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ORIGINAL RESEARCH PAPER

Stable 'Floating' Air Diffusion Biocathode Based on Direct Electron Transfer Reactions Between Carbon Particles and High Redox Potential Laccase

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Abstract

We report on the assembly and characterisation of a high potential, stable, mediator-less and cofactor free biocathode based on a fungal laccase (Lc), adsorbed on highly dispersed carbonaceous materials. First, the stability and activity of *Trametes hirsuta* Lc immobilised on different carbon particles were studied and compared to the solubilised enzyme. Based on the experimental results and a literature analysis, the carbonaceous material BM-4 was chosen to design efficient and stable biocatalysts for the production of a 'floating' air diffusion Lc-based biocathode. Voltammetric characteristics and operational stability of the biocathode were investigated. The current density of oxygen reduction at the motionless biocathode in a quiet, air saturated citrate buffer (100 mM, pH 4.5, 23 °C) reached values as high as 0.3 mA cm^{-2} already at 0.7 V *versus* NHE. The operational stability of the biocathode depended on the current density of the device. For example, at low current density (20 μA cm^{-2}), the biocathode lost only 5× of its initial power after 1 month of continuous operation. However, when the device was polarised at 150 mV it lost more than 32× of its initial power in just 10 min. We also found that co-immobilisation of Lc and peroxidase on highly dispersed carbon materials could protect the biocatalyst from rapid inactivation by hydrogen peroxide produced during electrocatalytic reactions at high-current densities.

Keywords: Direct Electron Transfer, Dispersed Carbon-Based Material, Floating Gas Diffusion Biocathode, Laccase, Peroxidase

1 Introduction

Successful realisation of many important electrochemical processes, including development of fuel cell (FC) technology, requires generation of novel highly effective catalysts, which are active at ambient temperatures and physiological pH values. Optimal technological solutions can be achieved by using biocatalysts heterogeneously arranged in an electroconductive matrix, e.g. redox enzymes immobilised on properly selected highly dispersed materials. For cathodic processes occurring in FCs, blue multicopper oxidases (BMCO) have been identified as very promising bioelements. BMCO efficiently catalyse the reduction of O_2, a very common electron acceptor because of its high-redox potential and its ready availability, at very low overpotentials [1–5].

At the end of the 1970s, direct electron transfer (DET) based bioelectrocatatalytic reduction of O_2 by a fungal laccase (Lc), an enzyme from the BMCO family, was discovered [6, 7]. Later this occurrence was identified in many other redox enzymes including different BMCO, e.g. ascorbate oxidase [8, 9] and bilirubin oxidase [10, 11], as well as fungal [12–15], plant [12, 16] and bacterial [17, 18] Lcs.

BMCO contain four copper ions which are historically classified into three types according to their spectroscopic characteristics, *viz*. the T1, T2 and T3 sites (Figure 1, bottom) [19].

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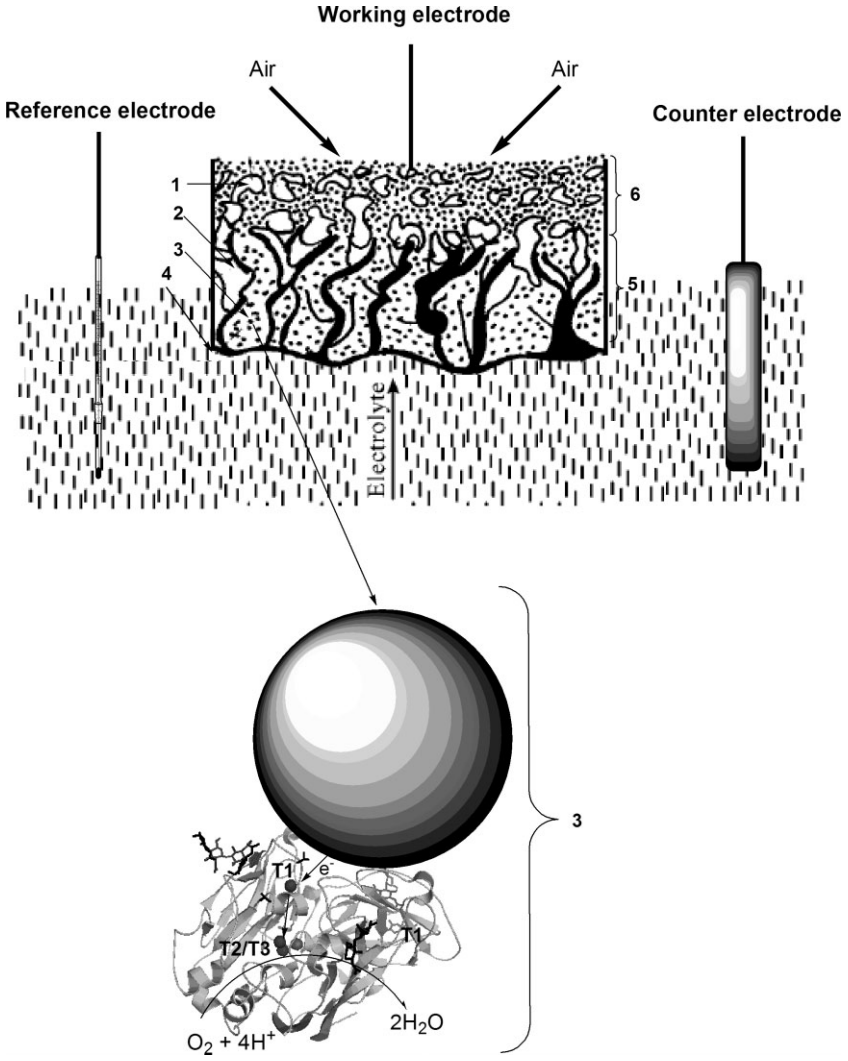

Figure 1 A principal scheme of an electrochemical setup. Working electrode – "floating" air diffusion electrode (1 – pores filled with air, 2 – pores filled with electrolyte, 3 – biocatalysts based on Trametes hirsuta laccase adsorbed on dispersed carbon materials, 4 – Nafion layer, 5 – active layer, 6 – hydrophobic layer); Reference electrode – Ag|AgCl|KCl_{sat}, electrode; Counter electrode – platinum mesh electrode.

The detailed mechanism of DET reactions during BMCO function on electrodes was discussed in several publications, e.g. Ref. [14]. Briefly, electrodes, on which enzymes are appropriately adsorbed with the T1 site in close proximity to the electrode surface (Figure 1, bottom), can serve as electron donors for the active centre of the enzyme, where molecular O_2 is reduced to H_2O without formation of highly reactive oxygen species. This important feature, i.e. the absence of formation of H_2O_2 during bioelectrocatalytic four-electron reduction of O_2, was confirmed using the rotating ring-disk electrode method already in 1979 [7]. Detailed fundamental bioelectrochemical investigations of BMCO were performed using various planar electrodes [14]. It was discovered that carbon was superior, providing efficient O_2 bioelectroreduction at high potentials, whereas DET-based bioelectrocatalysis at metal electrodes seems to be much more complicated [20–22]. Thus, regarding the very efficient and stable BMCO-based biocathodes assembled and characterised so far, carbonaceous electrodes are preferred (e.g. Refs. [23, 24]).

For more than 30 years, Russian electrochemists have been studying O_2 bioelectroreduction by Lcs adsorbed on different electrode materials [6, 7, 25–29]. In many cases bioelectrochemical results from the Russian school are presented as polarisation curves with positive cathodic currents and potentials expressed *versus* the NHE, which causes some difficulties for other scientists to compare results thus gathered with data already available.

In order to increase the efficiency of Lc-based cathodes different carbon-based dispersed materials, some of them nowadays called carbon (nano)particles were used, *viz*. channel carbon black (AD-100), furnace butyric carbon blacks (PM-100, PM-105) and different coals (KM-2, AG-3, T-39 and BM-4). The main physical–chemical and electrochemical properties of these materials can be found in Refs. [25, 26, 30, 31]. Usage of particulate carbonaceous materials with particle sizes varying from 4 up to 1000 nm and above (the average size of Lc is ca. 5 nm) increased the concentration of electronically connected (i.e. biocatalytically active) Lc up to 20 pmol cm^{-2}, resulting in the realisation of very efficient and stable biocatalysts. For comparison, the maximal surface concentration of electrochemically active Lc on planar carbon electrodes is estimated to be about 2 pmol cm^{-2} [13]. Basic properties of biocatalysts based on Lc adsorbed on dispersed carbon materials were studied and some results were published in Russian journals [25–27], including the very first report on DET-derived BFCs based on carbon particles [29]. As early as 2002, suggestions for exploitation of 'air diffusion electrodes, which ensure high overall current density in a biofuel cell' were given. However, such an electrode was produced only recently, *viz*. a motionless air diffusion biocathode, which reached current densities as high as 20 mA cm^{-2} at 200 mV in a citrate buffer at room temperature [32]. The biocathode was designed based on BMCO from *Escherichia coli* (CueO) adsorbed on carbon particle-modified electrodes [32].

One of the most promising applications of enzymatic FCs is their usage as a power source for implantable and 'semi-implantable' devices operating in blood, saliva, tears, sweat and different fluids of the digestive tract. It should be emphasised that the power density of actual implanted biofuel cells will, in all likelihood, be limited by O_2 supply to the electrode surface, given the low solubility of O_2 (~0.25 mM in air saturated buffers) and its small diffusion coefficient (~2 × 10^{-}

^{5} cm^2 s^{-1}). One approach to overcome the mass transfer problem in useful biodevices for semi-implantable applications is to construct an air diffusion biocathode utilising O_2 directly from the air [29, 33]. Obviously, the power of a FC depends on the current densities and operating voltages of both electrodes. Taking into account, the achieved operating voltages of potentially implantable DET-based bioanodes, which usually are not less than 200 mV [3, 5, 34–36], one can speculate about the power density of an implanted BFC based on the recently developed CueO Lc cathode [23, 32]; in spite of the impressive maximal current density of a CueO Lc-based device, its power density will be limited because of the low redox potential bioelement used, *viz*. CueO Lc (480 mV *vs.* NHE [37]). To overcome this problem, high redox potential BMCO, e.g. fungal Lc and bilirubin oxidase, should be exploited.

Another significant problem of current BFCs is the limited operational stability, owing to a number of factors, including insufficient stability of the bioelements used, i.e. adsorbed redox enzymes. In addition, at high-current densities the amount of H_2O_2, electrochemically produced on carbon materials due to two-electron reduction of O_2, might be enough to inhibit the Lc, and thus deactivate potentially very efficient biodevices. However, the stability of the fabricated air diffusion Lc-based biocathode is yet to be investigated [32].

Below we describe the fabrication and characterisation of a very stable air diffusion 'floating' (ADF) biocathode based on a high redox potential fungal BMCO, i.e. *Trametes hirsuta* Lc, adsorbed on carbon particles.

2 Experimental

2.1 Materials

Unless otherwise specified, all chemicals were purchased from Sigma–Aldrich GmbH (Germany). All solutions were prepared using water (18 MΩ) purified with a Milli-Q system from Millipore (USA). The ADF electrodes were fabricated using acetylene black (Russia), analogous to XC-72 produced by Cabot (Belgium). Two different carbon sorbents, *viz*. furnace butyric black PM-100 and carbon BM-4 (Russia), were used as carriers in the immobilisation of redox enzymes.

2.2 Enzymes

Trametes hirsuta Lc was obtained from the basidiomycete *Trametes hirsuta* (*Wulfen*) *Pilát*, strain *T. hirsuta* 56, provided by the laboratory collection of the Moscow State University of Engineering Ecology (Russia). The basidiomycete was grown by submerged cultivation as described in Ref. [38]. The Lc was isolated and purified from a culture medium according to Ref. [39]. The homogeneous preparation of the enzyme, as judged from SDS–PAGE, with a protein concentration of about 15 mg mL^{-1} was stored in 100 mM phosphate buffer, pH 6.5 at –20 °C.

Amoracia rusticana peroxidase (Type VI-A, HRP) isolated from horseradish roots was obtained from Sigma–Aldrich GmbH and used without further purification. The lyophilised powder of the enzyme was stored at +4 °C.

2.3 Catalytic Assay

The specific activity of Lc and HRP measured towards 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) was not less than 200 and 500 U mg^{-1}, respectively. The activity of both enzymes, in homogeneous solutions and in an adsorbed state, was determined spectrophotometrically in 100 mM citrate–phosphate buffer, pH 4.5, at different ionic strengths (addition of KNO_3). Five millimolars of ABTS was used as the first enzyme substrate, i.e. electron donor. The concentrations of the second substrates of the enzymes, O_2 and H_2O_2 for Lc and HRP, were 0.25 mM (air-saturated buffer) and 1 mM, respectively. The absorbance change at 405 nm (ε = 36,000 M^{-1} cm^{-1}) was measured for 60 s using a UV-mini 1240 spectrophotometer from Shimadzu Europe GmbH (Germany). For both enzymes, 1 U of activity was defined as the amount of enzyme oxidising 1 µmol of ABTS per minute under standard conditions. Specific activity is expressed as units of activity per mg of protein.

2.4 Preparation and Investigation of Biocatalysts

The immobilisation of pure Lc on two different carbon materials was performed by direct physical adsorption of the enzyme from a 100 mM citrate–phosphate buffer solution, pH 4.5, with a total protein concentration of about 0.1 mg mL^{-1}. For this purpose, 5 mg of carbon BM-4 or carbon-black PM-100 was dispersed in 5 mL of the buffer and the dispersions were sonicated for 10 min. After that the enzyme was added and incubated for 4 h. For the Lc–peroxidase–BM-4 biocatalyst, simultaneous immobilisation of both enzymes from a citrate–phosphate buffer solution with a total protein concentration of about 0.1 mg mL^{-1} (90× Lc/10× HRP w/w) was also performed.

2.5 Electrode Fabrication

The air diffusion electrodes (discs, Ø 1.5 cm) were produced from dispersed acetylene black using F-4D (Russia), an aqueous suspension of a fluorocarbon polymer powder, as a binding and water-repellent agent. As a current conductor, a NP-2 nickel mesh (Russia) was pressed into the fabricated porous electrodes. Suspensions of bare BM-4 particles or particles modified with Lc or a Lc–peroxidase mixture (i.e. two biocatalysts, *viz*. Lc–BM-4 and Lc–peroxidase–BM-4) were dropped onto the electrodes. After that, electrodes were dried and covered by Nafion from Sigma–Aldrich GmbH by immersion in 1% w/w Nafion solution for a few seconds.

2.6 Electrolyte

The main buffer was a 100 mM Na–citrate–phosphate buffer, pH 4.5. All electrochemical studies were performed at room temperature, which varied insignificantly, from 20 up to 23 °C, during the measurements.

2.7 Electrochemical Measurements

Electrochemical measurements were performed using the three-electrode system presented in Figure 1. A galvanostatic method was used, i.e. measurements of the potential of the working electrode at constant current between counter and working electrodes. For this purpose, a dc (power) supply, a resistance set, an amperometer and a voltmeter, Ionomer I-130.2M from APK Energoservis (Russia), were used. In all experiments, a homemade one-compartment electrochemical cell (volume 10 mL), an Ag/AgCl/3 M NaCl reference electrode from Bioanalytical Systems (USA), and a platinum mesh counterelectrode from Sigma–Aldrich GmbH were used. The potential values were registered under air and O_2 saturated conditions created by bubbling air and O_2 through (before the measurements) and above (during the measurements) the electrolyte. All potentials in the manuscript are reported *versus* NHE.

3 Results and Discussion

A very high electroactive surface area confined to a small volume can be produced by employment of highly dispersed materials. Thus, in the case of O_2 electrocatalytic reduction, high-power densities limited only by O_2 diffusion to the electrode surface can be obtained. Based on information available from previous investigations regarding enzyme stability and enzyme capacity [25–27, 29], two carbon materials were selected, coal BM-4 and carbon black PM-100.

3.1 Investigations of Native and Immobilised Trametes hirsuta Laccase

The activity and stability of *T. hirsuta* Lc adsorbed on the carbon materials were studied and compared to the solubilised enzyme. The specific activities of the enzyme adsorbed on BM-4 and PM-100 were measured to be 110 and 48 U mg^{-1}, respectively, *versus* 200 U mg^{-1} for Lc in homogeneous solution. It should be emphasised that significant differences in specific activities regarding homogeneous and heterogeneous reactions is quite common for many enzymes, and it might reflect diffusion problems in the heterogeneous case rather than simple enzyme denaturation. Also, as protein flexibility is essential for Lc biocatalysis [40, 41], any restriction to protein movements as a consequence of its adsorption to the carbon surface, most likely will influence the catalytic values obtained.

An important factor affecting both activity and stability of Lc is the buffer composition. As was shown previously for *Polyporus versicolor* Lc, a citrate–phosphate buffer is preferred compared to acetate, sodium tetraborate–succinic acid and hydrophthalate buffers [26]. Based on those results, a 100 mM citrate–phosphate buffer was selected as the electrolyte in our studies. Moreover, usage of this buffer allowed us to directly compare our results with recently published work concerning the air diffusion Lc-based biocathode [32].

Not only the electrolyte composition but also the overall ionic strength can affect the activity of the enzyme. To reduce Ohmic losses, a biocathode should function in a high-ionic strength electrolyte, which can be achieved by adding a base electrolyte to the main buffer. Taking into account, BMCO inhibition by halide ions, *viz*. F^- and Cl^- ions, $NaClO_4$ was usually used as base electrolyte [13, 14, 24, 28], but other base electrolytes such as KNO_3 and Na_2SO_4 can also be employed. In the present study, the activity dependence of native and immobilised Lc on solution ionic strength adjusted using KNO_3 was investigated. It was found that immobilised Lc is about 10× less sensitive to variations of the ionic strength, compared to the solubilised enzyme. Moreover, in 3 M solutions the native enzyme still showed more than 90% of its maximal activity. As was also suggested by Kano and coworkers [32], proton supply to the surface of the electrode could be the rate limiting step in the biocathode function and thus, high concentrations of the buffer might improve the proton availability.

Also, the long-term stability of native and immobilised Lc was investigated, the results of which for native Lc, as well as Lc–BM-4 and Lc–PM-100 biocatalysts are presented in

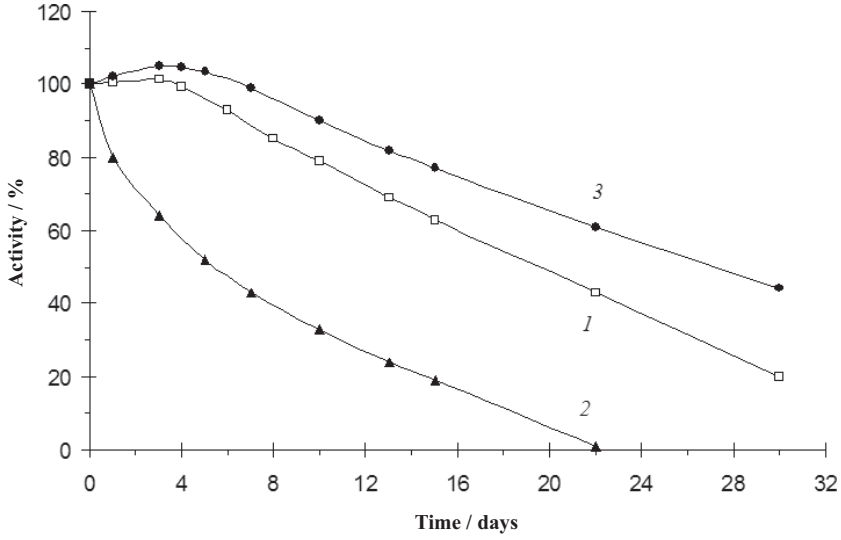

Figure 2 Comparison of long-term stabilities of Trametes hirsuta laccase in homogeneous solution and in the adsorbed state (100 mM citrate-phosphate buffer, pH 4.5, 20–23°C): 1 – native enzyme; 2 – enzyme immobilised on carbon-black PM-100; 3 – enzyme immobilised on carbon BM-4.

Figure 2. From the Figure, it is evident that immobilisation of the enzyme on BM-4 improved the stability of Lc compared to the enzyme in homogeneous solution and especially compared to the Lc–PM-100 biocatalyst. Moreover, Lc–BM-4 retained 55% of its initial activity after storage in the citrate–phosphate buffer for a month. Thus, Lc–BM-4 was selected for the fabrication of 'floating' air diffusion cathodes.

3.2 Electrochemical Characterisation of Air Diffusion 'Floating' Electrode

A schematic view of the fabricated ADF electrode is shown in Figure 1. The electrode, which can be represented as a sandwich membrane separating the electrolyte and air, comprises two layers, the active and the hydrophobic, respectively. The layer adjacent to the electrolyte, i.e. the active layer, is hydrophilic and it carries the biocatatalysts, i.e. redox enzymes adsorbed on carbonaceous materials; the air-facing layer is hydrophobic. During operation of the ADF electrode, atmospheric O_2 diffuses through the hydrophobic layer and is brought into contact with the electrolyte when penetrating through the active, hydrophilic layer. Therefore, the O_2 bioelectroreduction is localised at the electrode–electrolyte–air interface. When a gaseous bio-oxidant, e.g. molecular O_2, is supplied, electrochemical processes occur in specific volume elements of porous electrodes, which are available for both oxidant and electrolyte. Thus, the efficiency of ADF electrodes depends on the distribution of biocatalyst, O_2, and electrolyte inside the pores of the electrode. Establishing proper electrochemistry conditions is essential, but to ensure real world functionality, many other parameters of this complex heterogeneous system need to be considered [32, 33]. Two of the most important factors are (i) the potential established on the electrode, and (ii) the operational stability.

The open-circuit (stationary, steady-state) potential of the ADF electrode was measured to be very high, 850 mV *versus* NHE (Figure 3). This value is in agreement with open-circuit potentials of carbon electrodes modified with high redox potential *Trametes* Lcs, reported to be in the range from 670 up to 910 mV depending on electrode material, Lc source, solution pH and amount of enzyme on the electrode surface [3, 7, 13, 14]. We also found that potentials established were almost independent of O_2 concentrations. The redox equilibrium potential of the O_2/H_2O couple at pH 4.5 is +960 mV, e.g. only 110 mV higher than the starting potential of O_2 electroreduction on the ADF electrode (Figure 3).

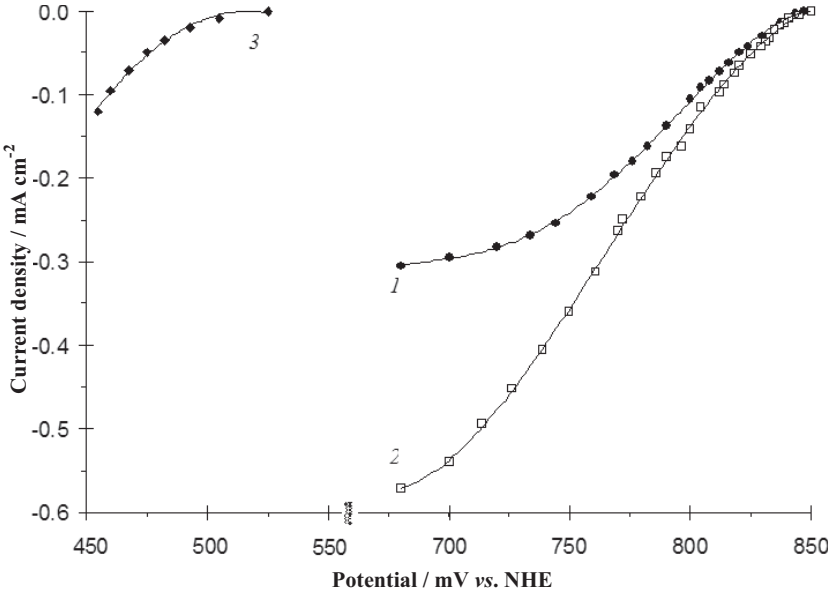

Figure 3 Polarisation curves of oxygen electroreduction on a "floating" air diffusion electrode with and without Trametes hirsuta laccase immobilised on carbon BM-4 (100 mM citrate-phosphate buffer, pH 4.5): 1 – in air; 2 – in oxygen; 3 – in the absence of enzyme.

Polarisation curves recorded on air diffusion electrodes with and without biocatalysts are also presented in Figure 3. As one can clearly see from the Figure, Lc significantly decreases the overpotential needed for O_2 electroreduction (compare curve 3 with curves 1 and 2). In accordance with the mechanism of Lc on carbon electrodes [3, 9, 14], the half-

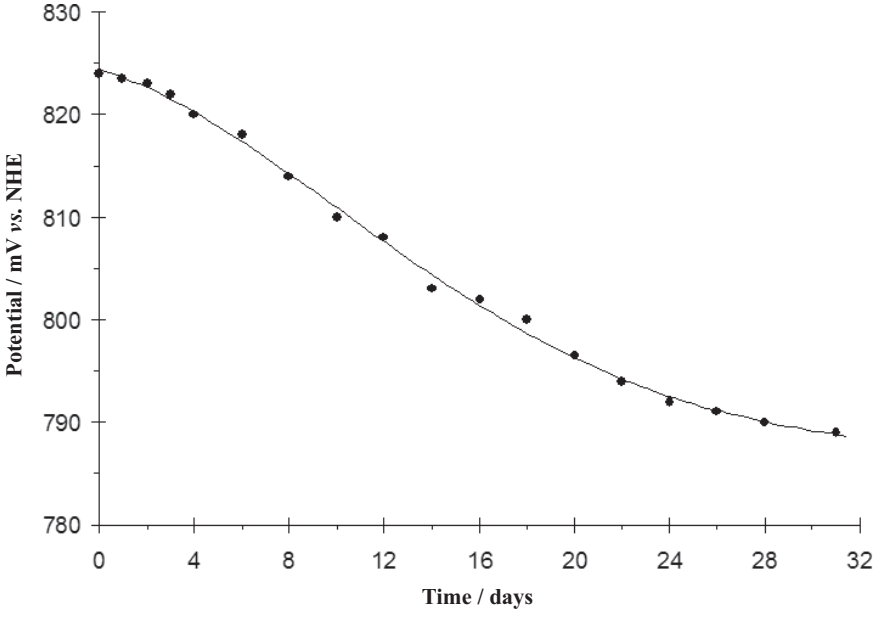

Figure 4 Changes in potential of a "floating" air diffusion electrode based on Trametes hirsuta Lc-BM-4 catalyst at a constant current density of 20 mA cm^{-2} (100 mM air-saturated citrate-phosphate buffer, pH 4.5).

wave potential of O_2 bioelectroreduction should coincide with the potential of the T1 site, the initial electron acceptor of the enzyme during heterogeneous electron transfer. Obviously, half-wave potentials registered in our studies (Figure 3) coincide well with the redox potential of *Trametes hirsuta* Lc, measured to be 780 mV ± 10 mV *versus* NHE at pH about 6 [39, 42]. We also noticed that the initial parts of both curves, 1 and 2, are almost identical, pointing to the fact that at low overpotentials, the bioelectrochemical reaction of O_2 reduction occurs in the kinetic mode, i.e. the process is limited by DET between carbon particles and the redox enzyme. However, at high overpotentials, the bioelectrochemical reaction has, in all likelihood, mixed kinetics as indicated by a current doubling when a +670 mV potential is applied to the electrode under O_2-saturated conditions, compared to air-saturation (cf. curves 1 and 2 in Figure 4). The very high open-circuit potentials of the device (850 mV *vs.* NHE) along with fast equilibration of the ADF electrode resulted in the device delivering relatively high power, in spite of the low maximal current densities obtained, *viz*. 0.3 and 0.6 mA cm^{-2} under air and O_2 saturated conditions, respectively. For example, a power density of 0.21 mW cm^{-2} for the air diffusion cathode can be calculated at potential as high as 0.7 V, whereas the biodevice based on low redox potential CueO Lc had zero power at this voltage [32]. The fast equilibration of the Lc–BM-4-based ADF electrode is, most likely, due to fast DET between carbon particles and the T1 site of Lc, i.e. the overpotential needed to achieve the current plateau under air saturated conditions was only 120 mV (Figure 3).

The reproducibility of ADF electrodes was also addressed and mean values were used to draw the conclusions. Hence, three different electrodes were examined and showed small deviations regarding the main electrochemical parameters, i.e. differences in current densities and open-circuit potentials did not exceed 5%.

The ADF electrode showed excellent stability at low current densities; the biodevice lost less than 5% of its initial operating potential after 1 month of continuous operation in 100 mM citrate–phosphate buffer, pH 4.5. In other words, the biocathode power density degraded from 16.5 to 15.8 μW cm^{-2} after 31 days of operation (Figure 4). However, at higher currents, i.e. when significant overpotentials were applied to the biocathode, the bioelectrochemical activity deteriorated sharply (Figure 5). To appreciate the possible reasons for the inactivation of the biodevices, additional voltammetric studies were performed.

It is widely accepted that when relatively low potentials are applied to carbon electrodes (450 mV *vs.* NHE and below), H_2O_2 can be electrochemically produced in a two-electron reduction of molecular O_2 on the electrode surface. We speculated that when significant overpotentials were applied to the biocathode, the amount of electrochemically produced H_2O_2 might be enough to inhibit the Lc immobilised on BM-4, and hence drastically deactivate the biodevice. In order to evaluate our assumption, as well as to find a possible solution to the problem, two biocathodes based on different biocatalysts were prepared, as described in the experi-

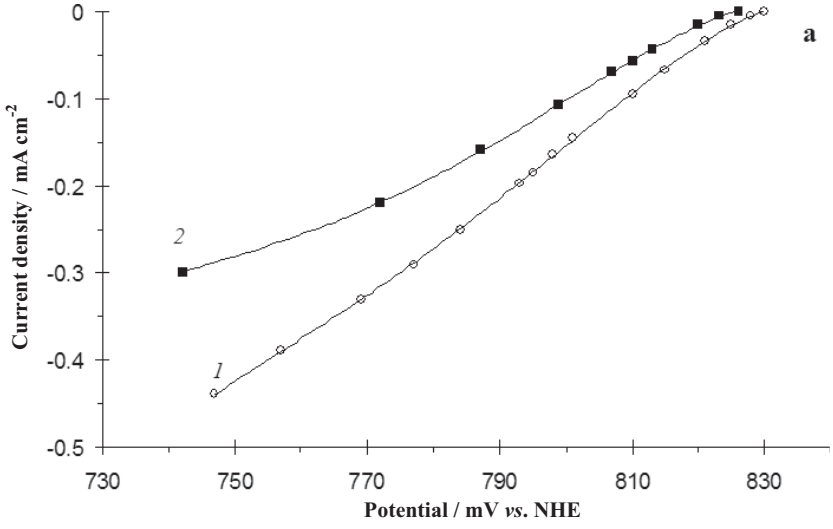

Figure 5 Changes in polarisation curves of a "floating" air diffusion electrode based on Trametes hirsuta Lc-BM-4 catalyst (100 mM O_2-saturated citrate-phosphate buffer, pH 4.5). 1 – initial polarisation curve; 2 – polarisation curve recorded after 10 min of electrode polarisation at 150 mV versus NHE.

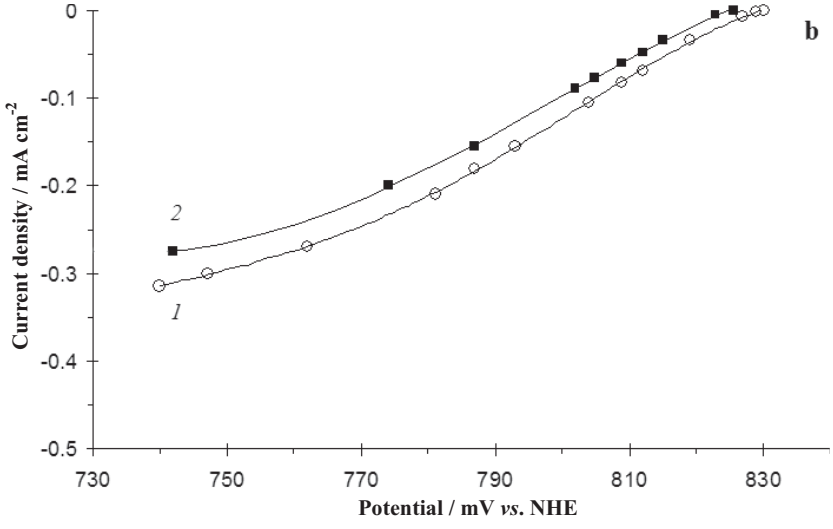

Figure 6 Influence of peroxidase on the voltammetric characteristics of oxygen electroreduction on a "floating" air diffusion electrode (100 mM O_2-saturated citrate-phosphate buffer, pH 4.5). 1 – initial polarisation curve, 2 – polarisation curve recorded after 10 min of electrode polarisation at 150 mV versus NHE.

mental section. Horseradish peroxidase immobilised on carbon electrodes catalyses bioelectroreduction of H_2O_2 to H_2O [43, 44], and co-immobilisation of which could possibly prevent deactivation of Lc immobilised on the same electrode. Indeed, it was found that the operating stability of the air diffusion biocathode based on a combined Lc–peroxidase–BM-4 biocatalyst was significantly improved compared to the Lc–BM-4 biocathode. For example, when polarised at 150 mV, the Lc–BM-4-based air diffusion biocathode lost more than 32× of its initial power in just 10 min of continuous operation (Figure 5), whereas the combined Lc–peroxidase–BM-4-based biocathode retained more than 87% of its initial power under the same conditions (Figure 6). However, co-immobilisation did reduce the maximal current density of the biocathode (cf. curves 1 and 2 in Figures 5 and 6) starting at 13% reduction at low overpotentials, and reaching 32% and more at high overpotentials.

3.3 Short Discussion and Further Perspectives

We have shown that the efficiency of a Lc-based biocathode dependeds on many factors, such as the nature of the bioelectrocatalyst, the properties of the electroconductive matrixes for enzyme immobilisation, the composition and ionic strength of electrolytes, etc. The ADF electrode is very stable at low current densities. The duration of continuous operation of the device at significant overpotentials depends on the biocatalysts, as well as the buffer capacity of the electrolyte used. Both efficiency and operational stability of the designed device might be further improved, e.g. by optimising the composition of Lc–peroxidase–carbon particles-based biocatalysts and by optimising the hydrophobic layer to enhance O_2 supply to the biocatalysts by usage of different polymers.

4 Conclusion

Herein we report the fabrication and characterisation of a mediatorless and soluble cofactor free, stable, high potential air diffusion biocathode, which might be useful to construct very efficient and non-toxic cathodes of BFCs. The latter can be pasted or otherwise attached and operate at air–liquid interfaces, e.g. in sweat, saliva and tear fluids. The designed biocathode showed a maximal current density of more than 0.5 mA cm^{-2}, already at 0.7 V *versus* NHE. The operational stability of the biocathode depended on the current density of the device. At low current density the biocathode is stable for 1 month of continuous operation, whereas when polarised at high overpotentials, the device looses a significant fraction of its initial power in just 10 min. The most likely reason for the rapid deterioration at high overpotentials is electrochemically produced H_2O_2, which deactivates Lc-based biocathodes. Thus, co-immobilisation of Lc and peroxidase on carbon particles protects the biocatalyst from rapid inactivation at high current densities.

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