Enzyme and Microbial Technology 86 (2016) 127–133

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Ethanol/O_2 biofuel cell using a biocathode consisting of laccase/HOOC-MWCNTs/polydiallyldimethylammonium chloride

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ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 6 October 2015
Accepted 9 October 2015
Available online 22 October 2015

Keywords:
Biofuel cell
Polydiallyldimethylammonium chloride
Carboxylated multiwall carbon nanotube
Laccase
Alcoholdehydrogenase
Polymethylene green

ABSTRACT

In the present report we focused on the substitution of metallic catalysts by biocatalysts to develop a high efficient biofuel cell. A bioanode and a biocathode were designed using ADH and laccase, respectively. Carboxylated multiwall carbon nanotubes (HOOC-MWCNTs) and polydiallyldimethylammonium chloride (PDDA) were used for immobilizing the enzymes on either polymethylene green (PMG) modified glassy carbon or graphite electrodes. In this way, an ethanol–oxygen biofuel cell was designed in which PDDA/ADH/PDDA/HOOC-MWCNTs/PMG/GC and PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr operated as bioanode and biocathode, respectively. In the optimized condition of O_2 saturated PBS (0.1 M, pH 7.5) containing 1 mM ethanol and 1 mM NAD^+ the open-circuit voltage reached to a plateau at 504 mV based of which the power density of 3.98 mW cm^{-2} was obtained.

1. Introduction

Enzymes are good substitute to metallic catalysts in the case of biofuel cells (BFC) that convert energy [1–3]. Palmore et al. reported the dehydrogenase fuel cells for oxidation of methanol, ethanol, glucose, malate and cellobiose, etc. [4–8]. Biodevices that use the enzymes as catalyst, represent an interesting strategy for operating at low temperatures and neutral pHs [9–12]. Although these devices show favorable features and represent several advances over the years, many challenges still have to be confronted before they find practical applications [13]. Applying enzymes on solid electrode surfaces to make bioelectrodes often require immobilization steps. Several strategies involve membranes, adsorption, encapsulation or chemical binding have successfully been used to immobilize enzymes on the surface of solid electrodes [14,15]. Some papers have described the use of Nafion to prepare alcohol or sugar biofuel cells, where the immobilized enzymes activity are preserved and the prepared bioanodes remained stable [16,17]. In some enzyme fuel cells, the catalytic oxidation of NADH is one of the most appropriate reactions occurring at the electrode surface [3,12]. So that dehydrogenases that use NAD^+ as cofactor represent the largest group of redox enzymes that is applicable in many bioelectronics devices [18–20]. However, electrochemical NAD^+ regeneration normally occurs at high overpotentials [21]. The importance of NAD^+ regeneration in bioelectrochemistry has fortified the search for improving the conditions that facilitate NADH oxidation. These efforts led to produce the bioelectrodes which catalyze NADH oxidation with high conversion rates [22–27]. For example, NADH oxidation has an overvoltage of nearly 0.7–1 V at glassy carbon electrodes and higher values at platinum surfaces [28]. When a regeneration system is not available, the use of NAD^+-dependent dehydrogenases is impossible. Many authors have prepared mediators that can reduce the overpotential of the NADH oxidation [29–32]. For instance, phenazine dyes can lower the overpotential of the NADH oxidation reaction. This permits the reaction to occur at the surface of bioelectrode [33–38]. Among the various phenazine dyes, organic compounds such as methylene green (MG) and methylene blue oxidize NADH professionally. By electropolymerization of MG, polymethylene green (PMG) is formed on electrode that provides long stability during biofuel cell elucidation [39,40]. In the past years, some research teams have achieved good results by using a stable PMG layer to electrocatalyze NADH oxidation in alcoholic biofuel cell using different dehydrogenase enzymes [41–44]. Application of carbon nanostructures [45–47] for modifying the bioelectrodes is another strategy to proficiently regenerate NAD^+ in bioelectronics devices involving dehydrogenase enzymes. In this case, carbon nanotubes (CNTs) make a good environment in which the target reaction takes place at lower overpotentials [48–51]. CNTs have brilliant electronic properties such as high electron transfer rate, large surface area

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http://dx.doi.org/10.1016/j.enzmictec.2015.10.004
0141-0229/

and high catalytic activity which make them attractive for bioelectrode modification. They also improve the conductivity of both bioanode and biocathode. Based on the results obtained by Liu et al. the impedance of the CNTs modified glassy carbon electrode decreased to the lowest R_{ct} (~120 Ω) in comparison with unmodified glassy carbon electrode (R_{ct} ~230 Ω) [51,52]. Therefore, the high electrical conductivity and biocompatibility of functionalized CNTs allow their use as electrode materials in biofuel cell. For example, Pang et al. showed that 69.3% of current density of the laccase/CNTs/glassy carbon electrode can be retained for seven days [53]. Liu et al. showed that the current response of laccase/CNTs/glassy carbon electrode toward catechol, and O_2 is less than 1% after 15 days [54]. Aquino Neto et al. studied the direct electron transfer of a biofuel cell in the presence and absence of CNTs [55]. They showed that the prepared enzyme electrode indicated an oxidation peak at about 265 mV vs. Ag/AgCl, but a lower oxidation peak was obtained at around 85 mV (vs. Ag/AgCl) for the bioelectrode containing enzyme and multi-wall carbon nanotube (MWCNTs). Also, it seems that the immobilization of enzyme on functionalized CNTs closes the enzyme molecules to electrode surface. In addition, the functionalized CNTs may orient the enzyme molecules at bioelectrode surface and consequently make the electron transferring process easier and lower the oxidation potential [56–58].

Here, by using PMG, functionalized CNTs and ADH, we focused on the preparation of an active layer that catalyzes NADH oxidation on bioanode. It seems that CNTs improve the electronic conductivity of bioanode while both PMG and functionalized CNTs play effective role in reducing the oxidation potential of NADH.

The cathodic reaction is also very important in biofuel cells performance since it can significantly increase electric potential and current of biofuel cell. Various strategies were applied to maximize the performance of cathodes in biofuel cells. It seems that the application of laccase is very helpful for this purpose [59–61]. It was demonstrated that some carbon-based nanomaterials may significantly improve the current of laccase-based biofuel cell cathode [62].

Polydiallyldimethylammonium chloride (PDDA), a cationic polyelectrolyte, can improve the dispersibility of MWCNTs in water and form a uniform thin film for modification of bioelectrodes. PDDA usually acts as a positively charged colloid when it is dissolved in aqueous solution. This positively charged layer is easily covered by the negatively charged functionalized carbon nanotube through electrostatic interaction [63–67].

In the present report, a bioanode and a biocathode were designed using ADH and laccase, respectively. HOOC-MWCNT and PDDA were used for immobilizing the enzymes and improving the BFC efficiency. The prepared PMG/HOOC-MWCNT/PDDA nanocomposite in the presence of NAD^+ and ethanol showed an acceptable power output in comparison to other BFCs reported before.

2. Experimental

2.1. Materials

NADH, NAD^+, ADH (from *Saccharomyces cerevisiae*, pI: 6.8, >300 units mg^{-1}) and l accase (from Rhus vernicifera, pI: 4.1–4.7, E.C. 1.10.3.2, ≥50 units mg^{-1}) were purchased from Sigma–Aldrich. Functionalized MWCNTs (contaning 0.49% wt. of —COOH group, outside diameter: 50–80 nm, inside diameter: 5–15 nm, length: 10–20 nm, and >95% purity) were attained from US Research Nanomaterials Inc. The sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$), sodium tetraborate ($Na_2B_4O_7$), sodium nitrate ($NaNO_3$), PDDA, MG and ethanol were obtained from Merck. All the enzyme and coenzyme solutions were freshly prepared and rapidly used. All the solutions were prepared with double distilled deionized water.

2.2. Instrumentation

Electrochemical investigations were done using a Potentiostat/Galvanostat EG&G 263A (controlled by a Power Suite software package and a GPIB interface), equipped with a three electrode electrochemical cell including an Ag/AgCl (KCl sat.) reference electrode, a platinum rod as counter electrode and a glassy carbon (GC, ϕ = 3 mm, bare or modified with nano composite film) and graphite (Gr, ϕ = 3.3 mm, bare or modified with nanocomposite film) electrodes from Azar Electrode (Uromia, Iran) as working electrodes. All experiments were carried out inside a Faraday-cage. pH of solutions were measured using a pH electrode coupled to a Metrohm model 691 pH meter.

2.3. Electrochemical polymerization of MG

The GC and Gr electrodes were polished with 0.3 and 0.05 μm alumina slurries and sonicated in water and ethanol, respectively. Electrochemical polymerization of MG was carried out according to the method described by Zhou et al. [68].

2.4. Preparation of bioanode and biocathode

The matrix used for immobilization of enzymes on anode or cathode is important since it is responsible for preserving the activity of enzymes and also it helps the enzymes to communicate with electrode surfaces electronically, allowing a fast electron transfer. For immobilization of enzymes on either anode or cathode, at first using an ultrasonic bath, 1 mg of HOOC-MWCNTs was dispersed in 1 mL of ethanol to give a black suspension. Then, the PMG coated electrodes (PMG/GC as anode and PMG/Gr as cathode) were treated by dropping HOOC-MWCNT suspension on electrode surfaces and drying at air. Thereafter, 0.5% PDDA solution was dropped on either HOOC-MWCNTs/PMG/GC or HOOC-MWCNTs/PMG/Gr electrodes and left to dry. Then, the anode and cathode surface were covered by 2 μL of related enzyme (ADH for anode and laccase for cathode: 10 mg mL^{-1}, in 0.1 M PBS, pH 7.5) and dried at room temperature. Finally, the dried electrodes were covered by 2 μL of 0.5% PDDA, again.

2.5. BFC Assembling

As shown in Scheme 1, for BFC assembly the PDDA/ADH/PDDA/HOOC-MWCNTs/PMG/GC electrode was used as bioanode and PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr was used as biocathode. The cell with the volume of 1 mL was made of Plexiglas. The bioanode and biocathode were connected to each other via a metallic wire. The cell was filled out with oxygen saturated phosphate buffer (pH 7.5, 0.1 M) containing 1.0 mM NAD^+ and 1.0 mM ethanol. Before starting the cell to work it was equilibrated for 2 to 4 h (Scheme 1).

3. Results and discussion

3.1. Cyclic voltammetry of bioelectrodes

The electropolymerization of MG on GC and Gr electrodes occurs by cycling the voltage from −0.7 to +1.3 V (vs Ag/AgCl). Due to the difference between the polarity of MG and either graphite or glassy carbon, the MG molecules do not absorb on electrode surfaces but by applying a positive voltage (+1.3 V vs Ag/AgCl) the electrode surfaces (graphite or glassy carbon) is functionalize. This modification facilitates the absorption of MG molecules on electrode surface and