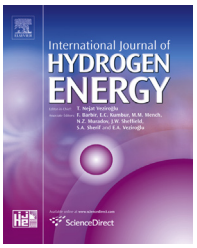
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2015) 1–8

Available online at www.sciencedirect.com

ScienceDirect

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



Multi walled carbon nanotube and polyaniline coated pencil graphite based bio-cathode for enzymatic biofuel cell

Diwakar Kashyap [a], *Chulmin Kim* [b], *Sung Yeol Kim* [b], *Young Ho Kim* [c], *Gyu Man Kim* [b], *Prabhat K. Dwivedi* [d], *Ashutosh Sharma* [d,e], *Sanket Goel* [a,*]

[a] *University of Petroleum and Energy Studies (UPES), Dehradun 248007, India*
[b] *School of Mechanical Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea*
[c] *Medical Device Development Centre, Daegu-Gyeongbuk Medical Innovation Foundation, 88 Dongnae-ro, Dong-gu, Daegu, Republic of Korea*
[d] *Center for Nanoscience, Indian Institute of Technology, Kanpur 208016, India*
[e] *Department of Chemical Engineering, Indian Institute of Technology, Kanpur 208016, Indiaauthor-group*

ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form
17 May 2015
Accepted 18 May 2015
Available online xxx

Keywords:
Enzymatic biofuel cell (EBFC)
Electro-polymerization
Bio-cathode
Polyaniline
Mediated electron transfer
Multi-walled carbon nanotube (MWCNT)

ABSTRACT

In the present work, two types of cost-effective bio-electrodes were fabricated by immobilizing Laccase on commercially available pencil graphite leads, and their electro catalytic activity have been examined. The first type of electrode (E_1) was fabricated by electro polymerization of polyaniline on pencil graphite leads on which multi walled carbon nanotubes (MWCNT) was immobilized by covalent bonding, while the second type of electrode (E_2) was fabricated by direct coating of MWCNT on pencil graphite leads. Two types of bio-cathodes were fabricated by immobilizing Laccase on the two types of electrodes by covalent bonding. The scanning electron microscopy (SEM) revealed three dimensional porous networks providing large surface area for enzyme immobilization. The maximum open circuit potential (OCP) for E_1 and E_2 bio-cathodes were measured 0.58 V and 0.60 V respectively vs Ag/AgCl reference electrode. The maximum current density observed for E_1 and E_2 electrodes were 295.7 μAcm^{-2} and 228.94 μAcm^{-2} respectively. Both electrodes exhibited excellent stability, conserving more than 75% of its optimum activity during the period of measurement.

Introduction

Enzymatic Bio Fuel Cell (EBFC) utilizes organic molecules as fuel to generate electricity with high efficiency. It employs enzymes to catalyse redox reactions [1,2]. Replacing traditional noble metal based catalyst with biocatalysts have several advantages; such as comparatively low cost, operation under mild condition (room temperature and neutral pH) and no emission of greenhouse gases [3–5]. Being a biological catalyst, enzymes have high specificity and selectivity to the catalysing reaction. These properties of enzymes allow anodic and cathodic redox

* *Corresponding author.*
E-mail addresses: sgoel@ddn.upes.ac.in, sanketgoel@gmail.com (S. Goel).
http://dx.doi.org/10.1016/j.ijhydene.2015.05.120

Please cite this article in press as: Kashyap D, et al., Multi walled carbon nanotube and polyaniline coated pencil graphite based bio-cathode for enzymatic biofuel cell, International Journal of Hydrogen Energy (2015), http://dx.doi.org/10.1016/j.ijhydene.2015.05.120

reaction to take place in the same chamber and remove the requirement of ion exchange membrane [6,7]. Glucose oxidase and Glucose Dehydrogenase are most extensively examined anodic enzymes for oxidation of glucose, while Laccase, Bilirubin Oxidase and Tyrosinase are widely studied cathodic biocatalysts for electro catalytic oxygen reduction reaction. Glucose is the favoured fuel because of high energy density and abundance in nature, whereas high redox potential and limitless availability of oxygen makes it an ideal electron acceptor [8–12]. EBFCs have also been envisioned as power source for small portable devices, wireless sensor network and self-powered sensors. The glucose and oxygen is abundantly available in body fluid that could be used to generate electricity for implantable devices such as deep brain simulator, pacemaker, and cochlear implant [2,13–15].

Like other types of fuel cells, EBFCs have their own advantages as well as disadvantages. The low power output and instability over time are the main obstacles in commercialization of these devices. The power output from EBFCs is still far from the required to run small scale/implantable devices [7,16]. Being a biological molecule, enzymes are engineered by nature to catalyse redox reaction in biological system where electrons are transfer by redox active molecule (mediator). The active site of enzymes is buried in deep pockets that hinder significant electron transfer to the conductive support, primarily because of the electrical insulation of the bio-catalytic site by the surrounding protein shells [17,18]. To facilitate electron transfer between enzyme and electrode, various mediators are usually employed that efficiently shuttle electrons between the enzyme active site and the electrode. $ABTS^{2-}/ABTS^{-}$ (2, 2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)) redox couple is commonly used as diffusional mediator that could easily oxidized and reduced at the electrode with formal potential of 700 mV vs. Ag/AgCl. This value is close to the formal potential of T_1 site of laccase (780 mV vs. NHE) [19–22].

Laccase is the preferred cathodic enzyme for electro catalytic oxygen reduction reaction because of high open circuit potential (OCP) in comparison to Bilirubin Oxidase and Tyrosinase [11]. However, high over potential and sluggish kinetics at neutral pH makes oxygen reduction energetically expensive [23]. As power is potential times current, the power output of EBFCs is enhanced by increasing open circuit potential (OCP) as well as current. The current may also be enhanced by increasing the enzyme loading on the bio-electrodes and efficient collection of electrons [24–26]. Carbon based electrodes such as carbon nanotubes, graphene, carbon paste, glassy carbon, and graphite electrodes have been extensively examined. The Pencil Graphite (PG) has many advantages such as low cost, commercial availability, and high stability [30]. But to the best of our knowledge there is no report on the pencil graphite electrodes modified with poly-aniline and multi-walled carbon nanotubes (MWCNT) bio-fuel cell electrode. Modified PG based electrodes have been used for fabrication of biosensor in the recent years. Glucose bio-sensor based on glucose oxidase on modified PG electrodes have been reported by Dervisevic et., al and Sehat et [31,32]. An acrylamide biosensor based on PG modified with multi walled carbon nanotube, copper nanoparticles and polyaniline has been examined by Batra et al. [33].

In the present work, we investigate the fabrication and testing of polyaniline and MWCNT coated pencil graphite (PG) based cost effective bio-electrodes. Here, the surface area of graphite rod was enhanced by electro-polymerization of conductive polymer polyaniline and immobilization of MWCNT on PG. Multi copper oxidase enzyme, Laccase was covalently immobilized by amide bond on MWCNT using EDC/NHS to activate carboxyl group sacctio2.

Material and methods

Materials

Ultrapure water with resistivity of 18.2 MΩ cm (at 25 °C) was used for all the experiments. The chemicals, Laccase from *Trametes versocolor* (24 units/mg), disodium phosphate (Na_2HPO_4), monosodium phosphate (NaH_2PO_4), dimethyl sulfoxide (DMFO), hydrochloric acid (HCl), N hydroxy succamide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS, aniline ($C_6H_5NH_2$), acetone and carboxylated multi walled carbon nanotubes (MWCNT) were purchased from Sigma Aldrich. The length of pencil graphite lead was 60 mm and diameter was 1.4 mm. The supporting electrolyte (phosphate buffer-0.1 M, pH 5.0) for electrochemical testing of bio-cathode was prepared by dissolving required salts in deionized water. The electro polymerization aniline and electro-analysis of bio-cathode were performed in conventional three electrode system using platinum mesh counter electrode and Ag/AgCl reference electrode. The electrolyte solution for aniline polymerization was prepared by dissolving 5 ml of aniline into 50 ml of 1.0 M HCl solution. All electrochemical testing was performed on potentiostat/Galvanostat (CH Instruments 660E and Bio-logic). The morphology of electrode was examined using scanning electron microscope (SEM) model JSM-6700F from Jeol, USA.

Methods

Fabrication of E_1 electrode (PG/PA/MWCNT/Lac)

The commercially available Pencil Graphite (PG) leads were cleaned in ultrasonic bath for 2 min in 0.5 M HCl solution. The leads were washed several times with deionized water and again sonicated in acetone for 2 min. Finally, the PG leads were dried in hot air oven for 2 h at 90 °C. Using cyclic voltammetry, electro-polymerization of aniline was performed on PG in potential range −0.2–1.0 V at scanning rate of 50 $mVsec^{-1}$. The electrodes were washed with deionized water after polymerization to remove excess of unbound aniline. The polyaniline coated PG leads were dipped into activated MWCNT solution (1 mg/ml MWCNT in 1 mg/ml EDC/NHS) for 2 h. The carboxylated MWCNTs were covalently linked to the free amine group of polyaniline to by amide bond. The bio-cathode was formed by dipping polyaniline and MWCNT coated PG leads into enzyme solution (3 mg/ml laccase in 0.1 M phosphate buffer, pH 5.0) and kept at room temperature for 24 h. The enzymes got covalently linked with activated carboxyl group of MWCNT by amide bond. Finally the bio-cathodes (PG/PA/MWCNT/Lac) were washed with

Please cite this article in press as: Kashyap D, et al., Multi walled carbon nanotube and polyaniline coated pencil graphite based bio-cathode for enzymatic biofuel cell, International Journal of Hydrogen Energy (2015), http://dx.doi.org/10.1016/j.ijhydene.2015.05.120