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

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Highlights

- PMG could reduce the over potential of NADH oxidation and oxygen reduction.
- HOOC-MWCNTs beside PDDA made a suitable microenvironment to preserve the activity of ADH and laccase.
- In the optimized condition, BFC produced the power density of 3.98 $mW.Cm^{-2}$.

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.

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

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 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) [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]. AquinoNeto 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 hrs (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 initiates the electropolymerization [73]. The progressive nature of PMG was shown by the consecutive cyclic voltammograms (CVs) represented in Fig. 1 (A, B). As seen, by progressing the cycle numbers both anodic and cathodic peak currents were increased, too. At the same time the anodic peak potential shifted in the positive direction and the cathodic peak potential in the negative direction. Increasing the electric current indicated the increase in quantity of electroactive species such as methylene green radical cations ($MG^{+\cdot}$) on the electrode surfaces. The oxidation potential is an important factor for the electropolymerization of MG since it causes the formation of $MG^{+\cdot}$ radical cation which is necessary for initiating the electropolymerization [74-76]. After continuous cycling (Fig. 1, A and B, Cycles from **a** to **j**), at first the redox peaks heights are gradually increased then they tend to decrease. This indicates that in the first cycles much concentration of MG is adsorbed on electrode surface and electropolymerizatione is speed up but after a while the concentration of MG monomer on electrode surface is gradually decreased while formation of PMG reached to an entire step. By removal of the electrode from the MG-containing solution, a golden film was seen on the electrode surface which confirms the completion of electropolymerization. When the filmed electrode was washed and examined in phosphate buffer (pH 7.5), a wide oxidation peak was observed that confirmed the presence of PMG on electrode (Fig 2, Cycle a).

For biocathode preparation, after PMG formation on Gr electrode, HOOC-MWCNTs were dropped on modified electrode. As seen in Fig 2, the modification of PMG/Gr electrode by HOOC-MWCNTs, caused an enhancement in electric current (Fig. 2, Cycle b) in comparison with the CV obtained for PMG/Gr electrode (Fig. 2, Cycle a). In next step, by immobilization of laccase on modified electrode, biocathode (PDDA/Lac/PDDA/HOOC-MWCNT/PMG/Gr) with relatively high resistance is formed. Due to the insulating characteristic of enzyme the peak current is comparatively decreased (Fig. 2, Cycle c).

To verify whether the reduction of O_2 due to the catalytic activity of laccase contribute in reduction current of PDDA/Lac/PDDA/HOOC-MWCNT/PMG/Gr electrode the CVs in buffer solution in two conditions (N_2 or O_2 saturation) were compared. As seen in Fig. 3 Cycle b, a large cathodic wave was observed in oxygen saturated PBS (pH 7.5, 0.1 M). This indicated that laccase immobilized on electrode surface acted as an efficient biocatalyst for O_2 reduction. According to some reports in literature [77, 78], the process of electron transfer between O_2 and laccase at PMG surface can be illustrated as follows. In the beginning laccase reduces the O_2 dispersing from the solution:

$$\text{laccase}_{(red)} + O_2 \longrightarrow H_2O + \text{laccase}_{(OX)}$$

Then the oxidized laccase oxidizes PMGH to PMG^+:

$$\text{laccase}_{(OX)} + \text{PMGH} \longrightarrow PMG^+ + \text{laccase}_{(red)}$$

This total reduction reaction of laccase ($_{OX}$) includes two separate steps [77, 78]:

$$\text{PMGH} + \text{laccase}_{(I)} \longrightarrow \text{laccase}_{(II)} + PMG^{\bullet} \quad (1)$$

$$PMG^{\bullet} + \text{laccase}_{(II)} \longrightarrow \text{laccase}_{(red)} + PMG^+ \quad (2)$$

Where one electron is given at a time, laccase (I) is laccase $_{(OX)}$ and PMG• represented the free radical formed during the reaction. Subsequently oxidized PMG is reduced to PMGH to produce the cathodic catalytic current at the electrode:

$H^+ + PMG^+ + 2e^- \longrightarrow PMGH$

On the other hand, the T1 Cu site of laccase (E_{redox} = -237 mV versus Ag/AgCl), which is the most acceptable electron-accepting site of laccase, is located within the O_2 reduction potential window (from -183 to -340 mV) as shown in Fig. 3A (Cycle b). Therefore one may conclude that the laccase immobilized on electrode surface could act as an efficient biocatalyst for O_2 reduction.

To control the electrochemical behavior of bioanode (PDDA/ADH/PDDA/HOOC-MWNTs/PMG/GC electrode) it was inserted in PBS and in the presence of NAD^+ (1 mM) and EtOH (1.6 μM) the CV was recorded (Fig. 3B). During this process, ethanol passes through the contacting solution and reaches to the electrode surface, where it is enzymatically oxidized to acetaldehyde and NAD is reduced to NADH. Then, the generated NADH is re-oxidized by PMG^+ to form NAD^+ and MGH (Scheme 1). These CVs shown in Fig. 3 (A and B) indicates that both enzymes (ADH and laccase) immobilized on the anode and cathode is active and they work satisfactorily.

Since, the cathodic process of BFC is a proton exchanged reaction; therefore, the pH of buffer solution has a considerable effect on the performance of the biocathode. To control this effect, the voltammetric responses of biocathode were obtained at different pH (from 6.2 to 7.8) of PBS (100 mM). As shown in Fig. 4, the highest current response was observed at pH 7.5.

The storage stability of the ADH and laccase modified electrodes were also examined. When the ADH modified electrode in PBS was stored at 4 °C, it retained 88% and 48.6% of its initial

current response for ethanol after two and four weeks, respectively (data not shown). The stability of laccase modified electrode, when the enzyme electrode in O_2 saturated PBS was stored at 4 °C, was elucidated for two and four weeks and showed 93.2% and 53.2% of its initial current response, respectively. For operational stability, the cyclic voltammetric responses of ADH and laccase modified electrodes were recorded and after 50 continuous cycles the current responses remained ~86% and 73% of the original signals, respectively.

The reproducibility of electrochemical response for biocathode and bioanode were examined by cyclic voltammetry. By comparing the current responses of 6 prepared biocathodes a relative standard deviation (RSD) of 6.20% was obtained in 100 µL O_2 saturated PBS (0.1 M, pH 7.5) (data not shown). The reproducibility of bioanode was determined by comparing 5 modified ADH electrodes. The RSD of ADH modified electrode response toward 1 mM ethanol and 1 mM NAD^+ in 0.1 M PBS pH 7.5 was 1.84% (data not shown).

3.2. Biofuel cell performance

The BFC was characterized while PDDA/ADH/PDDA/HOOC-MWCNTs/PMG/GC and PDDA/Lac/HOOC-MWCNT/PDDA/PMG/Gr were used as bioanode and biocathode, respectively. The BFC characterization was done by plotting the open-circuit voltage (OCV), polarization curve and power density curve.

The OCV indicates the loss in voltage from the theoretical cell voltage. The measured OCV is the difference between the onset potentials for catalysis at the bioanode and biocathode [79]. The OCV was determined using a Potentiostat/Galvanostat over time until reach to a plateau. The voltage was measured in O_2 saturated PBS (0.1 M, pH 7.5) containing 1 mM ethanol and 1 mM NAD^+ and the OCV was recorded. As shown in Fig. 5, the OCV reached to a plateau at 504 mV. This voltage was used as initial point to record the polarization curve, as shown in Fig. 6. Each

polarization curve has three zones that are related to activation (charge transfer) loss, ohmic loss and mass transfer loss which result BFC overpotential [80-82]. But as seen in Fig 6, only two regions can be distinguished: a) activation loss in the initial part and b) ohmic loss and mass transfer loss which are merged in the same zone. For increasing the BFC efficiency, the activation, Ohmic and mass transfer losses should be decreased. The activation loss is dominant at low current densities when the rate of the electrochemical reaction at the electrode surface is controlled by sluggish electrode kinetics. The processes involving adsorption of reactant species, transfer of electrons across the double layer, desorption of product species, the number and distribution of active sites, and the nature of the electrode surface can all contribute to activation loss. Ohmic losses vary directly with current, increasing over the entire range of current density. These are due to the electrode materials, interconnects current collector plates and constant resistance between various interfaces. The Ohmic losses can be reduced by reducing contact resistances at various interfaces. The concentration losses occur due to the mass transport limitation of reactants/products to or from the electroactive sites. These voltage losses occur over the entire range of current density, but become prominent especially at high currents, when it is difficult to provide enough reactant transferring to the enzyme active sites. The mass transport voltage losses can be reduced by making the higher porosity of the electrode without losing conductivity, or a right combination of the hydrophobic and hydrophilic properties of materials used to construct electrode layer [83-85]. The use of HOOC-MWCNTs can decrease all overpotentials because of high electrical conductance and more enzymes loading and therefore promoting the enzyme-substrates interaction [86].

For further characterizing of BFC the power density curve was also determined. The maximum power density was obtained to be 3.98 $mW.Cm^{-2}$ (Fig 6). The power density is affected by two

factors, current density and cell voltage. The cell voltage is enhanced by adding substrates concentration and decreasing the BFC resistance, but the current density is affected by electrode surfaces, pH of buffer, substrates concentration and enzymes activities.

Increasing the current density of BFC could be obtained via increasing in enzyme concentration (activity), however this may causes agglomeration of enzymes. Here, in both bioanode and biocathod PDDA was used to disperse the enzymes on HOOC-MWCNTs and consequently decreases the agglomeration of enzymes. Another factor for increasing the power density of BFC is immobilization of laccase on PMG modified electrodes and its application as biocathode. This could speed up the oxygen reduction as shown in Fig. 3A. Optimizing the the pH of buffer and substrates concentration are also affective on increasing the power density of BFC, since the pH is responsible for proton exchanging and substrates are necessary for improving the anodic and cathodic reactions. Therefore, in the present work pH 7.5 for O_2 saturated PBS (0.1 M) and concentrations of 1mM for ethanol or NAD^+ were the optimum conditions. Moreover, The electrode surfaces area is another factor for enhancing the current density and earning the optimum power density. The HOOC-MWCNTs modified GC or Gr electrodes are suitable supports with sufficient surfaces area for immobilization of enzymes [87]. All these parameters were the factors affected on the efficiency of the presented BFC. A comparison between the characteristics of the present BFC and those reported in the literature is presented in Table 1. In addition to these factors, the superiority of this BFC was indebted for the unique design used for this BFC as compared in Table 2.

4. Conclusion

The procedure introduced in the present report indicated that both laccase and ADH could be immobilized on PDDA/HOOC-MWCNTs/PMG/Gr or GC electrodes. The combination of

HOOC-MWCNTs and PDDA made a suitable microenvironment to preserve the activity of immobilized ADH and laccase and facilitated the electron transferring process from redox species (ethanol and oxygen) to the electrodes and vice versa. Moreover, the electropolymerized layer of PMG on electrodes' surfaces not only could help the absorption of the HOOC-MWCNTs via electrostatic interaction but also could reduce the over potential of NADH oxidation and oxygen reduction. It seems that all the mentioned factors in this procedure were contributed to obtain a BFC with higher power density relative to those reported in the literature.

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Figure Captions

Scheme 1. Principle of operation of the ethanol-oxygen biofuel cell using PDDA/ADH/PDDA/HOOC-MWCNTs/PMG/GC as bioanode and PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr as biocathode.

Fig. 1. The CVs of MG electropolymerization on Gr (A) and GC (B) electrodes. The electropolymerization was carried out in solution consisting of MG (4×10^{-3} M), $NaNO_3$ (0.1 M) and $Na_2B_4O_7$ (10^{-2} M) at the scan rate of 50 $mV.s^{-1}$. **a** to **j** stands for first to 10^{th} cycles.

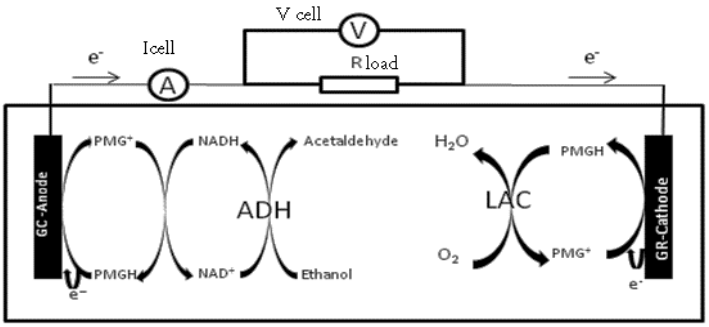
Fig. 2. CVs of modified electrodes: (a) PMG/Gr, (b) HOOC-MWCNTs/PMG/Gr and (c) PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr electrodes. The experiment was carried out in O_2 saturated PBS (0.1 M, pH 7.5) at scan rate of 50 mVs^{-1}.

Fig. 3. A: The CVs of PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr electrodes in N_2 saturated (a) and O_2 saturated (b) PBS (0.1 M, pH 7.5). B: CVs of PDDA/ADH/PDDA/HOOC-MWCNTs/PMG electrode in the absence (a) and presence (b) of NAD^+ (1 mM) and ethanol (1.6 μM). The scan rate was 50 $mV.s^{-1}$.

Fig. 4. Effect of pH on cathodic (a) and anodic (b) current response of PDDA/Lac/PDDA/HOOC-MWCNT/PMG/Gr electrode. The electrode was inserted in PBS (0.1 M) with different pHs and the CVs were recorded.

Fig. 5. OCV of BFC in O_2 saturated PBS (0.1 M, pH 7.5) in 1 mM ethanol and 1 mM NAD^+. The OCV was stable at 504 mV for at least 1 hour.

Fig. 6 Power and polarization curve of BFC at scan rate of 1 mVs^{-1}. The experimental conditions were the same as Fig 5. The maximum power density was 3980 $mW.Cm^{-2}$.



Poly methylene green = PMG Alcoholdehydrogenase = ADH
Laccase = LAC Nicotinamide adenine dinucleotide = NADH

Scheme 1

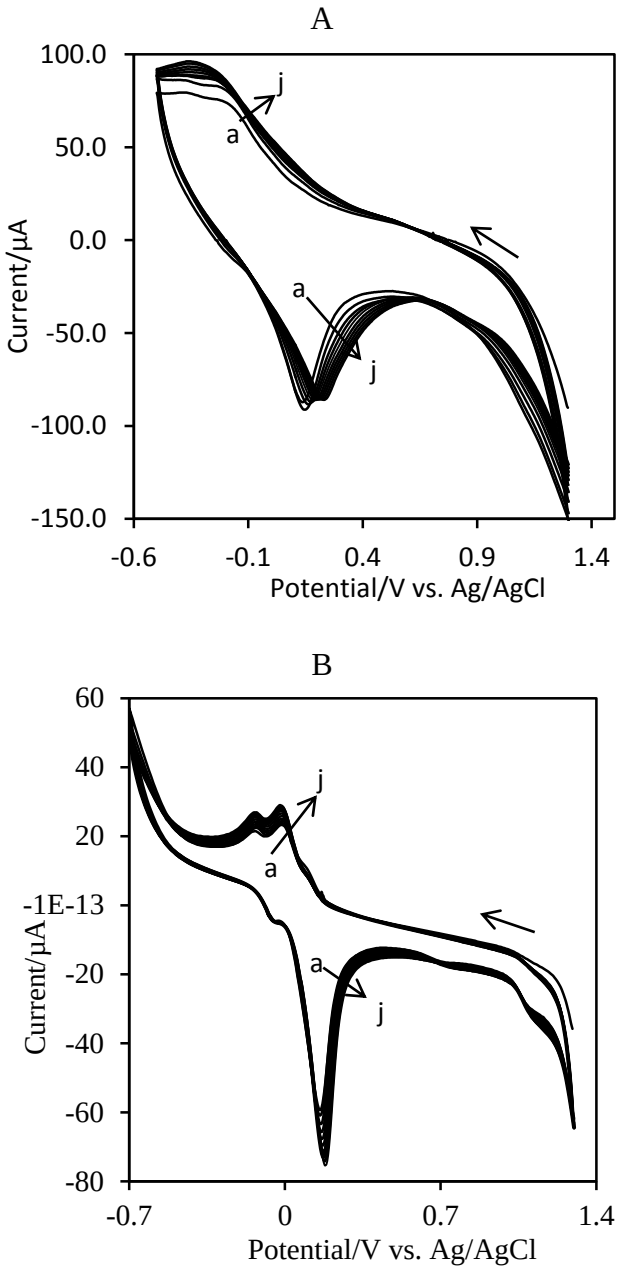


Fig. 1

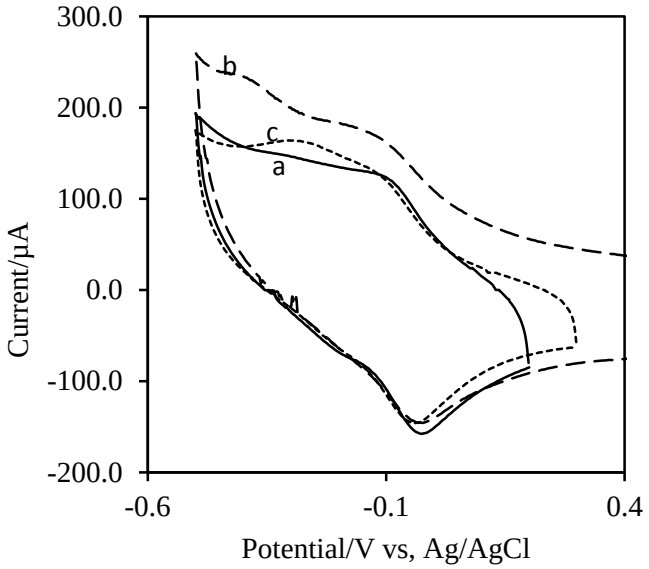


Fig. 2

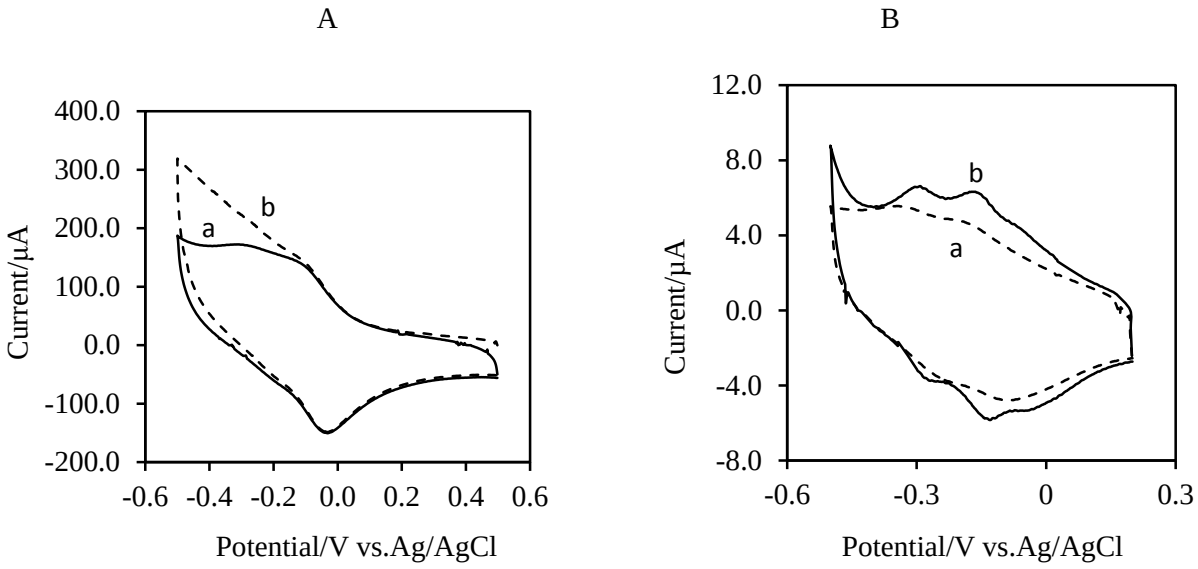


Fig. 3

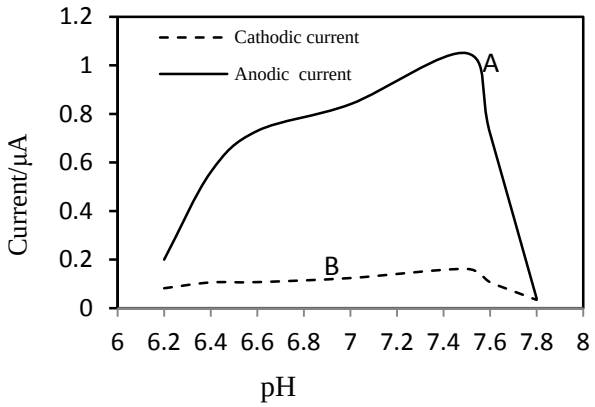


Fig. 4

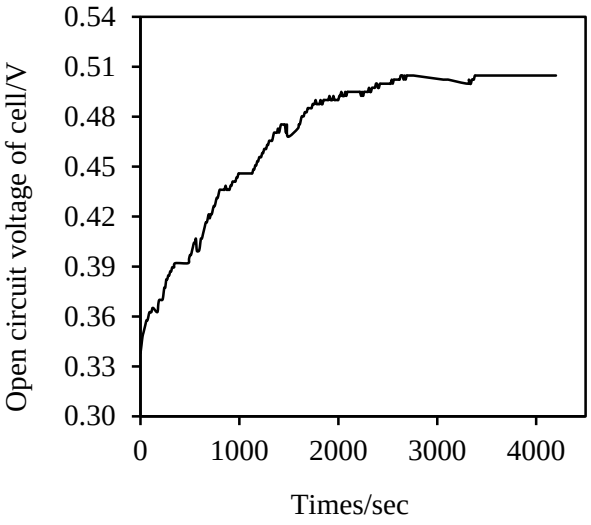


Fig. 5

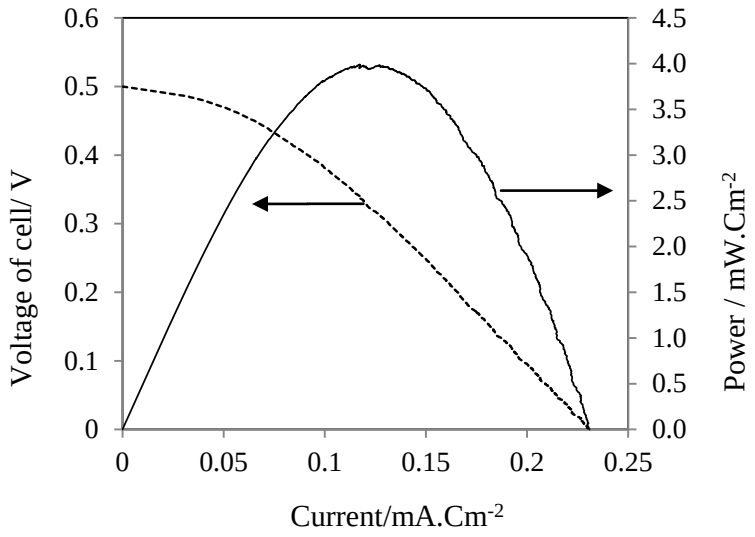


Fig. 6

Tables

Table 1- Comparison between the characteristics of different biofuel cell

Anode composition	Cathode composition	Concentration of enzymes on anode (mg/mL) / cathode (mg/mL)	Fuel/Oxidant	Power density ($\mu W.Cm^{-2}$)	Cell Voltage (V)	References
Os(N,N`-dialkylated-2,2`bi-imidazole)$_3$]$^{2+/3+}$ /GDH	Laccase/Os(N,N`-dialkylated-2,2`bi-imidazole)$_3$]$^{2+/3+}$	-/-	Glucose/O_2	4.3	0.52	87
PMG entrapped in MWCNTs/chitosan scaffold/ADH/ NADH	1-pyrenebutyric acid N-hydroxy succinimide ester/Laccase	5/4	Ethanol-NAD^+/O_2	~26	0.372	88
sulfonated (3-mercaptopropyl)-trimethoxysilane sol–gel/chitosan/Meldola's blue/gold nanoparticles/ADH	sulfonated (3-mercaptopropyl)-trimethoxysilane sol–gel/ chitosan/ gold nanoparticles/ Laccase	5/5	Ethanol-NAD^+/O_2	3210	0.68	89
PMG/COOH-MWCNT/PDDA/ADH/ PDDA	C-Pt	10/-	Ethanol-NAD^+/O_2	1713	0.281	Our non published data
PMG/COOH-MWCNT/PDDA/ADH/ PDDA	PMG/COOH-MWCNT/PDDA/Laccase/ PDDA	10/10	Ethanol-NAD^+/O_2	3980	0.504	Present work

Table.2 Comparison between different enzyme-modified electrodes

Modified electrode composition	**Usage of modified electrode**	**References**
GC/cellulose acetate/toluidine blue O/ADH/ glutaraldehyde/BSA	biosensor	58
Nafion/ADH–PDDA–SWNTs/GC	"	51
ADH/MWCT-Meldola blue/GC	"	90
Poly(nile blue A)/ADH/SWCNT/GC	"	91
Poly(6-acrylamidohexanoic acid)-Ruthenium complexes/PDDA/ADH	"	92
PDDA-modified CNTs/ADH/GC	"	93
Graphene/PDDA/ polystyrenesulphonate/ ADH	"	94
GC/HOOC-MWCNT/PDDA /Actylcholine esterase/PDDA	"	66
GC/HOOC-MWCNT/PDDA /choline oxidase/PDDA	"	67
PDDA/ADH or laccase/PDDA/HOOC-MWCNT/PMG/GC or Gr	Biofuel cell	Present work