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Modification of porous carbon tubes with enzymes: application for biofuel cells

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Abstract

Enzyme biocatalyst assemblies on electrode surfaces can be used to construct efficient membrane-less biofuel cells. The present study reports on the construction of biocatalytic oxygen electrodes based on the modification of porous carbon tubes with laccase and 2,2′-azinobis-(3-ethylbenzothiazoline−6-sulfonate) ($ABTS^{2-}$) as mediator, co-immobilized within electrochemically polymerized films. Porous carbon tubes are used as original conducting support for enzyme incorporation and for the transport of dissolved dioxygen solution via diffusive flow through the porosity. The activity of the laccase immobilized on the porous carbon support is determined. Performance of the biocathodes towards the reduction of dioxygen to water is demonstrated by chronoamperometry at + 0.4 V vs. Ag/AgCl in citrate/phosphate buffer pH 3. Moreover, the current–voltage behaviour of the biocathodes show high current densities at pH 3 (a current peak equal to 280 μA cm^{-2} for the biocathode based on both entrapped laccase and mediator) and a low oxygen reduction overvoltage compared to platinum electrode.

1. Introduction

Biological fuel cells utilize microorganisms or enzymes as biocatalysts to reduce and oxidize organic molecules, producing electrical energy [1–4]. Dioxygen is the common fuel used in the cathode compartment because it is readily available and a good oxidant [5]. Electrochemical reduction of dioxygen to water has been demonstrated with electrocatalysis metal complexes [6, 7] and redox enzymes [8]. In particular, much attention has been paid to laccase, an oxidoreductase containing four copper ions [Cu (II)] that catalyzes the four-electron reduction of dioxygen to water [9]. To enhance the slow rate of electron transfer between the enzyme and the electrode, dioxygen reduction is carried out in the presence of an electrochemical mediator 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{2-}$), which shuttles electrons from the cathode to the catalytic site of the enzyme [10].

In order to construct miniature and implantable biofuel cells, a compartment-separating membrane is excluded. Immobilization of enzymes and mediators on the conducting support is thus required. A variety of immobilization methods on different supports have been used [11] for laccase applications in biotechnological and environmental applications. However, incorporation of laccase in electropolymerized films such as polypyrrole impregnated with electron transfer mediators has never been carried out. This technique, largely developed for a variety of biomolecules, provides very convenient and stable biocatalyst interfaces investigated for biosensor design [12, 13]. The bio-molecule immobilization on electrode surfaces can be carried out either by entrapment [14, 15] or by covalent binding to electrochemically polymerized films [16].

The aim of this work was to produce operational biocathodes based on laccase entrapment within electrochemically polymerized films for electrocatalytic reduction of dioxygen in biofuel cells. Porous carbon tubes were used as original conducting support for enzyme incorporation and for the transport of dioxygen via convective flow through the pores. We present an immobilization method, which consists of laccase entrapment during electropolymerization of pyrrole monomer formed at the electrode surface.

Activity of the laccase immobilized on the carbon porous support was determined. The performance of the biocathodes towards the reduction of dioxygen to water

was demonstrated by chronoamperometry at +0.4 V vs. Ag/AgCl in citrate/phosphate buffer pH 3. Current–voltage curves of the different biocathodes were compared under different operating conditions for example pH 3 and 5. Indeed, the laccase exhibits a higher activity under acidic conditions but in the objective to associate this biocathode with a bioanode, which uses glucose oxidase as biocatalyst, the experiments were carried out also at pH 5 (intermediate pH between 3 and 7, which are pH optima for laccase and glucose oxidase activities, respectively).

2. Experimental

2.1. *Materials*

2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) diammonium salt ($ABTS^{2-}$) was purchased from Sigma-Aldrich and used without further purification. Pyrrole and sodium perchlorate $NaClO_4$,(H_2O) were supplied by Merck. The different buffers were prepared with sodium dihydrogen phosphate monohydrate (NaH_2PO_4, H_2O) and di-sodium hydrogen phosphate (Na_2HPO_4) salts (pH 7) from Merck, and from citric acid (Prolabo) and NaH_2PO_4, H_2O salt (pH 3). All the aqueous solutions were prepared using 18 MΩ cm MilliQ water (Millipore).

The 1 cm diameter carbon porous tubes were a gift from Novasep-Orelis. They are characterized by average pore diameter of 3 μm and an inner diameter of 0.6 cm.

2.2. *Enzyme extraction*

The organism used for laccase production was *T. versicolor* ATCC 32745. *T. versicolor* was initially cultivated on agar malt extract plates that were incubated for 7 days at 27 °C and then stored at 4 °C. 200 ml broth pre-cultures were prepared in a Roux flask by inoculating a nitrogen rich medium containing 1 g glucose, 0.2 g yeast extract, 0.4 g bactopeptone, 0.36 g ammonium tartrate, 0.46 g sodium tartrate, 0.27 g KH_2PO_4, 0.19 g $CaCl_2 \cdot 2H_2O$, 0.093 g $MgSO_4 \cdot 7H_2O$ and 6 ml mineral salt solution with five agar culture plugs (6 mm diameter) from the growing edge of the mycelium. The mineral salt solution contained 2.4 g $FeSO_4 \cdot 7H_2O$, 1.6 g $ZnSO_4 \cdot 7H_2O$, 1.21 g $MnSO_4 \cdot H_2O$, 0.24 g $CuSO_4 \cdot 5H_2O$ per litre. 0.2 ml of Tatum vitamins was finally added after filtration through 0.2 μm. Roux flasks containing the pre-culture were then incubated statically in darkness at 27 °C under a regime of passive aeration. After 6 days of growth, an uniform inoculum was obtained by homogenization of the biofilm in 200 ml of water with an Ultra-Turrax homogenizer. Four hundred millilitre culture medium inoculated with 50 ml of the homogenized pre-culture was then incubated in 2 l Erlenmeyer flasks at 27 °C and agitated at 150 rpm. The culture medium was modified for laccase production from that of Jönsson [17] to have the following composition: 20 g of maltose, 4 g of ammonium tartrate, 0.9 g of KH_2PO_4, 0.16 g of $NaH_2PO_4 \cdot 2H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.0066 g of $CaCl_2 \cdot H_2O$, 0.01 g of $CuSO_4 \cdot 5H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, 0.0005 g of $ZnSO_4 \cdot 7H_2O$ and 0.00001 g of thiamine per litre of demineralized water. The medium was buffered at pH 5 by 2 mM 2,2-dimethyl-succinic acid. 2,5 xylidine (0.3 mM) was added to the medium before inoculation to induce laccase production. After seven days of growth, the laccase-containing extracellular culture liquid was separated from the mycelium by filtration through a layer of glass-wool.

Extracellular polysaccharides were precipitated with 10% cold acetone, frozen at −80 °C, then separated by successive filtrations (final porosity 0.22 μm). The filtrate was concentrated by ultrafiltration (Millipore YM10, cut-off 10 000 Da), and laccase was recovered in 20 mM citrate-phosphate buffer (CPB) pH 5 by dialysis using the same membrane. The enzyme was then applied to a DEAE 52 anion exchange column equilibrated in the same buffer. Combined active non-retained fractions were pooled and concentrated by ultrafiltration on YM 30, then dialyzed against 50 mM phosphate buffer at pH 6.8, where the stability of the enzyme is maximal at 4 °C. After addition of glycerol (30% v/v final), this crude preparation of laccase (around 500 U ml^{-1} and 3 mg ml^{-1}) was stored at −20 °C. Enzyme activity is expressed in terms of enzymatic units (U). One enzymatic unit is the amount of enzyme required to transform 1 μmol of substrate per minute.

2.3. *Methods*

2.3.1. *Elaboration of the biocathode*

2.3.1.1. *Biocathode with entrapped laccase*. The biocathode was prepared by dipping a 1 cm-long bare carbon tube ($S = 3.15\ cm^2$) in phosphate buffer pH 7 (0.1 M) with laccase (5 U ml^{-1}), overnight at 4 °C. 0.1 M $NaClO_4$ and 0.02 M pyrrole monomer were added to the solution just before starting the anodic electropolymerization at +0.9 V vs. Ag/AgCl. The controlled-potential oxidation of the pyrrole was continued until a charge of 8 C was passed. The modified electrode was then rinsed for 3–4 h in 2 ml of 0.1 M phosphate buffer (pH 7). The prepared biocathode was stocked in 0.2 M phosphate buffer pH 7 at 4 °C.

2.3.1.2. *Biocathode with entrapped laccase/ABTS*. The bare carbon tube (1 cm long, $S = 3.15\ cm^2$) was soaked into a mixture of laccase (6.15 U ml^{-1}) and $ABTS^{2-}$ (6.6 mM) in 0.1 M phosphate buffer pH 7. The incubation lasted overnight at 4 °C. Pyrrole electropolymerization was then performed in the conditions described above for the laccase adsorption without $ABTS^{2-}$.

2.3.2. *Enzyme assays*

Laccase activity was measured by the rate of oxidation of 1 mM $ABTS^{2-}$ in 0.1 M CPB, pH 3 at 23 °C. The

substrate solution was aerated by air bubbling before the addition of the enzyme. The increase in absorbance, corresponding to the production rate of $ABTS^{2-}$ oxidation product, was followed by spectrophotometry at 420 nm (ϵ_{420} = 36,000 mol cm^{-1} l^{-1}). The amount of enzyme generating 1 μmole of product over 1 min was taken as one unit (U) of activity. The same procedure was used to determine laccase activity immobilized on the tube. Measurements were performed by soaking the tube in a 1 mM $ABTS^{2-}$ solution at pH 3 (V = 8 ml) that was circulated through a spectrophotometric cell. Activity measurements are the mean of at least three measurements. Experimental error can be estimated to around 10%.

2.3.3. *Electrochemical measurements*

Chronoamperometry studies were performed with a potentiostat EGG model 273A, using a three-electrode cell (20 ml) equipped with an Ag/AgCl reference electrode and a cylindrical stainless steel counter electrode. The working electrode was the carbon porous tube modified by entrapped enzymes. Experiments were performed in aerated (O_2 bubbling) 0.1 M CPB pH3 at +0.4 V vs. Ag/AgCl.

The performances of biocathodes (curves $I = f(E)$) were evaluated in an individed conventional three-electrode Pyrex cell (36 ml) (Figure 1). The modified carbon tubes used as biocathodes were electrically connected to a palladium-gold wire using conductive carbon paste. Two carbon plates and a saturated calomel electrode were used as the counter and reference electrodes, respectively. The electrodes (anode and biocathode) were immersed in an electrolyte consisting of 0.2 M CPB adjusted to pH 3.0 or 5.0. The reference electrode was separated from the solution by a Luggin–Haber capillary tip. The electrolyte was saturated with nitrogen or dioxygen gas. The electrochemical measurements were performed using a potentiostat VoltaLab10 (Radiometer Analytical) connected to a computer.

Fig. 1. Three-electrode cell used for electrochemical measurements.

2.3.4. *Scanning electron microscopy*

The scanning electron microscopy (SEM) observations of the cross section of carbon tubes were made using a 5 kV scanning electron microscope (HITACHI S4500). The samples were mounted with conductive glue to metal stubs with the fractured edge up and then coated with gold by sputtering. These samples were then viewed in the SEM at around 1000× magnification.

3. Results and discussion

3.1. *Characterization of entrapped laccase biocathodes*

The biocathodes were elaborated by entrapment of laccase within polypyrrole films on the electrode surface. As described in the experimental part, the bare carbon tube was first soaked in dissolved enzyme solution during a certain time to ensure laccase adsorption. Second, the pyrrole monomer was electropolymerized on the electrode surface. In order to determine the sufficient time for the adsorption equilibrium of laccase on the tube, a preliminary experiment was conducted. A bare carbon tube was soaking in a buffered solution pH 7 containing the dissolved enzyme (0.2 U ml^{-1}) at 4 °C. The enzyme activity in the supernatant was studied as a function of time (Figure 2, dashed curve). The activity decrease is linked to the amount of adsorbed laccase on the tube as compared to the activity of an enzyme solution reference (Figure 2, straight curve) that shows stable activity over 30 h in absence of carbon tube. From the experiments, about sixteen hours are sufficient to ensure adsorption equilibrium of the laccase on the carbon tube at pH 7.

We point out that a lower enzyme concentration was used (0.2 U ml^{-1}) for that experiment, compared to the concentration used for the elaboration of the biocathode (5 U ml^{-1}), in order to get detectable variation of adsorbance in spectrophotometry.

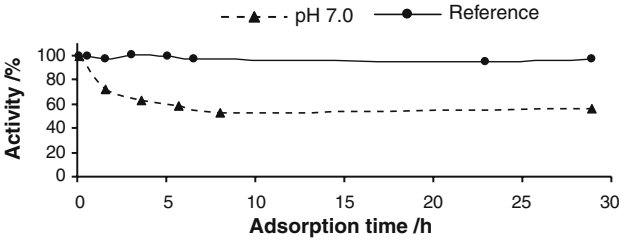


Fig. 2. Enzymatic activity of laccase solution vs. time in phosphate buffer pH7 at 4 °C in reference solution (•) and in presence of carbon tube (▲).

The actual activity of immobilized laccase on the tubes was estimated to 31 mU cm^{-2} by measuring the oxidation rate of $ABTS^{2-}$ (see experimental part). The biocathodes were characterized by SEM (Figure 3) to point out the homogeneous matrix of polypyrrole deposited on the carbon porous surface of the tubes.

3.2. *Electrolysis of dioxygen to water at the modified biocathode*

The combination of blue copper oxidases (laccase, bilirubin oxidase) and $ABTS^{2-}$ as an electron transfer mediator, has been reported by Palmore and Kim [10] showing a number of attractive features for oxygen-reducing fuel cell cathodes, in particular, a relatively high operating potential, and a direct 4-electron reduction mechanism, reducing O_2 completely to H_2O. By oxidizing the electrochemical mediator $ABTS^{2-}$, laccase accumulates the reducing equivalents necessary for the reduction of dioxygen to water. $ABTS^{2-}$ is thus oxidized to $ABTS^{\circ-}$ and must be reduced at the electrode at a potential more negative than its formal potential. On the carbon porous tubes used as original conducting support for enzyme entrapment, $ABTS^{2-}/ABTS^{\circ-}$ produces a first well-defined one electron reversible wave (E° = 0.46 V vs. SCE) by cyclic voltammetry at pH 5.0 (Figure 4). This value is closed to the reported values 0.505 V vs. Ag/AgCl at pH 4.0 [8] and 0.440 V vs. SCE at pH 4.0 [10].

In our conditions, the feasibility of the laccase biocathodes to the electrocatalytic reduction of O_2 to water was thus demonstrated by chronoamperometry in presence of $ABTS^{2-}$. The current corresponding to the bioelectrocatalytic reduction of dioxygen was measured at 0.4 V vs. Ag/AgCl for biocathodes immersed in citrate/phosphate buffer solution at pH 3 (Figure 5). In air-saturated solution, during the first 800 s differences in the transient behaviour of the current are observed due to the different nature of the electrodes. For entrapped laccase/ABTS biocathode, the oxidized form $ABTS^{\circ-}$ is initially present on the electrode following oxidation of $ABTS^{2-}$ by the enzyme, and it is immediately reduced at +0.4 V. However, for entrapped laccase biocathode, an unexpected oxidation current is observed probably due to oxidation of impurities at +0.4 V. After ca. 800 s, in absence of oxygen, the currents of the biocathodes reach a quasi-steady state and no reduction current of dioxygen was detected due to the depletion of O_2 in close to electrode surface. After 1600 s, the solution is bubbled with oxygen and a steady state cathodic current of −110 and −80 μA respectively for each electrode was measured and remained constant for 2500 s, corresponding to the electrocatalytic reduction of dioxygen. The importance of oxygen-saturated solution to observe the reduction of dioxygen was as well examined by Tsujimura et al. [8]. On carbon felt electrode, the authors observed a gradual decrease of the cathodic current at 0 V vs. Ag/AgCl/$KCl_{sat.}$ in air-saturated 0.5 mM $ABTS^{2-}$ (pH7) solution containing 0.11 μM bilirubin oxidase. Then the cathodic current increased to 500 μA cm^{-2} by bubbling O_2 in the solution.

In the case of co-immobilized laccase and ABTS biocathodes, a decrease in electrocatalytic activity by 30–50% was observed after ca. 60 h with successive

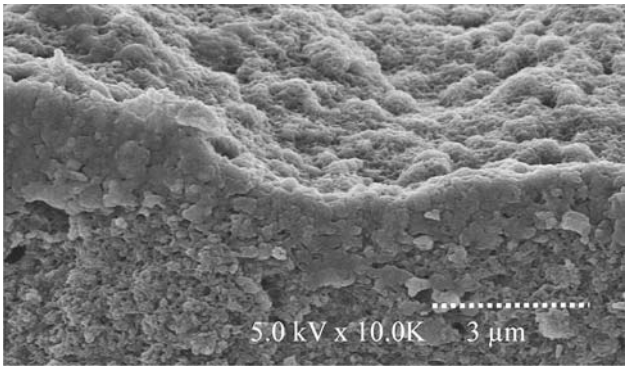


Fig. 3. SEM image of the cross section of the modified porous carbon tube.

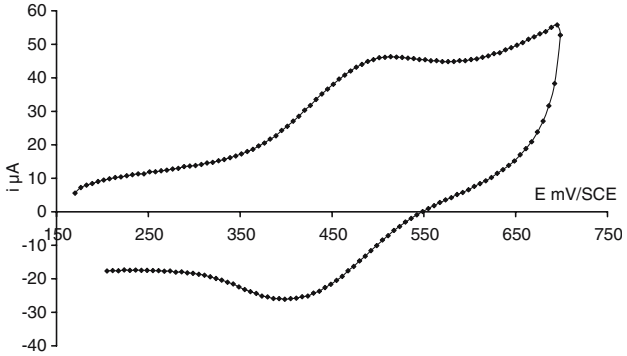


Fig. 4. Cyclic voltammetry of $ABTS^{2-}$ (0.5 mM) at porous carbon electrode, υ = 5 mV s^{-1} in pH = 5 phosphate buffer.

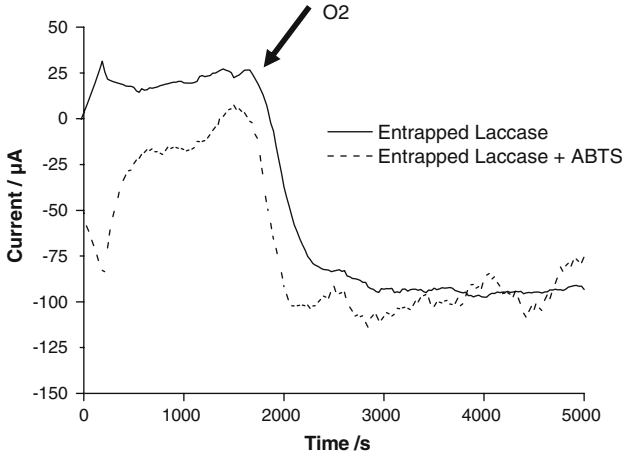


Fig. 5. Electrolysis of dioxygen to water in air-saturated pH 3 citrate phosphate buffer, 0.1 M $NaClO_4$, at +0.4 V vs. Ag/AgCl at entrapped laccase biocathode with 0.25 mM $ABTS^{2-}$ in solution (dash curve) and at entrapped laccase/ABTS biocathode (dotted curve). From 1600 s, oxygen gas was bubbled through the solution.

chronoamperometric runs (data not shown). This activity loss was associated with ABTS leaching as the electrolyse solution took a green coloration whereas the measured laccase activity was still constant. The same result was observed in the case of encapsulated laccase electrodes with silica or organic polymer binders on porous carbon paper supports [18]. The authors have demonstrated that silica microstructures were insufficiently impermeable to ABTS leaching as only 39% of the electrocatalytic activity was maintained into a second experiment.

3.3. *Biocathode electrochemical behaviour*

The cathodic current observed for the biocathodes establishes the feasibility of the co-immobilization of both enzyme and its mediator within electropolymerized films to achieve a biocatalytic electrode able to reduce dioxygen to water. A prototype biofuel cell has been used to evaluate the current density delivered by the biocathodes at room temperature and atmospheric pressure. The activity of biocathodes were determined before plotting the current density vs. voltage curves. Consequently we can consider that the observed values correspond to the initial condition of system. First, the efficiency of $ABTS^{2-}$ as electron mediator between the enzyme and the electrode was pointed out by comparing the current voltage curves obtained with and without mediator for a biocathode with only entrapped laccase on its surface (Figure 6).

The reduction of O_2 in presence of laccase begins at 0.43 V/SCE whereas it takes place at a more positive potential (0.55 V/SCE) in presence of $ABTS^{2-}$. The mediator $ABTS^{2-}$ is thus a good electron donor for use in the cathode compartment of a biofuel cell.

Figure 7 compares the current–voltage behaviour of two biocathodes to platinum. The biocathode A corresponds to the electrode with only entrapped laccase whereas the biocathode B is an electrode with both entrapped laccase and $ABTS^{2-}$. The current density vs. voltage curves in the case of the both biocathodes present a reduction current peak because the indirect reduction of oxygen is limited by the diffusion of the species to the electrode. The electrochemical characterization of the biocathode A, immersed in a solution pH 3 containing 0.222 mM $ABTS^{2-}$, has been carried out under N_2 or O_2 bubbling directly in the solution (Figure 7). After 15 min of N_2 bubbling, the open-circuit voltage was 0.48 V vs. SCE and increased with the bubbling of oxygen. Indeed, the open-circuit voltage reflects the presence of $ABTS^{2-}$ and $ABTS^{\circ-}$:

$$E\,(V/\mathrm{SCE}) = E^{\mathrm{O}}_{\mathrm{ABTS}^{\circ-}/\mathrm{ABTS}^{2-}} + \frac{RT}{F}\ln\frac{[\mathrm{ABTS}^{\circ-}]}{[\mathrm{ABTS}^{2-}]} \quad (1)$$

In presence of oxygen, the equilibrium shifts towards the formation of $ABTS^{\circ-}$: $4\,ABTS^{2-} + O_2 + 4\,H^+ \xleftrightarrow{\text{laccase}} 4ABTS^{\circ-} + 2H_2O$ as a result of the oxidation of $ABTS^{2-}$. The mediator oxidized and reduced forms concentrations are modified and thus the open-circuit voltage and diffusion current densities increase with oxygen bubbling (from $+0.48$ to $+0.55$ V and from -100 to $-140\ \mu A\ cm^{-2}$). The values of current densities of dioxygen reduction are not similar for both biocathodes as compared to chronoamperometry. This difference is linked to the measurement method. In current–voltage experiment, the electrode is scanning over a potential range and the current density is limited by mass transfer of $ABTS^{2-}$ and O_2 in solution. The entrapped laccase/$ABTS^{2-}$ biocathode is less affected and present higher current densities as the mediator is already in the vicinity of the enzyme and the electrode.

These values of current densities obtained with 31 mU cm^{-2} of immobilized laccase and 0.222 mM $ABTS^{2-}$ in solution are equivalent to 130 $\mu A\ cm^{-2}$ achieved by Palmore and Kim [10] with higher concentrations (2 mM $ABTS^{2-}$ and 350 mU laccase in solution). The time of oxygen bubbling has the same effect for the biocathode B immersed in CPB. For the same bubbling duration (3 h30), the open-circuit voltage is similar for both electrodes but the current densities are higher when enzyme and mediator are co-immobilized.

Consequently, this biocathode B was selected to evaluate the effect of pH. In the first part of the study, the biocathodes were immersed in a 0.2 M CPB adjusted to pH 3.0. At this pH, laccase from *T. versicolor* presents highest activity. However, in the objective to associate this biocathode with a bioanode, which uses glucose oxidase as biocatalyst, the next experiments were carried out at pH 5 (intermediate pH between 3 and 7, which are pH optima for laccase and glucose oxidase activities, respectively). As shown in Figure 7, the increase in pH slightly decreases the open-circuit voltage and the current densities remain attractive.

Finally the performances of these biocathodes were compared to platinum (Figure 7). In the case of platinum electrode, the current density vs. voltage curve shows a typical Butler–Volmer behaviour. The rate of oxygen reduction is controlled only by the rate of the electrochemical charge transfer process. The open-circuit voltage for the direct electroreduction of dioxy-

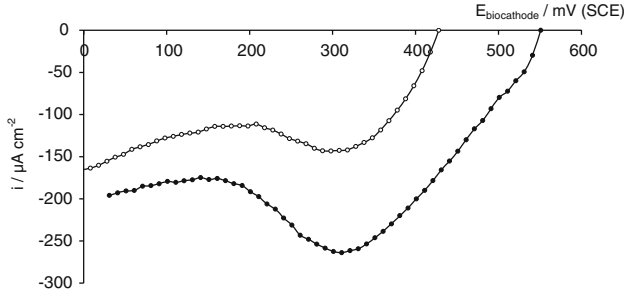


Fig. 6. Polarization curves of entrapped laccase biocathode in pH 3.0 CPB 0.2 M solution after 3 h30 O_2 bubbling (○) without $ABTS^{2-}$, (•) with 0.222 mM $ABTS^{2-}$.

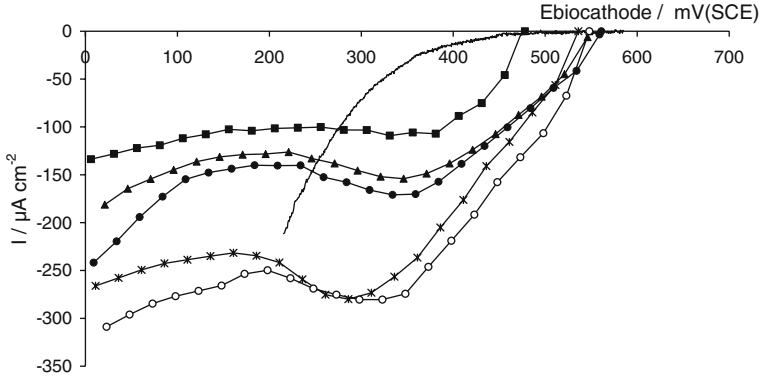


Fig. 7. Polarization curves of biocathodes and platinum electrode under different conditions of bubbling achieved directly in the electrolytic solution. Biocathode A with 0.222 mM $ABTS^{2-}$ in pH 3.0 CPB 0.2 M solution: (■) 15 min. N_2 bubbling, (▲) 30 min O_2 bubbling, (•) 3 h30 O_2 bubbling. Biocathode B: (○) 3 h30 O_2 bubbling in pH 3.0 CPB 0.2 M solution, (*) 3 h30 O_2 bubbling in pH 5.0 CPB 0.2 M solution. Pt electrode: (-) O_2 bubbling in pH 3.0 CPB 0.2 M solution.

gen at a cathode of platinum is 0.58 V vs. SCE and is thus superior to those observed for the biocathodes. But due to electron transfer slow kinetics, dioxygen is reduced at platinum several hundred millivolts lower than its formal potential. Consequently, significant oxygen reduction current densities are observed at a higher potential on a laccase biocathode than on platinum, which is in favour of the bioelectrode.

In the following experiment (Figure 8), dissolved oxygen was supplied to the system by diffusion through the inner cavity of the carbon tube instead of bubbling in the solution as depicted in Scheme 1.

At the beginning of the experiment, the biocathode was introduced in a non-degassed solution pH 5.0, allowing the immobilized mediator $ABTS^{2-}$ to react with oxygen traces and form $ABTS^{\circ-}$. Consequently as observed in Figure 8, the potential was well defined (0.48 V vs. SCE). After 15 min of N_2 bubbling, the open-circuit voltage had decreased to 0.41 V vs. SCE. With the elimination of oxygen by N_2 bubbling directly in the solution, the concentrations of $ABTS^{2-}$ and $ABTS^{\circ-}$ change and thus the open-circuit voltage decreases. In order to evaluate the potentialities of the carbon tube towards diffusion of dissolved oxygen, an oxygen flow was allowed to circulate inside the inner cavity of the tube. It is observed that the open-circuit voltage and the current densities increase (from 0.41 to 0.56 V/SCE and from −150 to −240 µA cm^{-2}) with the oxygen bubbling time, ensuring efficient oxygen diffusion through the porosity of the tube. This maximum of current density compares favourably with density currents of 280 µA cm^{-2} at pH 4.7 [19] and 330 µA cm^{-2} pH 5.0 [20] for laccase-based biocathodes using osmium-based redox polymers as mediators. In these experiments, dissolved oxygen was supplied to the system by bubbling in the solution.

4. Conclusion

The immobilization technique using a polypyrrole film generally provides homogeneous, chemically stable and adherent films. The significance of our work is the production of operational biocathodes based on laccase and $ABTS^{2-}$ co-immobilization within electrochemically polymerized films.

The electrochemical characteristics of the bioelectrodes satisfy the requirement of a cathode to achieve dioxygen reduction to water at pH 5 in a biofuel cell. High current densities (up to 280 µA cm^{-2} at pH 3) of

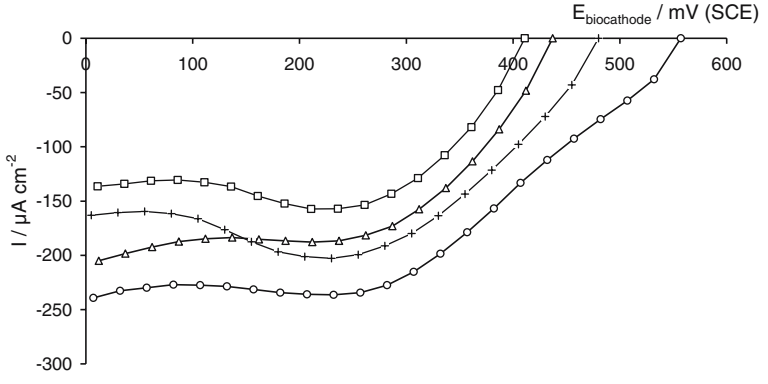
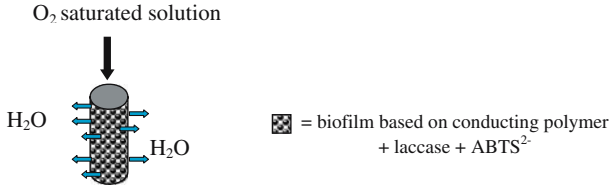


Fig. 8. Polarization curves of biocathode B in pH 5.0 CPB 0.2 M. The oxygen supply is carried out by the diffusion of dissolved oxygen through the carbon tube porosity. (+) no bubbling, (□) 15 min N_2 bubbling, (Δ) 30 min O_2 bubbling, (○) 3 h30 O_2 bubbling.



Scheme 1. Schematic representation of the carbon tube used as original conducting support for transport of dissolved oxygen solution.

dioxygen reduction have been reported and a lower oxygen reduction overvoltage was observed compared to platinum electrode.

Moreover we have demonstrated that the porous carbon tubes can be used as original conducting support for enzyme incorporation and for transport of dissolved dioxygen solution via diffusive flow through the porosity.

For the future, it is necessary to study different parameters such as the influence of amount of immobilized enzyme, the operational temperature, flow of oxygen solution through the carbon tube. Meanwhile, the efficiency of the electrode with entrapped mediator and enzyme could be improved by preventing mediator loss. Work is in progress to produce electrodes with grafted laccase and $ABTS^{2-}$ to substituted polypyrrole polymers. Finally we plan to construct a bioanode, with immobilized glucose oxidase in order to evaluate the performances of a complete biofuel cell.

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