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A one-compartment fructose/air biological fuel cell based on direct electron transfer

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ABSTRACT

The construction and characterization of a one-compartment fructose/air biological fuel cell (BFC) based on direct electron transfer is reported. The BFC employs bilirubin oxidase and D-fructose dehydrogenase adsorbed on a cellulose–multiwall carbon nanotube (MWCNT) matrix, reconstituted with an ionic liquid, as the biocathode and the bioanode for oxygen reduction and fructose oxidation reactions, respectively. The performance of the bioelectrode was investigated by chronoamperometric and cyclic voltammetric techniques in a standard three-electrode cell, and the polarization and long-term stability of the BFC was tested by potentiostatic discharge. An open circuit voltage of 663 mV and a maximum power density of 126 $\mu W\,cm^{-2}$ were obtained in buffer at pH 5.0. Using this regenerated cellulose–MWCNT matrix as the immobilization platform, this BFC has shown a relatively high performance and long-term stability compared with previous studies.

1. Introduction

Biological fuel cells (BFCs, including microbial and enzymatic fuel cells) and their potential uses have been attracting worldwide attention driven by the demands for clean and renewable energy resources; such devices directly convert chemical/biochemical energy into electrical energy (Bennetto et al., 1983; Bullen et al., 2006; Davis and Higson, 2007). Compared to conventional fuel cells, BFCs produce lower power density, but they have the potential of carrying out specific tasks such as the powering of implantable medical devices by enzymatic fuel cells (Barton et al., 2004; Minteer et al., 2007) or different wastewater treatment by microbial fuel cells (Zhao et al., 2008, 2009a).

Enzymatic fuel cells can be divided into mediated electron transfer (MET) and direct electron transfer (DET) types, which are the focus of most current research (Barton et al., 2004; Bullen et al., 2006). In MET-type systems, redox chemicals are added as mediators to enhance electron transfer processes; these mediators are, however, often toxic and present potential environmental problems; they also lead to voltage loss as there is a potential difference between the active site of enzymes and mediators. The DET-type BFCs, where a direct electron exchange between the active site of the enzyme and the electrode, possess very important advantages due to their simple construction allowing one-compartment membraneless BFCs, which has the potential benefit of miniaturization and low cost. The main drawback associated with DET is that this process is usually prohibited by the enzyme structure. A variety of attempts have been made to improve the electronic communication between the enzyme's active site and the electrode surface (Degani and Heller, 1989; Ghindilis et al., 1997; Ramanavicius et al., 2005; Sarma et al., 2009). However, there are only a few reported BFCs based on membraneless DET-type biocatalysts for both the cathode and anode reactions (Ramanavicius et al., 2005, 2008; Coman et al., 2008; Vincent et al., 2005; Kamitaka et al., 2007a; Tasca et al., 2008). The long-term stability is a key aspect of BFCs (Kim et al., 2006) and previously reported BFCs based on DET show relatively poor long-term stability.

Natural polymers provide unique characteristics for enzyme immobilization due to their abundance and especially the apparent biocompatibility which could minimize the possibilities of enzyme denaturation. Cellulose is the most abundant and renewable biopolymer on earth, has many advantages when used as an enzyme immobilization material, and provides a biocompatible environment to enhance the stabilization of immobilized proteins. The challenge to using cellulose as a material for enzyme immobilization is its insolubility in common solvents due to its high crystallinity. Some recent studies have showed that room

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temperature ionic liquids (RTILs), as environmental friendly solvents, can exhibit good dissolution power for cellulose, which can then be reconstituted into a variety of forms (Kosan et al., 2008; Hermanutz et al., 2008; Wu et al., 2009).

Carbon nanotubes (CNTs) represent an important group of nanomaterials with attractive geometrical, electronic and chemical properties (Katz and Willner, 2004; Zhou et al., 2009). The unique properties of carbon nanotubes make them attractive for the development of bioelectrochemical devices.

We demonstrate here a membraneless fructose/air BFC, using commercially available D-fructose dehydrogenase (FDH) and bilirubin oxidase (BOD) absorbed on the surface of electrodes modified with cellulose–MWCNT matrix, which is regenerated by RTIL, as bioanode and biocathode for D-fructose oxidation and oxygen reduction reactions, respectively. The long-term stability of the assembled BFC under continuous operation is also presented.

2. Experimental

2.1. Chemicals and reagents

Bilirubin oxidase (BOD; EC 1.3.3.5) from *Myrothecium verrucaria* and D-fructose dehydrogenase (FDH; EC 1.1.99.11) from *Gluconobacter industrius*, microcrystalline cellulose and the ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][CH_3COO]) were purchased from Sigma–Aldrich and used with no further purification. Multiwall carbon nanotubes (Nanocyl-3100 series with an average diameter of 10 nm) were treated as described previously (Liu et al., 1998). All other chemicals used in this study were of analytical grade. All solutions were prepared with ultra-pure water (>18.2 MΩ cm^{-1}) from a Purite purification system.

2.2. Preparation of the modified anode and cathode

The glassy carbon (GC) electrodes were polished successively with 0.3 and 0.05 μm alumina slurries, and then sonicated in ultra-pure water. The cellulose–MWCNT modified GC electrodes were prepared as follows: the cellulose–[EMIM][CH_3COO] solution was obtained by thoroughly mixing cellulose (3.0% mass) and [EMIM][CH_3COO], heating up to 70 °C for 1 h in an ultrasonic bath until an optically clear solution was obtained. The MWCNT (3.0% mass) were then suspended in [EMIM][CH_3COO]–cellulose solution by grinding in an agate mortar for 15 min under high purity nitrogen to prevent the [EMIM][CH_3COO] from absorbing moisture. The resulting materials were evenly spread on the GC surface using a doctor blade and the modified electrode was then immersed in ultra-pure water, to remove the [EMIM][CH_3COO] by dissolution, leaving the cellulose–MWCNT matrix on the electrode surface.

Bioelectrodes were prepared by placing an aliquot of 0.01 cm^3 of enzyme solution (i.e. ~0.1 mg of BOD for cathode or of FDH for anode) on the electrode surface, and allowing the solution to dry at the surface of the electrode in air at 22 °C. The electrodes were rinsed with deionized water to remove weakly adsorbed enzymes before electrochemical measurements. When not in use, the bioelectrodes were stored dry at 4 °C.

2.3. Bioanode and biocathode electrochemical measurements

Chronoamperometric and cyclic voltammetric measurements were carried out by using a computer-controlled Autolab potentiostat/galvanostat (EcoChemie, Netherlands) in a three-electrode cell with a 25 cm^3 volume and consisting of working electrode, a Pt wire counter electrode and an Ag/AgCl reference electrode (BASi, 3.0 mol dm^{-3} NaCl, +0.196 V *vs.* SHE at 298.2 K). Control experiments using unmodified GC and enzyme-free cellulose–MWCNT coated electrodes were carried out. The electrolyte was 0.2 mol dm^{-3} citrate buffer, which was purged with high purity nitrogen (BOC UK) or air (air pump) for at least 15 min prior to experiments to obtain a nitrogen- or air-saturated solution, respectively.

2.4. Biological fuel cell measurement

The FDH anode, BOD cathode and Ag/AgCl reference electrode were placed in a one-compartment configuration (see Scheme 1 in Supporting information). The biological fuel cell was operated in citrate buffer (pH 5.0) containing 200 mmol dm^{-3} D-fructose under continuous air-bubbling conditions (using an air pump). The potentiostatic discharge polarization performances and the durability behavior at constant voltage of 0.35 V were measured using a battery Test System (Arbin Instrument Corp.). The current density and power density were calculated based on the geometrical surface area of the electrode. The potentials of the cathode and the anode *vs.* Ag/AgCl as a function of time were individually recorded using a digital multi-meter (Integra 2700 series equipped with 7700 multiplexer, Keithley Instruments Inc.) interfaced to a personal computer for data collection (Zhao et al., 2009a,b). The internal ohmic resistance of the BFCs was determined by electrochemical impedance spectroscopy using a Solartron Analytical 1260 frequency response analyzer operating in conjunction with a Solartron Analytical 1287 potentiostat/galvanostat in the frequency range 1 MHz–0.1 Hz and with a potential perturbation signal of 10 mV rms (Zhao et al., 2009b). All electrochemical experiments and BFC operations were carried out at 22.0 ± 1.0 °C.

3. Results and discussion

3.1. The electrocatalytic behavior of the FDH anode

D-Fructose dehydrogenase is a membrane-bound enzyme with a molecular weight of ca. 140 kDa and contains flavin and heme c as prosthetic groups (Ameyama et al., 1981). This enzyme shows high substrate specificity for D-fructose and can catalyze the oxidation of D-fructose to 2-keto-D-fructose, which is therefore used extensively in food and clinical analyze (Matsumoto et al., 1986; Nakashima et al., 1985). In this study, the electrocatalytic activity of FDH towards D-fructose was investigated by chronoamperometry in a standard three-electrode electrochemical cell. In the absence of D-fructose (i.e. 0–140 s), the measured current density levels were around 30 μA cm^{-2} (Fig. 1a). A significant increase in current was obtained when 0.5 mmol dm^{-3} D-fructose was added into the buffer, and a maximum stable current density of 280 μA cm^{-2} was achieved by the direct electron transfer between the enzyme and the electrode. Control experiments were performed on a blank glassy carbon and enzyme-free cellulose–MWCNT modified electrode; no current change was observed when fructose was added in solution (data not shown). Oxygen had no effect on the electrocatalytic reaction of the FDH anode since no observable current change was obtained when air purging the buffer during 360–500 s.

Fig. 1b shows the current as a function of D-fructose concentrations. The current increased significantly to 892 μA cm^{-2} when the concentration of D-fructose reached 15.0 mmol dm^{-3}. This relationship between the current change and the concentration can offer advantages for the development of a D-fructose biosensor. A plateau response was observed at high D-fructose concentration, indicating a characteristic Michaelis–Menten kinetics mechanism. The apparent Michaelis–Menten constant K_m, which relates to an enzyme's affinity for a substrate, for D-fructose is 2.3 mmol dm^{-3} and is smaller than the reported values 11 ± 1 mmol dm^{-3} (Tominaga et al., 2009) and 9–10 mmol dm^{-3} (Ameyama et al., 1981; Kamitaka et