Chemical Engineering Journal 165 (2010) 524–528

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

A novel laccase-catalyzed cathode for microbial fuel cells

Haiping Luo [a], Song Jin [b,c,*], Paul H. Fallgren [c], Hee Joon Park [a], Patrick A. Johnson [a,**]

[a] Department of Chemical and Petroleum Engineering, University of Wyoming, 1000 E University Avenue, Laramie, WY 82071, USA
[b] School of Resources and Environmental Engineering, Hefei University of Technology, Hefei, PR China
[c] Department of Civil and Architectural Engineering, University of Wyoming, Laramie, WY 82071, USA

ARTICLE INFO

Article history:
Received 12 June 2010
Received in revised form
25 September 2010
Accepted 27 September 2010

Keywords:
Cathode
Enzymatic electrode
Microbial fuel cell (MFC)
Laccase

ABSTRACT

A microbial fuel cell (MFC) cathode was developed by immobilizing the enzyme, laccase, in polymer matrix (Nafion) on carbon paper together with carbon nanoparticles and a redox mediator (2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt). The results from characterization of the immobilized laccase on the electrode indicate that the immobilization preserved laccase activity. Electrochemical measurements demonstrate that the Nafion film can facilitate electron exchange between the electroactive domain of laccase and the electrode. The electrocatalysis capabilities of the laccase-coated electrode were characterized by cyclic voltammogram (CV), which showed the electrode facilitated direct and surface-controlled redox reactions in a 0.1 M citrate buffer solution (pH 5.0). Additionally, when installed in a two-chamber MFC, the laccase-coated cathode demonstrates a strong catalytic potential that is comparable to a Pt-catalyzed cathode.

1. Introduction

The high cost of noble metal-electrodes such as platinum (Pt) coated cathodes, is considered one of the limiting factors for scaled-up applications of microbial fuel cells (MFC) [1]. Utilization of other metal-based catalysts such as lead dioxide (PbO_2) [2], pyrolyzed-Fe(II) phthalocyanine (pyr-FePc) [3,4], and semi-conductive rutile (TiO_2) [5] have been reported; however, these metal-based catalysts are generally susceptible to adverse conditions that may occur in MFCs as a result of chemical reactions, biological activities, and changes in catholyte composition. An example of such adverse effects is catalyst poisoning by elevated sulfide or chloride [6].

Another type of MFC cathode catalyst relies on microorganisms attached to the electrode surface. These microorganisms serve as "biocatalysts" and the cathodes are commonly referred to as "biocathodes" [7–9]. Despite the lag in catalytic performance when compared to noble metal catalysts, the cost of biocatalysts and their compatibilities with the MFC operating conditions are appealing and deserving of further investigations. The performance of biocatalysts tends to be constrained by high cathodic activation overpotentials [7]. The accumulation of metabolites and limited ions transferred through cell membranes can also hinder bacterial activity. In addition, a carbon source must be present in the catholyte to maintain microbial populations.

A high-performance and cost effective cathode catalyst is desired to decrease the activation energy barrier and improve the reactive kinetics at the cathodic electrode surface [10]. Enzymes as cathode catalysts can potentially eliminate limiting factors in biocathodes, such as decreasing efficiency due to accumulation of metabolites, carbon source requirements, and specificity in catalyzing electron transfer. These types of enzymatic cathodes have been investigated in miniature enzymatic biofuel cells [11]. Studies on enzymes for electron interactions mainly focused on copper-containing oxidoreductases laccase and bilirubin oxidase, which include fungal laccases from *Coriolus hirsutus*, *Trametes versicolor*, *Coriolopsis gallica*, and *Pleurotus ostreatus*, tree laccase from *Rhus vernicifera*, bacteria laccase from *Streptomyces coelicolor* and fungal bilirubin oxidase from *Myrothecium verrucaria* [12–15].

Mediated electron transfer (MET) electrodes were developed by using an enzyme coated cathode and mediator buffer to achieve electron transfer. Barton et al. first reported carbon paper composite enzyme electrodes, using laccase of *C. hirsutus* and producing 5.0 mA/cm^2 at 0.62 V (vs. SHE, standard hydrogen electrode) in a 0.2 M (pH 5) oxygen-saturated citrate buffer at 37 °C [16]. Mano et al. constructed a bilirubin oxidase coated cathode producing 4.5 mA/cm^2 at 0.57 V (vs. SHE) in an oxygenated phosphate buffered solution (PBS, pH 7.4) at 37.5 °C[12]. Gallaway et al. reported an *S. coelicolor* coated electrode resulting in 1.3 mA/cm^2 at 0.43 V (vs. SHE) in 0.1 M pH 4 oxygenated citrate buffer at 40 °C [17]. All of these MET examples used a cross-linked osmium-based redox

* Corresponding author at: School of Resources and Environmental Engineering, Hefei University of Technology, Tunxi Road, Hefei, PR China.
Tel.: +1 970 449 9422; fax: +1 970 377 9406.
** Corresponding author. Tel.: +1 307 766 6524; fax: +1 307 766 6777.
E-mail addresses: sjin@uwyo.edu (S. Jin), pjohns27@uwyo.edu (P.A. Johnson).

1385-8947/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2010.09.061

mediator, producing the appropriate redox potential for mediation and resulting in a stable electrode film construction.

There are few studies on the application of enzymes on MFC anodes [18] and cathodes [19,20]. Schaetzle et al. demonstrated that MFC performance was improved by coating a cathode with laccase [20]; however, the laccase was coated on an existing coating of Pt, essentially making a hybrid or modified Pt cathode. Distinctively, our study investigated an enzyme serving as the sole cathodic catalyst in an MFC. Laccase was selected as the enzyme catalyst for cathode, considering its capability of catalyzing the four-electron reduction of O_2 to H_2O coupled to the one-electron oxidation of different substrates. A modified laccase electrode was prepared by using Nafion polymer as the binding material. Performance of the modified-laccase was evaluated in terms of enzymatic and electrochemical activities. Finally, we evaluated the enzyme coated electrode in MFC performance by comparing to a Pt-coated cathode.

2. Materials and methods

2.1. Electrode preparation

The anodes consisted of non-wet-proof carbon cloth (type A, E-TEK) and had a surface area of 10 cm^2. For Pt-coated cathodes, a commercial Pt catalyst (10 wt% Pt/C, E-TEK) was mixed with 5% Nafion solution to form a paste (7 μL of binder/mg of Pt/C catalyst). The paste was applied to one side of a carbon paper cathode (10 cm^2, 30 wt%, type B, E-TEK), and dried at room temperature (~25 °C) for 24 h. The Pt content on the surface of cathode was 0.5 mg/cm^2 based on the calculation, which was consistent with other MFC studies.

The laccase-modified cathodes were prepared by using laccase from *Trametes versicolor* (Sigma–Aldrich) without further purifications (8.6 U/mg). The redox mediator was 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) purchased from Fluka. To prepare the enzyme-coated cathode, 1.5 mg of laccase was dissolved in 300 μL of phosphate buffer (pH 7.0) containing 3.0 mg of carbon nanoparticles (diameter < 50 nm). An aliquot of 50 μL was mixed with 5.0 μL of Nafion in 0.2 M phosphate buffer. The Nafion solution served as an insoluble solid polymer electrolyte and a binder to hold laccase on the electrode surface. The volume of the solution was fixed at 100 μL with the addition of phosphate buffer and the concentration of ABTS was 1.45 mM. An aliquot of 50 μL was spread on the surface of the carbon paper and the cathode was dried at room temperature (~25 °C), then rinsed with DI water. All prepared electrodes were stored at 4 °C before use.

2.2. MFC setup

The two-chambered MFC consisted of two glass bottles (Corning Inc.; 300 mL capacity). Anode and cathode chambers were joined by a glass bridge containing a proton exchange membrane (PEM; Nafion TM 117, Dupont Co.) held by a clamp between the flattened ends of the two glass tubes (inner diameter 2 cm) fitted with rubber gaskets. The electrodes were attached using copper wire with all exposed metal surfaces sealed with a nonconductive epoxy (Dexter Corp., NJ, USA). The anode and cathode chamber were filled with 200 mL of medium and PBS solution, respectively, and operated at room temperature (~25 °C). The catholyte was sparged with air using an aquarium membrane pump.

For start-up of the MFC, the MFCs were inoculated with the river water from Laramie River (Laramie, Wyoming). The anodic medium consisted of (per liter of deionized water): CH_3COONa 1 g, Na_2HPO_4 4.0896 g (28.9 mM), NaH_2PO_4 2.544 g (21.2 mM), NH_4Cl 0.31 g (5.8 mM), KCl 0.13 g (1.7 mM), trace metals solution 12.5 mL, vitamin solution 12.5 mL [21]. The solution in the cathode chamber was phosphate buffer solution (PBS; Na_2HPO_4, 2.75 g/L; $NaH_2PO_4{\cdot}H_2O$, 4.22 g/L). Air was purged into the cathode compartment in order to supply the oxygen needed for the electrochemical reaction.

2.3. Enzymatic activity measurements

Laccase activity was determined by the oxidation of ABTS to ABTS*, characterized by an increase in the optical density of the reaction solution with time. A solution of 1.45 mM ABTS in 0.1 M citrate–phosphate buffer (pH 5.3) was used as the substrate [22]. The enzyme activity unit (U) was defined as the amount of enzyme required to oxidize 1.0 mmol ABTS/min at 25 °C. The color change was monitored by measuring the absorbance at 415 nm [23].

The activity of immobilized enzyme was measured in a reactor at 30 °C in a batch regime. The immobilized enzyme was suspended in the buffer (pH 7.0) and placed into the reactor at 30 °C. The preheated ABTS solution was added (1.45 mM end substrate concentration) and samples were collected from the reactor at 5 min intervals. After absorbance (415 nm) was measured, the sample was returned to the reactor.

2.4. Electrochemical activity measurements

The cathode potential was measured by applying a constant current in potentiometry tests (chronopotentiometry) using a three-electrode single-chamber electrochemical cell containing a working electrode (cathode electrode with 0.64 cm^2 surface area), a platinum wire counter electrode, and an Ag/AgCl reference electrode (EE009 no-leak electrode, Cypress Systems).

Cyclic voltammograms (at sweep rates varying from 1 to 1000 mV/s) of the Pt modified and laccase-modified electrodes were produced using a conventional three-electrode system connected to a Gamry Ref 600 Potentiostat (Gamry Instruments, Warminster, PA) with Gamry Framework (Gamry Software, Gamry Instruments, Warminster, PA) and a one-compartment electrochemical cell (10 mL). An Ag|AgCl|KCl salt (201 mV vs. NHE) reference electrode and a Pt counter electrode were used in these measurements.

2.5. MFC performance

Electrical potentials across the resistor were measured using a multimeter and data were automatically recorded by a data acquisition system. Area power density (P_A, W/m^2) and volumetric power density (P_V, W/m^3) were calculated using the following equations,

$$P_A = \frac{IU}{A} \quad (1)$$

$$P_V = \frac{IU}{V} \quad (2)$$

where I is the current (A), U is the voltage (V), A is the cross surface area of the anode or cathode (m^2), and V is the non-packing volume of the anodic compartment (i.e., the volume of the liquid medium) (m^3). The volumetric power density indicates how much power is generated from unit volume of wastewater. The Coulombic efficiencies (CE) (%) were calculated by using the formula below:

$$CE\,(\%) = \frac{\sum_{i=1}^{n} U_i t_i}{RFb\,\Delta SV} M \times 100 \quad (3)$$

where U_i is the output voltage of MFC at time t_i, R is external resistance, F is Faraday's constant (96 485 C/(mol e^-)), b is the number of moles of electrons produced per mol of chemical oxygen demand (COD, 4 mol e^-/mol COD), ΔS is the removal of COD concentration

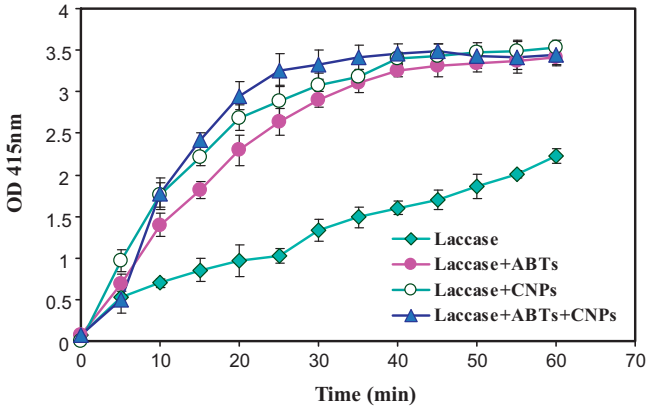

Fig. 1. Activities of immobilized laccase of various laccase catalysis electrodes using ABTS, carbon-nanoparticle, and ABTS + carbon-nanoparticle, respectively, as the surface-modified components. Average values were shown and error bars present the value range.

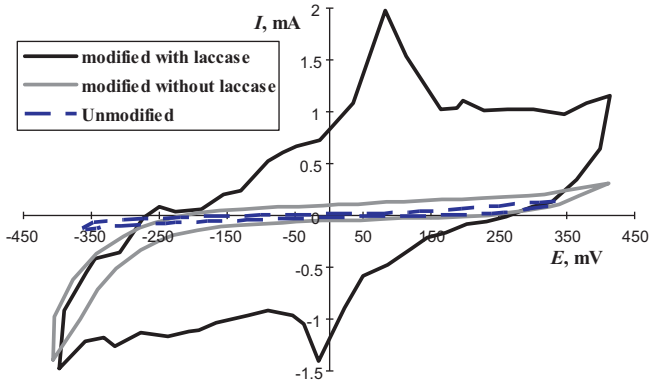

Fig. 2. Cyclic voltammograms of unmodified carbon paper electrode and CNTs + ABTs modified carbon paper electrodes with and without the laccase costing in 0.1 M citrate buffer solution, pH 4.0, under N_2 purging. Scan rate: 50 mV/s. The geometrical surface area was 10 cm^2.

(g/L), V is the liquid volume (L), and M is the molecular weight of oxygen (32 g/moL). The maximum power density was determined by adding fresh substrate to the MFC and establishing constant power, changing the external resistance over a range of 50–5000 Ω, and recording the voltage (typically 5–10 min for each resistance). The power was then calculated for each resistance as a function of the current. Samples of the anode solutions were taken every 12 h for measurements of total organic carbon (TOC) concentrations.

3. Results and discussion

3.1. Enzymatic activity of immobilized laccase

In this study, carbon nanoparticles were used in the preparation of Pt/C cathode in order to enlarge the activation area of the electrode [1]. We employed carbon nanoparticles to improve the enzyme loading on the carbon paper electrode. To determine the enzymatic activity of the immobilized laccase, the oxidation of ABTS to ABTS* was measured. The slope of the initial linear region of the absorbance versus time curve is proportional to the activity of laccase [24]. The changes in the UV absorbance spectrum during the reaction of ABTS and modified electrode were followed to identify the enzymatic activity of the immobilized laccase (Fig. 1). An increase in absorbance at 415 nm was observed (e.g. from 0 to 3.417 within 60 min for the Nafion/nanoparticle/C electrode) (Fig. 1), such an absorbance change has previously been attributed to ABTS oxidation [24]. It has been reported that enzyme activity may be markedly reduced as a result of covalent immobilization [25]. Results from this study indicate that the laccase immobilized on the Nafion/nanoparticle/C electrode retained its functional activity, which also suggest that the Nafion polymer could be used as an alternative binding agent for enzyme immobilization to electrodes.

Comparisons of various modified electrodes were conducted measuring the oxidation of ABTS to further elucidate the effects of the presence of carbon nanoparticles and ABTS in the polymer film on enzymatic activity. As observed in Fig. 1, ABTS (which was used as the electron mediator) and carbon nanoparticles enhanced the enzymatic activity of the immobilized laccase. For the nanoparticles, this enhancement is attributed to facilitated laccase immobilization from solution due to the increased surface area of the modified electrode.

Attempts to compare the specific activities of immobilized laccase and free laccase were unsuccessful. The functional mass of laccase is difficult to quantify in the immobilized constructs, because after the laccase was immobilized, the enzyme configuration would have been altered. In addition, the surface exposure of laccase and its functional moieties were close to impossible to determine and quantify. Therefore, we were not able to compare the activities between the native and immobilized states. However, the catalytic efficiency of laccase in facilitating electron transfer from cathode to terminal electron acceptor of oxygen was the focus of the study, rather than a comparison of the enzymatic activities in the immobilized and free phases.

3.2. Electrochemical activity of laccase modified electrode

Fig. 2 shows the cyclic voltammogram (CV) obtained for an unmodified carbon paper electrode, from which no electrochemical activity was observed. Fig. 2 also shows the CVs obtained for treated carbon paper electrodes with and without laccase immobilization. The control electrode without immobilized laccase was only modified with carbon nanoparticles and ABTS, with Nafion as the binding material. In the absence of laccase, no significant electrochemical activity was detected. In contrast, asymmetric anodic and cathodic peak shapes were observed in the CV of the laccase modified electrode in potentials ranging between −50 and +150 mV. The oxidation peak current was higher than the reduction peak. The substantial shift of currents demonstrates that active catalysis of the enzyme-coated cathode, indicating that active laccase was adsorbed to the carbon paper electrode with Nafion as the binding material. Since laccase is a multicenter enzyme, intermediate redox states are expected. We assume that the pair of anodic and cathodic redox peaks in the potentials between −50 and +150 mV can be attributed to the process of direct electrical transfer between the T2/T3 redox copper center of the laccase and the carbon paper.

Fig. 3 shows the CVs of a laccase-modified electrode in 0.1 M citrate buffer solution (pH 4.0) at different scan rates. With increasing scan rates, the anodic peak potential and the cathodic peak potential of laccase did not shift (estimated at 70 mV vs. Ag/AgCl). Both the cathodic and anodic peaks currents were linearly proportional to the scan rate in the scan range from 50 to 120 mV/s (linear regression equations: pa-$y = 0.0175x + 1.1029$, $r^2 = 0.9999$; pc-$y = -0.0194x - 0.4628$, $r^2 = 0.9972$), suggesting a surface-controlled electrode redox process of laccase [26,27].

The results thus far suggest the observed electrochemical activity is due to the laccase immobilized on the carbon paper surface. Laccase is a family of enzymes that catalyze the four-electron reduction of O_2 to H_2O coupled to the one-electron oxidation of different substrates. In nature these electrons are supplied by several phenols, amines, and lignins, as well as inorganic ions [28,29]. In a MFC, electrons were replenished to the cathode from the anode,

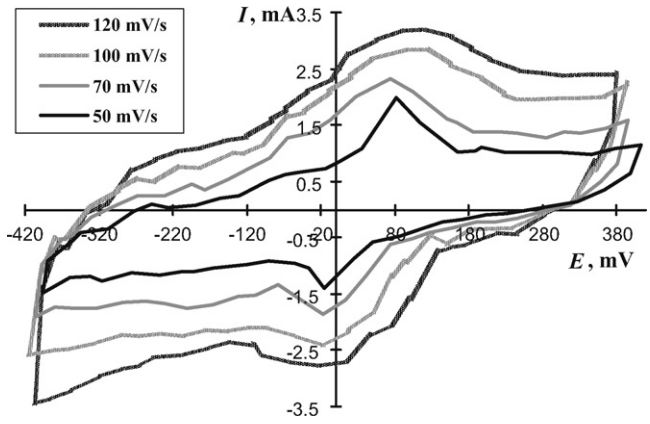

Fig. 3. Cyclic voltammogram of laccase-modified carbon paper electrodes in N_2-saturated 100 mM pH 4.0 citrate buffer, at various scan rates.

which accepts electrons from anode-respiring and other bacteria with cross-membrane electron transfer capabilities.

3.3. Power generation in the MFC using a Pt catalyst cathode

Power was generated in the MFC with a Pt-catalyzed cathode and glucose (1000 mg/L) as the substrate (Fig. 4). During five cycles of continuous electricity generation, the maximum voltage output varied between 410 and 438 mV each time the MFC glucose concentration was replenished. The maximum power densities achieved in the MFC ranged from 168.1 to 191.8 mW/m^2 (anode). Chronopotentiometry with a constant current of 400 μA was used to assess the performance of the Pt catalyst cathode. The cathode potential was approximately −192 mV for a current density of 40 μA/cm^2 (data not shown). This potential is consistent with the result reported in an MFC study by Chen et al. [30].

When a stable power output was generated in the MFC with a Pt catalyzed cathode, the cathode was switched to a laccase-modified cathode. The MFC using the laccase-modified cathode produced stable voltage around 7 h after the addition of the glucose as substrate. The maximum voltage was 402 mV for the first cycles, which is 40 mV lower than that obtained with the MFC with Pt cathode (Fig. 5). The maximum power density was 160 mW/m^2 (anode).

The TOC removal for anodic electrolyte solution in the MFC was in the range of 71–79%, either using the Pt/C cathode or the laccase modified cathode. However, the Coulombic efficiencies, which were calculated based on the total substrate concentration, were 13.7% for the MFC with Pt-coated cathode and 14.8% for the MFC with the laccase modified cathode.

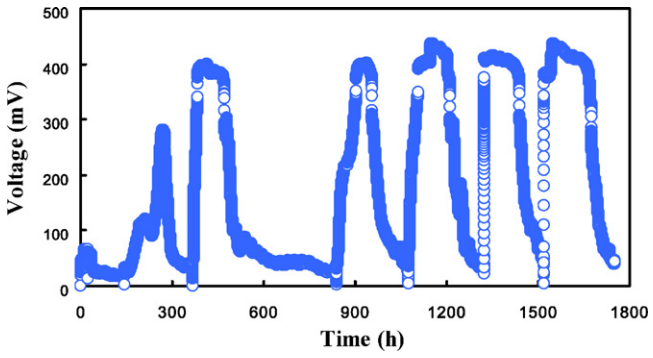

Fig. 4. Voltage outputs in the MFC using Pt catalyst cathode (with 1000 mg/L glucose as the fuel). The arrows show fuel replenishing events.

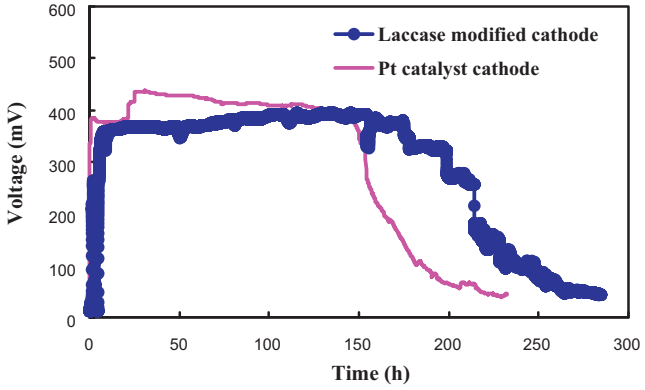

Fig. 5. Comparison of voltage–time curves in the MFCs using Pt and laccase as cathode catalysts.

3.4. Stability of laccase modified cathode

The long-term stability of the laccase-modified electrodes was examined by monitoring the change in the slope of UV absorbance of the ABTS solution, before and after it had reacted with the stored laccase-modified electrode. The electrodes were stored at 4 °C in 0.1 M PBS pH 7.0, and intermittently warmed to room temperature and tested for enzymatic activity. Decreased laccase activity was observed with increased storage times (Fig. 6), where after 15 days of storage in PBS solution, the slope of absorbance decreased by 50%, indicating loss of enzymatic activity of the immobilized laccase. There are reports that mineral-adsorbed enzymes are more stable than free enzymes during storage at ambient temperatures [31]. Compared to the free enzyme system in those studies, which had 90% loss of activity after 20 days of storage, the immobilized laccase in our study possessed a reasonable stability (50% activity loss in 15 days).

Continuous electricity generation by the MFC using a laccase-modified cathode is shown in Fig. 7. The maximum voltage outputs obtained in both the second (V_{max} = 210 mV) and the third cycle (V_{max} = 200 mV) of MFC operations were substantially lower than that from first cycle (V_{max} = 402 mV). This was probably caused by the aging of immobilized laccase during the operation of MFC. The long-term stability of the laccase-modified electrode, when it was used as a cathode in the MFC, performed better than the one in storage, of which enzymatic activity depleted completely after 30 days (Fig. 6). It is apparent that the laccase-modified cathode retained activity even after 800 h of MFC operation, suggesting that potential release of immobilized enzyme into the electrolyte was minimal during the system operation. The losing activity of the laccase-modified electrode, which was not used in an MFC, was most likely

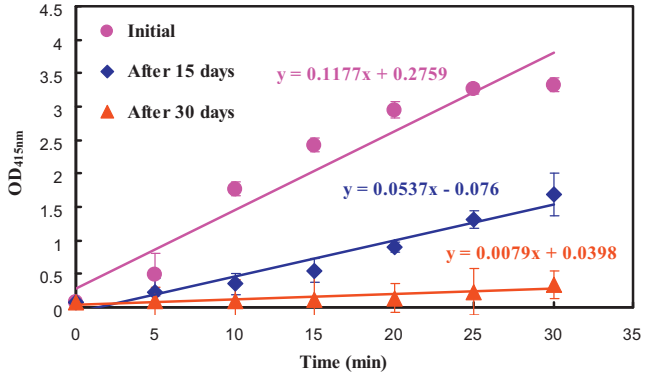

Fig. 6. Enzymatic activity of immobilized laccase of a laccase-modified electrode before and after its storage in 0.1 M PBS with pH 7.0.

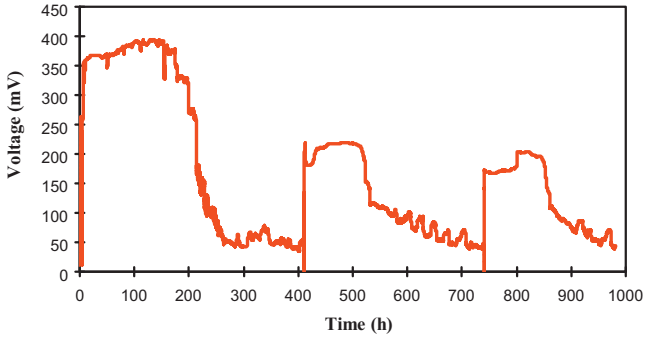

Fig. 7. Voltage outputs in the MFC using laccase-modified cathode (with 1000 mg/L glucose as the fuel).

attributed to the low temperature storage (4 °C). Factors contributing to the deactivation of laccase remains to be determined.

4. Conclusions

An MFC cathode was developed by immobilizing laccase on carbon paper that was modified with carbon nanoparticles and ABTS, using Nafion polymer as the binding material. The electrocatalytic capabilities of the laccase-coated electrode were characterized by cyclic voltammetry (CV), which showed the electrode facilitated direct and surface-controlled redox reactions in a 0.1 M citrate buffer solution (pH 5.0). Additionally, the enzymatic cathode achieved a comparable performance in an MFC, when compared to the Pt catalyzed cathode in terms of electricity production. Additional improvements for enzymatic electrodes, for example, should focus on increasing the efficiency of enzyme immobilization, retaining enzymatic activity in physiological conditions (i.e. pH 7 and chloride concentrations near 100 mM), as well as improving the stability of immobilized enzyme on the surface.

References

[1] B.E. Logan, J.M. Regan, Microbial fuel cell: challenges and technology, Environ. Sci. Technol. 40 (2006) 5172–5180.
[2] J.M. Morris, S. Jin, J.Q. Wang, C.Z. Zhu, M.A. Urynowicz, Lead dioxide as an alternative catalyst to platinum in microbial fuel cells, Electrochem. Commun. 9 (2007) 1730–1734.
[3] F. Zhao, F. Harnisch, U. Schrorder, F. Scholz, P. Bogdanoff, I. Herrmann, Challenges and constraints of using oxygen cathodes in microbial fuel cells, Environ. Sci. Technol. 40 (2006) 5193–5199.
[4] F. Zhao, F. Harnisch, U. Schrorder, F. Scholz, P. Bogdanoff, I. Herrmann, Application of pyrolysed iron(II) phthalocyanine and CoTMPP based oxygen reduction catalysts as cathode materials in microbial fuel cells, Electrochem. Commun. 7 (2005) 1405–1410.
[5] Y. Li, A. Lu, H. Ding, S. Jin, Y. Yan, C. Wang, C. Zeng, X. Wang, Cr(VI) reduction at rutile-catalyzed cathode in microbial fuel cells, Electrochem. Commun. 11 (2009) 1496–1499.
[6] T.J. Schmidt, U.A. Paulus, H.A. Gasteiger, R.J. Behm, The oxygen reduction reaction on a Pt/carbon fuel cell catalyst in the presence of chloride anions, J. Electroanal. Chem. 508 (2001) 41–47.
[7] A. Bergel, D. Feron, A. Mollica, Catalysis of oxygen reduction in PEM fuel cell by seawater biofilm, Electrochem. Commun. 7 (2005) 900–904.
[8] P. Clauwaert, K. Rabaey, P. Aelterman, L. De Schamphelaire, T.H. Ham, P. Boeckx, N. Boon, W. Verstraete, Biological denitrification in microbial fuel cells, Environ. Sci. Technol. 41 (2007) 3354–3360.
[9] A. Ter Heijne, H.V.M. Hamelers, C.J.N. Buisman, Microbial fuel cell operation with continuous biological ferrous iron oxidation of the catholyte, Environ. Sci. Technol. 41 (2007) 4130–4134.
[10] H. Rismani-Yazdi, S.M. Carver, A.D. Christy, O.H. Tuovinen, Cathodic limitations in microbial fuel cells: an overview, J. Power Sources 180 (2008) 683–694.
[11] A. Heller, Miniature biofuel cells, Phys. Chem. Chem. Phys. 6 (2004) 209–216.
[12] N. Mano, H.H. Kim, A. Heller, On the relationship between the characteristics of bilirubin oxidases and O_2 cathodes based on their "wiring", J. Phys. Chem. B 106 (2002) 8842–8848.
[13] S.C. Barton, M. Pickard, R. Vazquez-Duhalt, A. Heller, Electroreduction of O_2 to water at 0.6 V (SHE) at pH 7 on the 'wired' *Pleurotus ostreatus* laccase cathode, Biosens. Bioelectron. 17 (2002) 1071–1074.
[14] G. Gupta, V. Rajendran, P. Atanassov, Bioelectrocatalysis of oxygen reduction reaction by laccase on gold electrodes, Electroanalysis 16 (2004) 1182–1185.
[15] S. Shleev, M. Pita, A.I. Yaropolov, T. Ruzgas, L. Gorton, Direct heterogeneous electron transfer reactions of Trametes hirsuta laccase at bare and thiol-modified gold electrodes, Electroanalysis 18 (2006) 1901–1908.
[16] B.S. Calabrese, H.H. Kim, G. Binyamin, Y. Zhang, A. Heller, The "wired" laccase cathode: high current density electroreduction of O(2) to water at +0.7 V (NHE) at pH 5, J. Am. Chem. Soc. 123 (2001) 5802–5803.
[17] J. Gallaway, I. Wheeldon, R. Rincon, P. Atanassov, S. Banta, S.C. Barton, Oxygen-reducing enzyme cathodes produced from SLAC, a small laccase from Streptomyces coelicolor, Biosens. Bioelectron. 23 (2007) 1229–1235.
[18] M.V. Reddy, S. Srikanth, S.V. Mohan, P.N. Sarma, Phosphatase and dehydrogenase activities in anodic chamber of single chamber microbial fuel cell (MFC) at variable substrate loading conditions, Bioelectrochemistry 77 (2010) 125–132.
[19] S. Fishilevich, L. Amir, Y. Fridman, A. Aharon, L. Alfonta, Surface display of redox enzymes in microbial fuel cells, J. Am. Chem. Soc. 131 (2009) 12052–12053.
[20] O. Schaetzle, F. Barriere, U. Schroder, An improved microbial fuel cell with laccase as the oxygen reduction catalyst, Energy Environ. Sci. 2 (2009) 96–99.
[21] D.R. Lovely, E.J.P. Phillips, Novel mode of microbial energy metabolism: organism carbon oxidation coupled to dissimilatory reduction of iron and manganese, Appl. Environ. Microbiol. 54 (1988) 1472–1480.
[22] R.E. Childs, W.G. Bardsley, The steady-state kinetics of peroxidase with 2,2′-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) as chromogen, Biochem. J. 145 (1975) 93–103.
[23] C. Eggert, U. Temp, K.E. Eriksson, The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase, Appl. Environ. Microbiol. 62 (1996) 1151–1158.
[24] Y. Sun, S.C. Barton, Methanol tolerance of a mediated, biocatalytic oxygen cathode, J. Electroanal. Chem. 590 (2006) 57–65.
[25] C.M.F. Soares, M.H.A. Santana, G.M. Zanin, H.F.D. Castro, Covalent coupling method for lipase immobilization on controlled pore silica in the presence of nonenzymatic proteins, Biotechnol. Prog. 19 (2003) 803–807.
[26] C. Yu, J. Fan, B. Tian, D. Zhao, Morphology development of mesoporous materials: a colloidal phase separation mechanism, Chem. Mater. 16 (2004) 889–898.
[27] A.J. Bard, L.R. Faulkner, Electrochemical Methods, second ed., John Wiley & Sons, Inc, New York, 2001.
[28] A.I. Yaropolov, O.V. Skorobogatko, S.S. Vartanov, S.D. Varfolomeyev, Laccase: properties, catalytic mechanism and applicability, Appl. Biochem. Biotechnol. 49 (1994) 257–278.
[29] E.I. Solomon, U.M. Sundaram, T.E. Machonkin, Multicopper oxidases and oxygenases, Chem. Rev. 96 (1996) 2563–2605.
[30] S. Chen, H. Liu, B.E. Logan, Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells, Environ. Sci. Technol. 40 (2006) 364–369.
[31] L. Gianfreda, J.M. Bollag, Effect of soils on the behavior of immobilized enzymes, Soil Sci. Soc. Am. J. 58 (1994) 1672–1681.