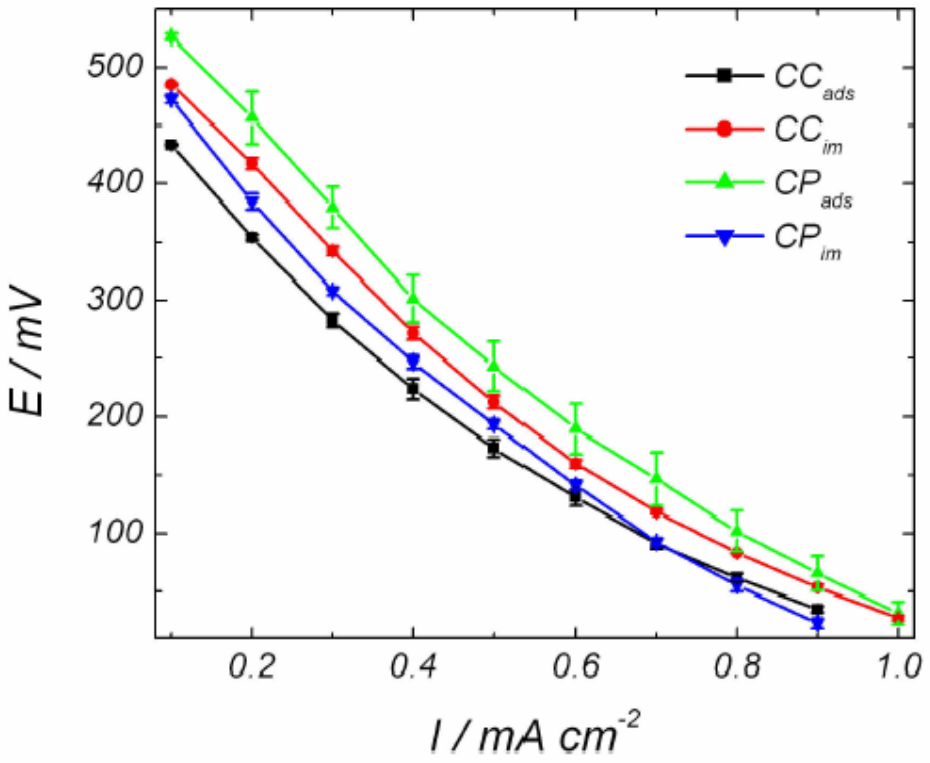

Figure 1 - Current density versus cell potential for a PEMFC. Anode: 0,4 mg Pt cm^{-2}, cathode: adsorbed (CC_{ads}) and immobilized (CC_{im}) peroxidase extract on carbon cloth, and adsorbed (CP_{ads}) and immobilized (CP_{im}) peroxidase extract on carbon powder. The error bars present the deviation observed for independent sets of experiments. Temperature 25ºC.

In contrast to the well-known anodic reaction [17], very little is known about the cathodic reaction, i.e. the, at least partial, oxygen reduction mediated by peroxidase. It is known that under some conditions, peroxidase can indeed act also as an oxidase, catalyzing the oxygen-peroxide reduction. A typical example is the role played by peroxidase in the cell wall biosynthesis [18]. In this case the enzyme acts initially as an oxidase reducing oxygen to peroxide coupled to the oxidation of NADH. This process is believed to occur via the generation of O^{2-} and then hydrogen peroxide [18], which can be further reduced to water. According to this scenario, the NADH required for peroxide formation is probably provided by a malate dehydrogenase also present in the cell wall. Therefore, in contrast to the previous reports [19], we believe that the peroxidase electroactivity observed here is attributed to its crude extract form, which, in turn, might include also some residues that contribute to the chemical oxygen reduction. Additionally to some residual NADH present in the crude extract, protons released

from the anodic compartment through the electrolyte membrane may also play a role in the peroxidase mediated oxygen reduction to hydrogen peroxide. Corroborating this conjecture, experimental evidence of direct electroctalytic activity of peroxidase towards the reduction of oxygen and hydrogen peroxide in a phosphate buffer (pH =7) has been reported by Dong *et al.*[13,14]. The specific differences among the biomaterial based cathodes are rationalized as follows.

The better performance of immobilized with respect to adsorbed biomaterial on carbon cloth can be ascribed to the better interaction between enzyme and carbon. Indeed, as far as the immobilization is achieved via cross-linking between the enzyme and the active sites of the carbon cloth, it might promote an improvement of the enzyme-support interaction [5]. Considering now the difference between the immobilized and adsorbed biomaterial on the carbon powder, the improved activity of the adsorbed material can be rationalized in terms of two different aspects: the eventual presence of residues or traces of diciclohexilcarbodiimida absorbed in the active carbon or the loss of electric contact between the carbon particle and the enzyme [11]. Overall, it can be concluded that the strong interaction between the enzyme and the active carbon is the key factor of the improved activity of the crude extract in this case.

Owing to the preparation method, it should be emphasized that carbon powder is more porous and has a larger active area than the carbon cloth, which makes it more advantageous due to the increased amount of adsorbed enzyme for a given area. In addition, the amount of adsorbed enzyme per unit area of support increases with the concentration of the crude extract up to a limit, according to a Langmuir isotherm [20]. The effectiveness and the efficiency of the adsorption of an enzyme on a substrate depends on factors such as the size of the protein, the superficial area of the support and, mainly, the porosity and the pore size.

Plots of power density as a function of the applied current density are presented in Figure 2 for the mean value observed in the four cases presented in Figure 1. As expected, the differences in the cell performance are better seen in this representation. A maximum in power density of 122 μW cm^{-2} is obtained when the CP_{ads} is used and a global improvement of about 36% is observed in this case in comparison to the CC_{ads} cathode. Noteworthy is the fact that the maximum in power density is obtained at a current density of 0.5 mA cm^{-2} in the case of the CP_{ads} cathode, whereas in the other three cases it is found at a current density of 0.4 mA cm^{-2}.

The power values presented here are considerably higher than those previously reported by Pizzariello *et al.* [21] of around 0.15 μW cm^{-2}, for a biochemical fuel cell using a peroxidase based cathode. In this system they fed the cell with 6.10^{-4} mol L^{-1} of glucose in the anode and $6,10^{-4}$ mol L^{-1} of H_2O_2 in the cathode. Power densities of the same order were also obtained by Mano *et al.* [22], using a compartment-less glucose- oxygen biochemical fuel cell. However, it is important to stress that this comparison should be seen more as an illustration, because in the mentioned references the authors worked with a *complete* biochemical fuel cell, in contrast to the present case where a *biocathode* is under evaluation.

To investigate the stability of the different cathodes, some experiments were carried out at a fixed current density [23,24]. Although preliminary, the results show that under operational conditions of the

fuel cell the cathode exhibits a reduced life. Typically, the cell reaches a slowly decreasing voltage in the range of 100 – 170 mV after c.a. 2.5 h of operation. These values can be considered low, roughly in the range of 50%, when compared to those presented in Figure 1.

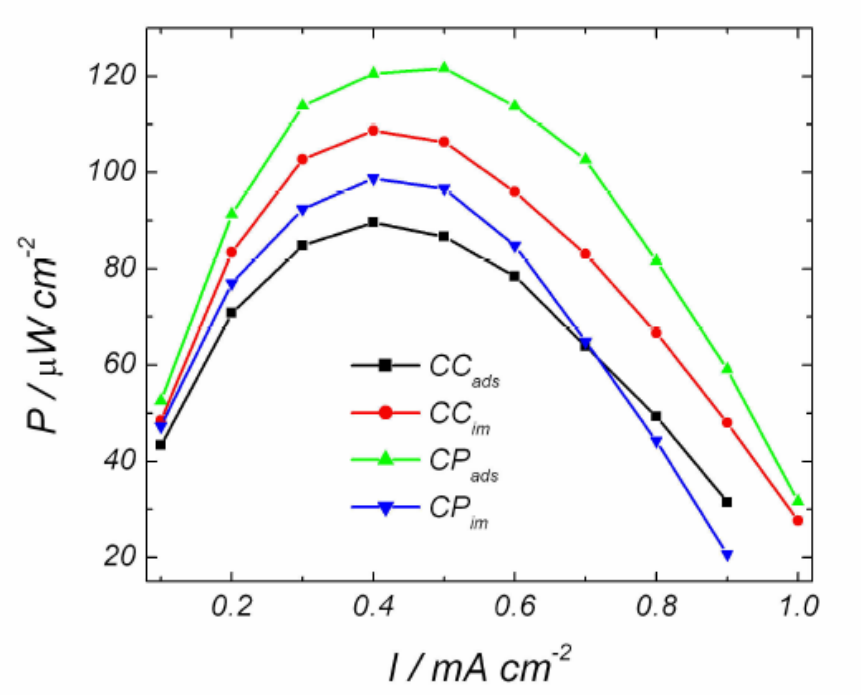

Figure 2 - Plots of power density versus cell current density for the four different configurations displayed in Figure 1.

Such deactivation process can be in principle attributed to the eventual loss of intimate contact between the biocatalyst and the substrate, leaching of the enzyme, or the decrease of the local pH near the catalytic layer during operation. Indeed, the optimum catalytic activity for this biomaterial is expected to occur in the pH range 4 – 8. An important factor for keeping the enzyme active is the presence of water at the cathodic compartment and, in the present case, the humidification of the oxygen stream was not very effective because it was carried out at room temperature. The role played by water is to help keeping the active three-dimensional conformation, because usually the covalent bond between the enzyme and substrate is known to facilitate a distortion of the optimum configuration [25]. Additionally, water also contributes to the three-dimensional structural integrity, polarity of the active site, stability of the protein, and might also limit the hydrophobic substrate solubility around the enzyme [18].

4. CONCLUSIONS

We have investigated a PEMFC fed with H_2/O_2 operating at 25 ^oC with a platinum based anode and a biocathode prepared with a crude enzymatic extract. This crude extract consists primarily of nonpurified peroxidase, which in turn might contain some residues that can help to catalyze the oxygen

reduction. Four different procedures were used for the cathode preparation, namely the adsorption and immobilization of the biomaterial directly on a carbon cloth (CC_{ads} and CC_{im}) and the same procedure but using carbon powder (CP_{ads} and CP_{im}). The cathode performance in the fuel cell decreases in the following sequence $CP_{ads} > CC_{im} > CP_{im} > CC_{ads}$. An improvement of 20% in the cell voltage and of about 120 mW cm^{-2} in the cell power density have been observed when the enzymatic cathode prepared with the biomaterial adsorbed on carbon powder was employed (CP_{ads}). Stability tests show that the cell reaches a slowly decreasing voltage in the range of 100 – 170 mV after c.a. 2.5 h of operation. Such deactivation was attributed to the eventual loss of intimate contact between the biocatalyst and the substrate, leaching of the enzyme, or the decrease of the local pH near the catalytic layer during operation. Considering the preliminary nature of our results, a less speculative interpretation requires more experimental work, mainly using the cathodic half cell. Such experiments are currently in progress in our laboratory.

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