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A Hybrid Enzymatic Zinc-Air Fuel Cell

(Sel Bahan Api Hibrid Berenzim Zink-Udara)

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ABSTRACT

A hybrid biofuel cell, a zinc-air cell employing laccase as the oxygen reduction catalyst is investigated. A simple cell design is employed; a membraneless single chamber and a freely suspended laccase in the buffer electrolyte. The cell is characterised based on its open-circuit voltage, power density profile and galvanostatic discharge at 0.5 mA. The activity of laccase as an oxidoreductase is substantiated from the cell discharge profiles. The use of air electrode in the cell design enhanced the energy output by 14%. The zinc-air biofuel cell registered an open-circuit voltage of 1.2 V and is capable to deliver a maximum power density of 1.1 mWcm^{-2} at 0.4 V. Despite its simple design features, the power output is comparable to that of biocatalytic cell utilising a much more complex system design.

Keywords: Biocatalyst; bioelectrochemical cell; enzymatic zinc-air cell; hybrid biofuel cell; laccase; metal biofuel cell

ABSTRAK

Sel bio-bahan api hibrid, sel zink-udara menggunakan lakase sebagai pemangkin bagi penguraian oksigen dikaji. Reka bentuk sel yang mudah diguna pakai: Ruangan tunggal tanpa membran dan lakase yang diampaikan secara bebas di dalam elektrolit pemampan. Pencirian sel adalah berdasarkan voltan litar terbuka, profil ketumpatan kuasa dan discas pada arus malar 0.5 mA. Aktiviti lakase sebagai enzim penguraian oksigen dibuktikan daripada profil discas sel. Penggunaan elektrod udara di dalam reka bentuk sel berhasil menambahkan keluaran tenaga sebanyak 14%. Sel bio-bahan api zink-udara memberikan voltan litar terbuka 1.2 V dan berupaya menghasilkan ketumpatan kuasa maksimum 1.1 mWcm^{-2} pada 0.4 V. Di sebalik ciri reka bentuk sel yang mudah, keluaran kuasa yang dihasilkan adalah sebanding dengan sel bio-pemangkin yang menggunapakai reka bentuk sistem yang jauh lebih rumit.

Kata kunci: Bio-pemangkin; lakase; sel bio-bahan api hibrid; sel bio-bahan api logam; sel bioelektrokimia; sel zink-udara berenzim

INTRODUCTION

One of the emerging areas of research in the renewable energy field is the development of biofuel cells. These devices employ biological catalysts for the oxidation and reduction of a fuel source. The main types of biofuel cells depend on the type of biocatalysts used. Microbial biofuel cells (MFC) employ whole living cells (microorganisms), whereas enzymatic biofuel cells (EFC) employ enzymes (functional proteins) (Minteer et al. 2007). MFCs have the advantage of long lifetimes (up to five years) (Moon et al. 2006) and are capable of completely oxidising simple sugars to yield carbon dioxide (Liu et al. 2004; Mano et al. 2003). They are, however, limited by low power densities due to slow transport across cellular membranes (Palmore & Whitesides 1994). On the other hand, EFCs have several positive attributes with respect to energy conversion, namely, the use of renewable catalysts, the flexibility of renewable fuels, the ability to operate at room temperature, and much higher power densities. In addition, enzymes have the added advantage of catalysing specific and defined reactions, eliminates the need for separating membrane (Bond & Lovley 2005; Bullen et al. 2006; Flexer et al. 2010).

In the present work, we studied a hybrid biofuel system which combines the well-understood zinc-air cell and the enzymatic biofuel cell. The electropositive zinc element is coupled with the biocatalytic activity of the laccase enzyme to produce an enzymatic zinc-air cell. As a result, the biofuel cell design is simplified since the anolyte components (enzyme, substrate, electron mediator and buffer solution) are replaced with a metallic zinc. Zinc is well known for its rapid electrokinetics and it is the most active metal that is relatively stable in aqueous solution. Laccase is an oxidoreductase that belongs to the copper-containing enzyme family that was first discovered in the Japanese lacquer tree *Rhus vernicifera* (Yoshida 1883). These enzymes demonstrate a specific affinity for oxygen as their electron acceptor. Laccase catalyses the removal of a hydrogen atom from the hydroxyl group of ortho- and para-substituted mono- and polyphenolic substrates and from aromatic amines by one electron abstraction to form free radicals capable of undergoing further laccase-catalysed oxidation or non-enzymatic reactions such as hydration of polymerisation (Thurston 1994; Yaropolov et al. 1994). The use of this enzyme is attractive because of its ability to reduce

molecular oxygen to form water at neutral pH (Murata et al. 2009).

The hybrid zinc-air biofuel system reported in this work is not similar with the zinc-laccase system published by Smolander et al. (2008). Basically they coupled a laccase-based biocathode with a zinc anode. The zinc-laccase cell was a closed system which operated under humidity controlled conditions and oxygenated electrolyte in double chamber design utilizing cellophane, Nafion or Whatman 1 filter paper as separator. By contrast, we studied a zinc-air system employing laccase as the oxygen reduction catalyst. Air electrode was utilized to permit direct air (oxygen) access to the system and the cell was left to operate under open ambient conditions. Besides, a much simpler design was employed: membraneless (single-chamber cell) and freely suspended laccase in the buffer electrolyte (not immobilized). The highly specific laccase reaction enables the use of a membraneless single compartment design (Atanassov et al. 2007). Despite its simple design features, the zinc-air biofuel system delivered power output of comparable performance with a much more complex biofuel system designs.

Experimental Details

Cell design, components and fabrication

The cell was a single-chamber design as illustrated in Figure 1. The reaction chamber measured 20 mm in diameter and 5 mm in depth with holding capacity of 1.5 mL. The anode was a zinc foil of 250 μm thick, selected because of ease of fabrication and rapid optimisation. A commercially available air electrode, an E4 electrode of Electric Fuel Ltd., was utilized as the cathode. The air electrode consists of laminated structures of fibrous carbon supported by a nickel mesh. The air side of the electrode is covered with a semi-permeable Teflon membrane. The membrane permits ambient air oxygen to diffuse into the system. Teflon hydrophobic characteristic maintains the crucial triple interface (air/oxygen- liquid/electrolyte-solid/conductor) requirement for an effective functioning of the air electrode (Chakkaravarthy et al. 1981).

The electrolyte consisted of laccase from *Rhus vernicifera* (120 Unit activity, U/mg, Sigma-Aldrich) and syringaldazine (0.216 mM) in potassium dihydrogen phosphate buffer, pH6.5 (Sigma-Aldrich, ≥99.0% purity). The syringaldazine solution was prepared in absolute methanol. The electrolyte was prepared fresh in cold deionised water before each run. Laccase served as the biocatalyst for oxygen reduction, whereas syringaldazine was the phenolic substrate for laccase. The final reaction mix contained 36.5 mM potassium phosphate, 0.01 mM syringaldazine, 10% methanol and 50 U laccase. A negative control cell was prepared consisting of only syringaldazine substrate in the buffer solution (without laccase) to substantiate the oxidoreductase activity. Cells were also prepared with lower laccase concentrations of 5 and 25 U. The enzyme was assayed spectrophotometrically as reported by Ride (1980) to estimate the unit activity of laccase prior to electrolyte preparation.

Electrochemical cell characterisations

The cell was characterised according to its open-circuit voltage (OCV), power density profile, polarisation curve and galvanostatic discharge capability. Computer controlled Eco Chemie Autolab potentiostat Model PGSTAT302N (Utrecht, The Netherlands) with General Purpose Electrochemical System (GPES) version 4.9 software was used for all experiments. All experiments were performed at room temperature.

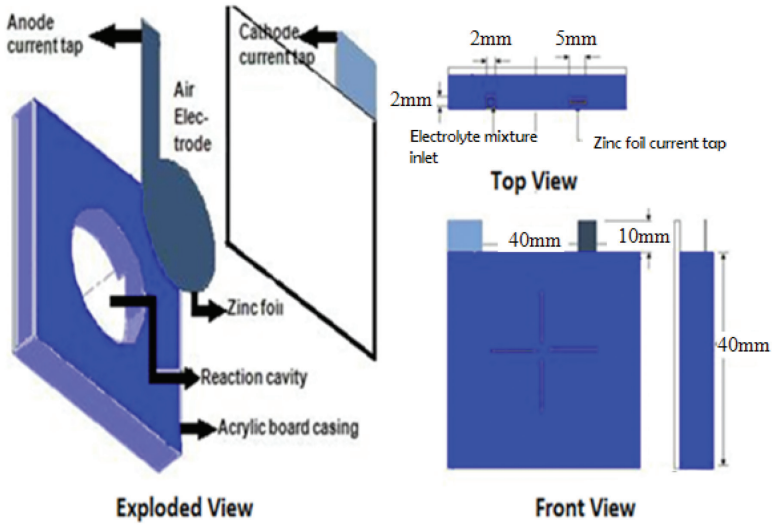

FIGURE 1. Single chamber cell design

LACCASE ACTIVITY AT ROOM TEMPERATURE

The activity of laccase at room temperature was monitored since the hybrid biofuel cell is intended for room temperature operation. At zero hour, 50 U/mL enzyme solution was prepared in potassium phosphate buffer of pH6.5 and left in room temperature. At an interval of 6 h, continuously for 5 days, the enzyme solution was assayed to identify the current unit activity of the enzyme with syringaldazine as the substrate. Details of the assay techniques can be referred in Ride (1980).

RESULTS

The zinc-air biofuel cell registered an average OCV of 1.2 V. Figure 2 shows the open-circuit voltage value of the cell over a period of 24 h. The voltage was fairly stable without any significant variations. Figure 3 displays the polarisation profile of the cell and its corresponding power density. The hybrid cell could deliver a maximum power density of 1100 μWcm^{-2} at discharge load of 2500 μAcm^{-2}. A discharge capacity test was performed at a constant current of 0.5 mA. This current load was chosen so as to obtain an operating voltage around 1.0 V. Figure 4 shows the discharge capacity of the zinc-air biofuel cell as compared to a control cell (without laccase). With the inclusion of laccase in the cell, its discharge capability was almost doubled. This result substantiates the contribution of freely suspended laccase as a biocatalyst for oxygen reduction. Current could be withdrawn from the control cell because phosphate buffer solution was slightly acidic (pH6.5); thus, the control cell was essentially an acidic zinc-air cell. Oxygen reduction activity has been observed even in quasi-neutral electrolyte (Jindra et al. 1973; Kozawa et al. 1970a, 1970b).

The role of laccase was further clarified when its concentration was varied, as displayed in Figure 5. As the laccase concentration was halved from 50 to 25 U, the cell discharge capacity was also reduced proportionately. However, at 5 U laccase, the cell performance was similar to that of the control cell; that is, at this concentration, laccase has no apparent contribution.

Another control experiment was conducted to ascertain the contribution of the air electrode towards the cell performance i.e. the semi-permeable Teflon membrane on the air side of the air electrode was blocked with a masking tape. As the ambient air access was blocked, the zinc-air biofuel cell was operating based on dissolved

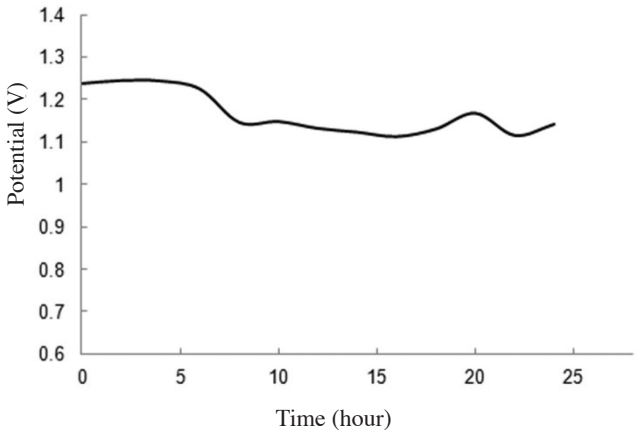

FIGURE 2. OCV of the hybrid cell monitored over 24 h

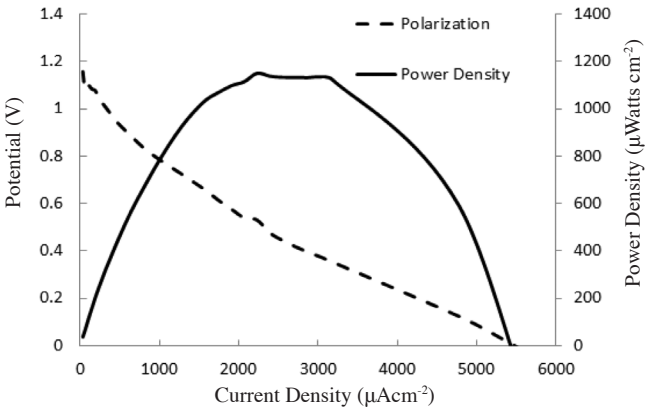

FIGURE 3. Polarization and power density profiles of the hybrid cell

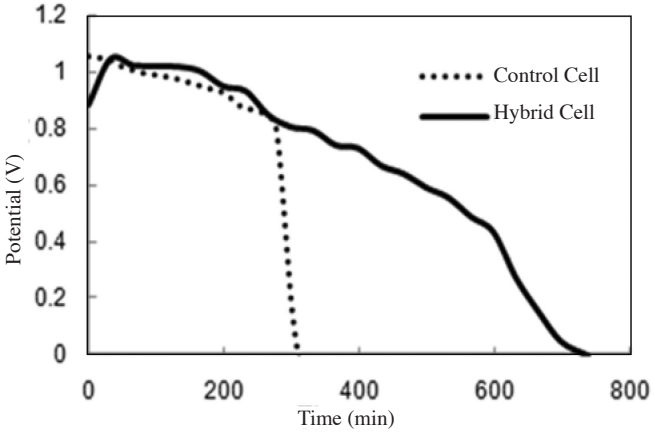

FIGURE 4. A comparison between discharge profiles of an enzymatic zinc-air cell and a negative control (without laccase) to substantiate the biocatalytic activity of laccase enzyme

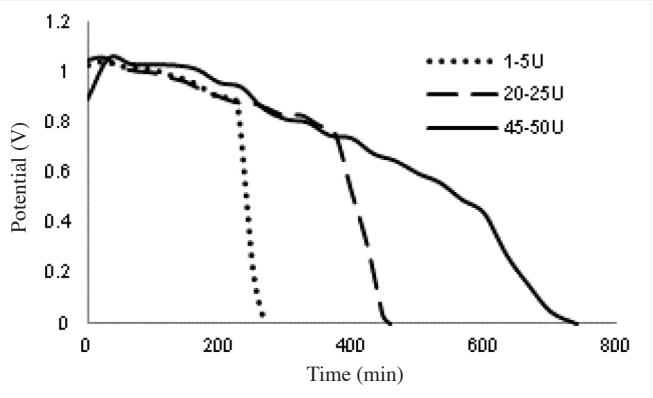

FIGURE 5. Cell discharge capacity with varying biocatalyst concentrations

oxygen mainly. Figure 6 shows the significant contribution of the air electrode on the cell discharge performance. The area under the discharge curve represents the energy output of the cell, Q

$$Q = I\int_{t_0}^{t_1} V(t)\,dt, \tag{1}$$

where $V(t)$ is the instantaneous operating voltage of the cell under galvanostatic discharge current I. The difference under the area of the discharge curve gives the cell power output gain due to the use of air electrode. Taking the discharge duration $\Delta t = t_1 - t_0 = 400$ min, the cell output has been enhanced by 14%.

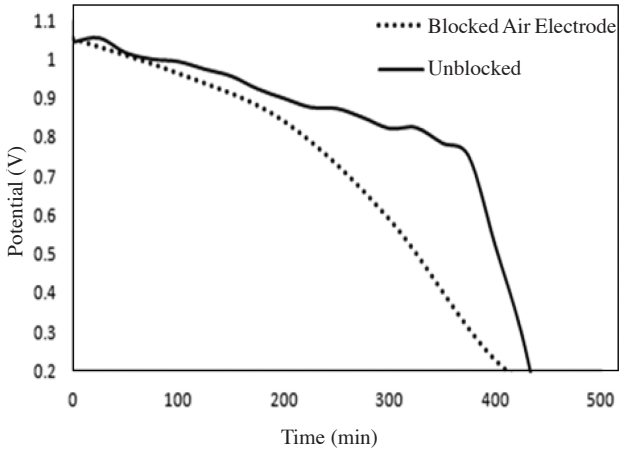

FIGURE 6. Enhanced discharge profile obtained by using the air electrode

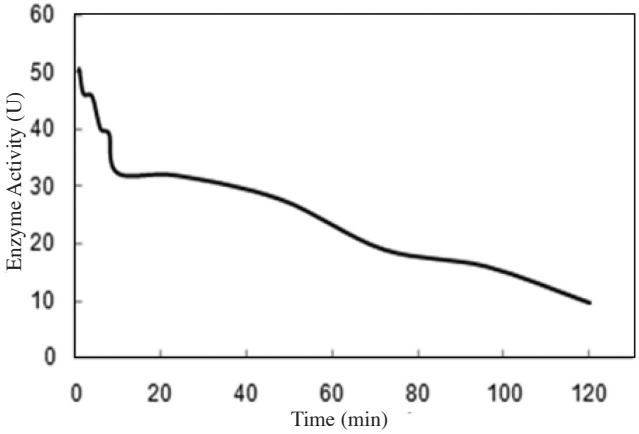

FIGURE 7. Laccase enzyme activity profile over 5 days

Laccase activity was monitored continuously for 5 days and the profile is depicted in Figure 7. Its initial activity corresponded to 50 U. Within 24 h, the activity dropped to 30 U. The activity of laccase declined by half in 55 h, as shown in Figure 7. After day 5 (120 h), 10 U remained. Unit activity of laccase was monitored at room temperature of 25°C and ambient conditions as the cell was intended for open ambient operation.

DISCUSSION

Besides the catalytic reduction of molecular oxygen by laccase enzyme, the chemistry of an enzymatic zinc-air cell is in principle that of the zinc-air system, summarized as follows (Othman et al. 2001):

At anode (negative):

$$2Zn \rightarrow 2Zn^{2+} + 4e^{-}. \quad (2)$$

At cathode (positive):

$$O_2 + 4H^{+} + 4e^{-} \xrightarrow{laccase} 2H_2O. \quad (3)$$

The complete reaction mechanisms of laccase catalyzed oxygen reduction to water are still under debate (Alcalde 2007). According to Thurston (1994) laccase operates as a battery; storing electrons from individual substrate oxidation which are then used to reduce molecular oxygen. Thus the oxidation of four reducing substrate molecules is necessary for a complete reduction of molecular oxygen to water. Figure 8 schematically illustrates the reaction mechanisms of single-chamber, freely suspended laccase of an enzymatic zinc-air cell: At anode, zinc metal oxidized to zinc ions, leaving electrons on its surface; at cathode, oxygen diffuses through semi-permeable membrane of the air electrode into electrolyte containing enzyme solution; laccase catalyzes the reduction of molecular oxygen to water and continuous flow of electron via the electrical circuit generates current. Note that an air electrode was utilized as the cathode in the present work to enhance the oxygen diffusion into the system.

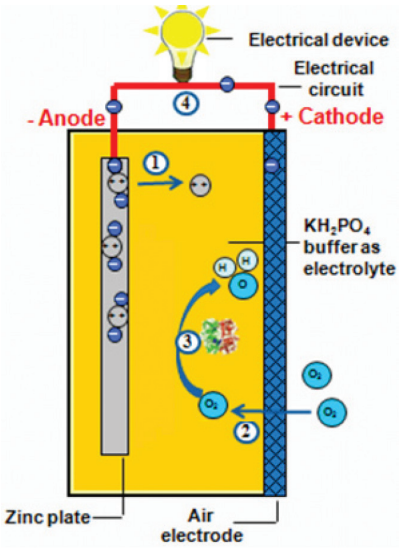

FIGURE 8. Reaction mechanisms of a membraneless, freely suspended laccase of an enzymatic zinc-air biofuel cell

The biocatalytic role of freely suspended laccase in the single chamber zinc-air system was evident from the discharge profiles of the cells containing various amounts of laccase. The discharge capacity of the control cell was extended by a factor of 2.4 and the energy output increased by 46% when 50 U of laccase was added to the system. The discharge profile of a zinc-air system is unique. It consists of a flat plateau and an abrupt drop that marks the end of the discharge capacity (Ramlen 1995). Zinc-air biofuel cells have similar features, particularly the abrupt drop at the end of discharge. However, the onset of discharge polarisation is apparent from the slightly slanted plateau. This slanted plateau is most likely due to the low conductivity of the near neutral electrolyte and to the lower catalytic activity

of laccase as compared to inorganic catalysts such as manganese oxide.

Despite its simple design features, the zinc-air biofuel system registered an OCV of 1.2 V and was able to produce a maximum power density of 1100 μWattscm^{-2} at discharge load of 2500 μAcm^{-2} and 0.4 V operating voltage. The zinc-laccase system was also reported by Martinez-Ortiz et al. (2011) and Smolander et al. (2008) but with a much complex system design. Smolander et al. (2008) reported a zinc-laccase system with a higher OCV of 1.45 V but the optimum maximum power density output was much lower than reported in the present work i.e. around 10 μW/cm^2 at discharge current of 39 μA/cm^2 which was obtained under humidity controlled condition and using a closed system. Martinez-Ortiz et al. (2011) reported a zinc-laccase system slightly better than our work. They reported a zinc-laccase system with an OCV of 1.667 V, a maximum power density of 1190 μWcm^{-2} and current density of 2977 μAcm^{-2} at 0.41 V. Martinez-Ortiz et al. (2011) utilized immobilized laccase onto a graphite electrode functionalized with a substrate-like molecule i.e. 4-[2-aminoethyl] benzoic acid hydrochloride.

Despite the extensive procedures to prepare the laccase biocathode, the reported power density is only around 8% higher compared to our simple design approach. Besides, they did not assess the discharge capacity of the zinc-laccase system which serves as true performance indicator of the cell. Sakai et al. (2009) reported among the highest biofuel cell output. They utilised a mediated NAD-dependent glucose dehydrogenase (GDH)–bilirubin oxidase (BOD) biocatalytic system. The enzymes, electron transfer mediators and other components were immobilised on carbon fibre electrodes. The GDH-BOD system gave an OCV of 0.8 V and was able to produce a maximum power density of 1450 μW cm^{-2} at 0.3 V, which is again comparable to our hybrid system. Among other examples that can be referred and compared with are the work of Habrioux et al. (2008), Mano et al. (2002) and Tan et al. (2009). In all these biocatalytic systems, the performance of the hybrid enzymatic zinc-air cell is comparable, if not better, to that of a much more complex system design reported by others.

Enzymes degrade upon prolonged exposure to the ambient environment. Thus, the activity of laccase in its unaltered form was monitored continuously for 120 h. Based on the activity of 50 U of laccase, we defined the T½ as the duration after which the enzyme's activity is halved to 25 U. T½ was estimated to be around 55 h, and we demonstrated that even at 25 U, laccase activity was still prevalent. Therefore, at 50 U laccase, it is possible to design a zinc-air biofuel cell that can be operated continuously for at least 55 h. This metal biofuel system possessed a high energy density and was able to operate with simple design features as described earlier. In fact, the hybrid system could also be utilized as a reference cell to gauge the efficacy of immobilizing an oxidoreductase enzyme on an electrode.

Conclusion

This work introduced a hybrid biocatalytic system that combines the zinc-air system and the enzymatic biofuel system. Replacing the customary glucose/glucose oxidase anolyte present in an enzymatic biofuel cell with cheap and abundant zinc simplified the design and reduced the cost. Though we employed system with simple design features: Membraneless single chamber cell and freely suspended or 'mobile' laccase enzyme, the performance of the hybrid biofuel is comparable to that of a much more complex biocatalytic system.

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