

RESEARCH PAPER

## Effect of Support on Power Output of Ethanol/O<sub>2</sub> Biofuel Cell

Farideh Gouranlou<sup>1,\*</sup>, Hedayatollah Ghourchian<sup>2</sup>, Mahdi Kheirmand<sup>3</sup>, Abdollah Salimi<sup>4</sup>

<sup>1</sup> Department of Bioscience and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran

<sup>2</sup> Laboratory of Microanalysis, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

<sup>3</sup> Department of Chemistry, School of Basic Science, Yasouj University, Yasouj, Iran

<sup>4</sup> Department of Chemistry, Faculty of Science, University of Kurdistan, Sanandaj, Iran

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### ABSTRACT

Enzymatic biofuel cells have many great usages as a small power source for medical and environmental applications. In this paper, we employed carboxylated multiwall carbon nanotube- (1-ethyl-3-methylimidazolium bis (trifluoromethyl sulfonyl) imide) ionic liquid nanocomposite on two different electrodes (glassy carbon and carbon felt) for immobilizing alcohol dehydrogenase. The properties of the two types of electrodes were characterized by cyclic voltammetry analysis. Polarization analysis and field emission scanning electron microscopy were used to show differences in the nanobiocomposite immobilization on two electrodes. Compared to glassy carbon, carbon felt achieved much more gains in electrochemical activity and power by catalyst coating. Power density of 10.027 μWcm<sup>-2</sup>, has been achieved by carbon felt, but glassy carbon showed 1.7 μWcm<sup>-2</sup> respectively.

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### INTRODUCTION

Biofuel cells (BFCs) are interesting energy conversion devices that use biocatalysts (either living cells or enzymes) to convert the chemical energy into electrical energy without pollution for environment. Because of higher catalytic activity of enzymes in comparison with microbes, these types of BFCs are noticed [1–9]. BFCs that use glucose and oxygen dissolved in body fluids as fuel and oxidant, respectively can serve as power source for implanted devices such as, pacemakers, drugs dispensers, and sensors [10]. They can operate at room temperature and at neutral pH. BFCs that could also be made in membraneless model can be easily miniaturized and, so, this allows the using of oxidant and fuel from the surrounding environment [11]. Some BFCs problems as, inadequate stability of the modified electrodes, transport limitations of the substrates and products

of redox reactions(not efficient direct electron transfer due to large distance between the electrode surface and the active center of the enzyme) and limited number of redox enzymes, are must be solved. For some redox reactions catalyzed by enzymes, electron transfer accelerators as carbon nanotube are usually used to facilitate electron transfer between the electrode and the active site of the redox center, that hidden inside the protein in the hydrophobic pocket arranged by amino-acid residues [11]. Immobilization of enzymes on electrode surface and modifying the electrode surface can easily improve the electron transferring too. Different ways were reported on literatures [12]. Recent studies have showed that a number of room temperature ionic liquids (RTILs), have good dissolution power for some materials [9, 10]. In many experiments, some reaction was done in ionic liquid (IL) to have better results as done by

\* Corresponding Author Email: [fgouranlou@alumni.ut.ac.ir](mailto:fgouranlou@alumni.ut.ac.ir)



Lozano *et al.* [13]. He showed that efficiency of reactions as trans esterification of vinyl butyrate with 1-butanol and the kinetic resolution (KR) of *rac*-1-phenylethanol with vinyl propionate in [emim][Tf<sub>2</sub>N]/scCO<sub>2</sub> and [bmim][Tf<sub>2</sub>N]/scCO<sub>2</sub> systems catalyzed by lipase at 100 °C and 15 MP<sub>a</sub> was above 99.9%, and the enzyme showed good activity and stability. The same group has tested the catalytic activity and stability of both free lipase and immobilized lipase in IL/scCO<sub>2</sub> systems composed of [emim][Tf<sub>2</sub>N] and [bmim][Tf<sub>2</sub>N] for the continuous KR of *rac*-1-phenylethanol [14]. The results showed that the enzyme shows excellent activity and stability under extremely denaturing conditions (*e.g.*, 150 °C and 10 MPa). [emim][CH<sub>3</sub>COO] has also been reported to be an enzyme-friendly solvent for organic reactions [11]. Turner *et al.* have developed cellulose-RTIL composite materials for the immobilization of laccase with the retention of catalytic activity using [bmim] Cl [15]. Due to their, carbon nanotubes (CNTs) have great physicochemical properties so they are attracting scientists attentions for the development of bioelectrochemical devices. However, the poor dissolving ability of carbon nanotubes has traditionally been a huge technical barrier to their application. Some researchers have shown that RTILs help in the dispersion of carbon nanotubes and impart useful properties in their application in bioelectronics [16-21]. Bare and modified electrodes made-up various materials used for creation “enzyme electrodes” as bioanodes for BFC construction. Also, many different electrodes have been used as bioanode or biocathodes, such as glassy carbon(GC), graphite (hard disc or felt), carbon fibers and carbon felt(Cfelt) [22- 27]. An electrode with larger surface area helps a higher enzyme loading and can also improve the stability of the immobilized enzyme; critical feature is high enzyme load to obtain high-performance enzymatic BFCs, vice versa. As an advantage, co-immobilization of the enzyme and nanostructures materials can also aid DET. As many enzymatic fuel cells, the main components of BFCs are electrodes, that classified to coarse two-dimensional (2D) and three-dimensional (3D) type, which corresponds to different BFC applications. Cfelt is usually used as 3D electrode, while carbon cloth, black carbon and GC can only be employed as 2D electrode. In BFC, Cfelt [28–30], black carbon [31] and GC [32] are the main electrode materials for the

aqueous ethanol/acetaldehyde couple and can be used for anode and cathode preparation. In the mid-1950s, Bernard Redfern discovered glassy carbon, in the laboratories of The Carborundum Company, Manchester, UK [33]. Obviously, the 2D and 3D electrode materials change much BFC performance. The difference between the two types of materials should be because of their various structural characteristics. On one hand, the specific surface area of GC is higher than that of Cfelt. On the other hand, the surface area of GC has pores in nanometer grade, while Cfelt in micron grade. The difference in physical structures changes their application performances. In this paper, Cfelt and GC based electrodes were compared to study the different BFCs application characteristics of the two types of materials. In order to evaluation, the electrodes (GC and Cfelt) modified by [emimTf<sub>2</sub>N], carboxylated multiwall carbon nanotubes (HOOC-MWNTs) and alcohol dehydrogenase (ADH) to make bioanodes. RTIL was physically attached to the sides and ends of HOOC-MWNTs and acted as an environment for enzyme and facilitate electron transferring; in addition it significantly improved the catalytic efficiency of the modified bioanode and prepare simple way to immobilize enzyme in a (HOOC-MWCNT) matrix reconstituted by [emimTf<sub>2</sub>N], and on the subsequent use of this matrix to modify bioanode with ADH [ADH/emim Tf<sub>2</sub>N/HOOCMWCNT]. The behavior of two modified electrodes as bioanode in biofuel cell was investigated by electrochemical techniques.

## EXPERIMENTALS

### Reagents and Solutions

Alcohol dehydrogenase, (>300 Units mg<sup>-1</sup>), nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and emimTf<sub>2</sub>N, were obtained from Sigma- Aldrich chemical company. Potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>), dimethylformamide (DMF), potassium phosphate monobasic monohydrate (KH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O), sodium tetraborate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>), sodium nitrate (NaNO<sub>3</sub>), ethanol (EtOH) were obtained from Merck. HOOC-MWCNTs (Content of -COOH: 0.49 wt %, Outside diameter: 50- 80 nm, Inside diameter: 5- 15 nm, Length: 10- 20 nm, and > 95% purity) were attained from US Research Nanomaterials Inc. All other chemicals were analytical grade and used without further purification. All solutions were prepared using distilled water.

### Instrumentation

All voltammetric measurements were carried out using a modified GC and Cfelt electrode as working electrodes, an Ag/AgCl reference electrode and a platinum rod auxiliary electrode. Cyclic voltammetry (CV) and polarization experiments were carried out using a Potentiostat/ Galvanostat EG&G 263A (Princeton, The USA). All the electrochemical measurements were carried out at room temperature ( $25 \pm 1$ ) and pH was measured using a pH electrode coupled to a Metrohm model 691 pH meter. Surface morphological studies were carried out using Hitachi S4160 field emission scanning electron microscope (FESEM) with 10- 20 nm gold deposition layer thickness and 30 kV voltages.

### Modification of electrodes

#### Fabrication of ADH/emim Tf<sub>2</sub>N/HOOC-MWCNT on glassy carbon electrode

GC (3mm diameter) was polished using alumina slurries and rinsed thoroughly with distilled water. The GC was cleaned by ultrasonic agitation for 5 min in ethanol and then washed with distilled water. The electrode was then dried using a stream of air. A stock solution of  $1 \text{ mg mL}^{-1}$  HOOC-MWCNTs-DMF was prepared by dispersing 1 mg of HOOC-MWCNTs in 1 mL DMF.  $2 \mu\text{L}$  of HOOC-MWCNTs-DMF solution was cast on the GC surface. The electrode was dried at room temperature to obtain HOOC-MWCNTs/GC. Then modified electrode was prepared by capping in pure emim Tf<sub>2</sub>N for 10 hours. After 10 hours, the modified electrode was washed with PBS pH 7.5 and then capped in solution of ADH (10 mg/mL) in 4°C for 10 hours. The ADH/ emim Tf<sub>2</sub>N/HOOC-MWCNT/GC then rinsed with PBS pH 7.5 and stored in 4 °C (Fig. 1).

#### Fabrication of ADH/emim Tf<sub>2</sub>N/HOOC-MWCNT on Cfelt electrode

Cfelt ( $1 \times 1 \text{ cm}^2$ ) was rinsed thoroughly with ethanol and then distilled water. The electrode was then dried using a stream of air. A stock solution of  $1 \text{ mg mL}^{-1}$  HOOC-MWCNTs-DMF was prepared by dispersing 1 mg of HOOC-MWCNTs in 1 mL dimethylformamide (DMF). The Cfelt was dipped in HOOC-MWCNTs-DMF solution. The electrode was dried at room temperature to obtain HOOC-MWCNTs/Cfelt. Then modified electrode was prepared by dipping in pure emim Tf<sub>2</sub>N for 10 hours. After 10 hours, the modified electrode was rinsed with PBS pH 7.5 and dried in air stream

then dipped in solution of ADH (10 mg/mL) in 4°C for 10 hours. The ADH/emimTf<sub>2</sub>N/HOOC-MWCNT/Cfelt then rinsed with PBS pH 7.5 and stored in 4 °C.

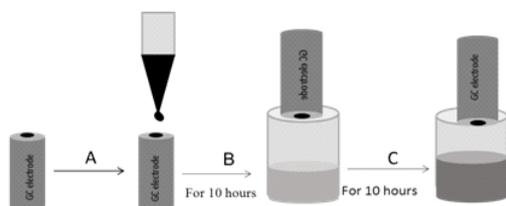
### BFC construction

As we explained in previous work [34], for assembling an ethanol/O<sub>2</sub> biofuel cell, the prepared bioanodes and the C/Pt cathode were placed into a 1mL cell containing 1mM ethanol and 1mM NAD<sup>+</sup> in oxygen saturated phosphate solution (0.10 M, pH 7.5) under ambient air.

Fig. 2 shows the cell assembly consisting of the Pt/C cathode and bioanode. Polarization curves were constructed for the complete biofuel cell. The biofuel cell consisted of two round-fabricated Plexiglas cabinets. The cathode and anode was in the discrete cabinets that were attached to each other membrane less. For connecting the Cfelt bioanode, the graphite rod was used.

## RESULT AND DISCUSSION

Via direct adsorption on surface of composite, ADH was immobilized onto the emim Tf<sub>2</sub>N/HOOC-MWCNT composite. In the immobilization step, first of all, HOOC-MWCNT coated on GC or Cfelt, which then dropped by emim Tf<sub>2</sub>N on the surface of the HOOC-MWCNT. The negative



A = Dropping  $2 \mu\text{L}$  of HOOC-MWCNT  $2 \text{ mg mL}^{-1}$

B = Capping of modified electrode with emim Tf<sub>2</sub>N

C = Capping of modified with Alcohol dehydrogenase

Fig. 1: modification of bioanode ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/GC



Fig. 2: Representation of BFC whole cell.

and positive parts of emim Tf<sub>2</sub>N adsorb directly with couple of them on charged surface of enzyme and HOOC-MWCNT then act as bridge between enzyme molecules and the HOOC-MWCNT. The resulting electrodes were examined in the biofuel cell as illustrated in Fig. 2. In the anode, NAD<sup>+</sup> cofactor was used to make possible the oxidation of ethanol by ADH. At the active sites of the ADH, NAD<sup>+</sup> accepts H atom from the ethanol and is reduced to NADH. NADH then diffuses to the surface of ADH where it is oxidized back to NAD<sup>+</sup> [35]. H<sup>+</sup> and electron are generated from this process. Protons are transferred to the buffer solution and finally reach cathode through the electrolyte. Electrons pass through the MWCNTs network, first to the backing electrode and finally reach cathode via external circuit. At the catalyst layer of the cathode (Pt/C), oxygen reacts with protons and electrons and water is generated.

Because this study was focused on the enzymatic anode, the influence of cathode polarization (potential drop with current density) on the biofuel cell performance was minimized by using a small anode (3 mm in diameter, surface area of 0.07 cm<sup>2</sup>) and a much larger cathode (surface area of 1 cm<sup>2</sup>).

*Electrochemical Properties of ADH/emim Tf<sub>2</sub>N / HOOC-MWCNT/GC or Cfelt electrodes*

The composite electrodes were typically prepared by casting solution containing ADH/emim Tf<sub>2</sub>N / HOOC-MWCNT onto backing materials such as glassy carbon and carbon felt. In order to establish successful modifying electrodes, each step of electrodes modifying was investigated by CV. As shown in Figs. 3A and 3B (in 0.1 M PBS (pH 7.5) at a scan rate of 50 mV s<sup>-1</sup> in the potential range from 0.5 to -0.5 V, composite-coated electrode showed higher peak height (enhancing electrochemical

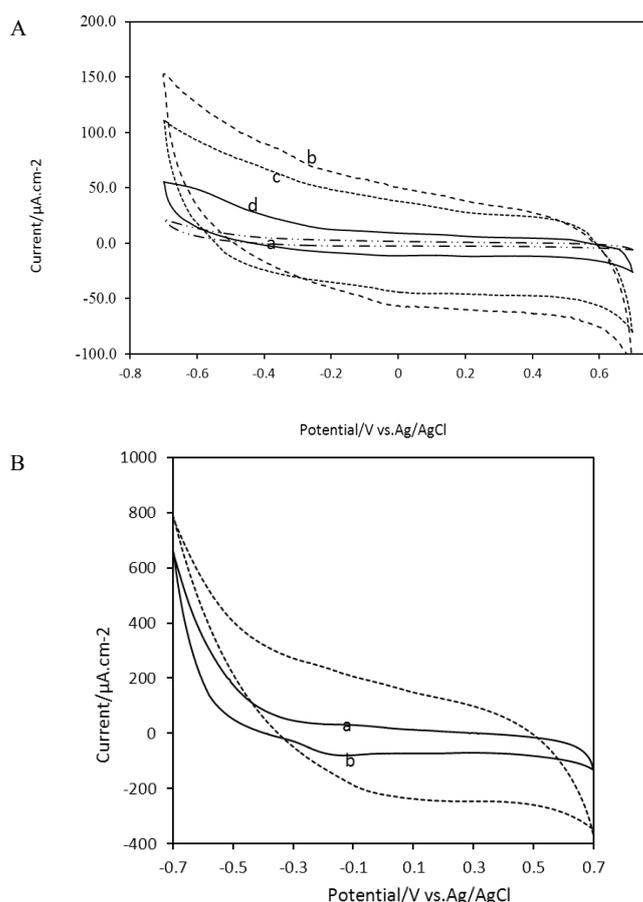


Fig. 3: (A) The CVs of naked GC (a), HOOC-MWCNT/GC (b), emim Tf<sub>2</sub>N /HOOC-MWCNT/GC (c), ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/GC (d) electrodes. (B) The CVs of naked Cfelt (a), ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/Cfelt (b) electrodes. The experiment was carried out in PBS (0.1 M, pH 7.5) at scan rate of 50 mV.s<sup>-1</sup>.

current) than the bare GC and Cfelt. The enhanced electrochemical current was most likely due to the larger surface area as a result of the use of HOOC-MWCNT. Only differing of intensity shows that immobilization steps are successful. The Fig. 3 (B) shows the same results for modifying Cfelt electrode because of increasing the intensity of CVs. Ethanol/Acetaldehyde redox couple typically displays quasi-reversible to irreversible behaviors on electrodes such as Pt, Au and glassy carbon and pyrolytic graphite electrodes [36]. Similar to graphite, HOOC-MWCNT-modified electrode surface also has a highly organized structure. That might contribute to the enzyme immobilization improvement. The effectiveness of emim Tf<sub>2</sub>N / HOOC-MWCNT was first examined by CV in the presence of NAD<sup>+</sup> and ethanol (data not shown). The data are agreed with Torabi Kachooangi results [37].

*FESEM characterization of ADH/emim Tf<sub>2</sub>N / HOOC-MWCNT/GC and ADH/emim Tf<sub>2</sub>N / HOOC-MWCNT/Cfelt electrodes*

Figs. 4(A) and 4(B) show the FESEM images of nanocomposite of ADH/emim Tf<sub>2</sub>N / HOOC-MWCNT/GC on GC and Cfelt respectively. As can be seen, in Fig. (4A, a), the surface of GC is bare and uniform. By dropping HOOC-MWCNT on GC surface (Fig. 4A, b) and adding [emim] Tf<sub>2</sub>N, the HOOC-MWCNT diameters increase greatly (As seen in Fig.4A, c). It is clear that [emim]Tf<sub>2</sub>N improves the dispersion of HOOC-MWCNTs, because the positive charged of emim could attract on the sidewall surface of HOOC-MWCNTs, resulting emim Tf<sub>2</sub>N /HOOC-MWCNTs /GC electrode. From Fig. 3A, d, it can be seen that after immersing in the ADH solution the hybrid film on modified electrode is still uniform and indicates the coverage of modified electrode surface with enzyme.

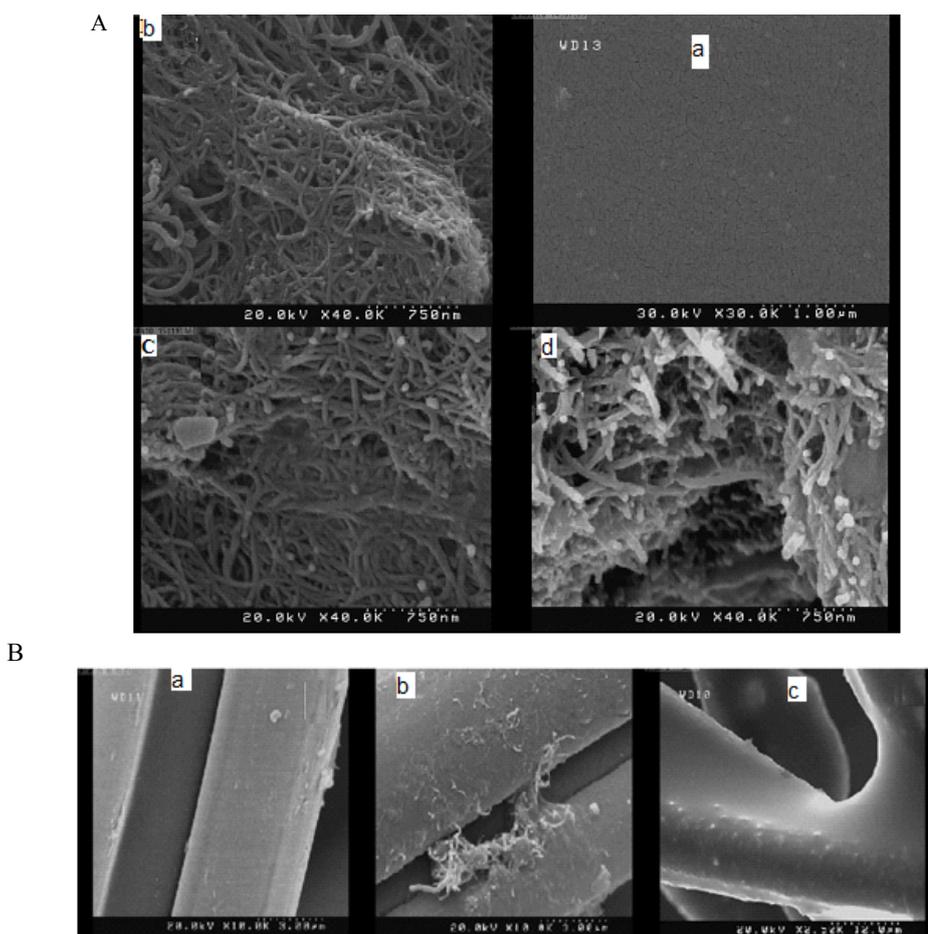


Fig. 4: (A) FESEM images of (a) bare GC, (b) HOOC-MWCNT/GC; (c) emim Tf<sub>2</sub>N /HOOC-MWCNT/GC and (d) ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/GC. (B) FESEM images of (a) bare Cfelt, (b) HOOC-MWCNT/Cfelt and (c) ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/Cfelt electrodes.

[emim] Tf<sub>2</sub>N adsorbs the enzymes via  $\pi$  interaction (Fig.4 A, d). Fig. 4(B) show the FESEM modification steps of Cfelt by ADH/emimTf<sub>2</sub>N/HOOC-MWCNTs. Comparison between the images of bare Cfelt (Fig. 4B, a) and emimTf<sub>2</sub>N/HOOC-MWCNTs/Cfelt (Fig. 4B, b) indicates the coverage of bare electrode surface with emimTf<sub>2</sub>N/HOOC-MWCNT nanocomposite. Since the isoelectric point of ADH is 6.8 therefore, in PBS (pH 7.5) it contains negative charges which can electrostatically interact with emim Tf<sub>2</sub>N and forms ADH/emim Tf<sub>2</sub>N/HOOC-MWCNTs nanocomposites. By immobilizing ADH the uniform film of enzyme is seen on CNTs (Fig. 4B, d).

#### Performance of the membrane less ethanol/O<sub>2</sub> biofuel cell assembly

By immobilization of ADH, without mediator, the enzyme oxidizes substrates and transfer electrons to electrode surface,; it is DET mechanism [38, 39]. Carrying out DET of ADH was established by cyclic voltammetry experiments that embedded in ADH/emimTf<sub>2</sub>N/HOOC-MWCNT/GC nanocomposite as bioanode in presence and absence of substrate (ethanol) (data not shown). After adding ethanol stepwise, the more current is raised and it showed that ADH has retained good activity toward ethanol in this nanocomposite. We saw that the ADH in nanocomposite emimTf<sub>2</sub>N/HOOC-MWCNT was able to provide electrical contact with GC or Cfelt surfaces. The GC surface is hard in compare with Cfelt electrode, so it can provide better electrical contact than Cfelt electrode. Tobalina et al showed that Cfelt is good electrode for immobilizing ADH [40], but it is not sufficient in fabricating bioanode for biofuel cell. Based on researchers' claim, the

ionic liquids can provide good environment for enzymes and stabilize them in different temperature and prevent of denaturation [41, 42]. Also, the ionic liquid makes an environment for successful DET too. These results clearly reveal that the electrocatalytic oxidation of NADH to NAD<sup>+</sup> takes place efficiently via an electron transfer mediation of the molecular assembly (emimTf<sub>2</sub>N/HOOC-MWCNTs, composed of emimTf<sub>2</sub>N as supporting material and functionalized MWCNTs as electron transfer mediator) and that the ADH immobilized on this nanocomposite, acts as a dehydrogenase enzyme for ethanol. An ethanol/O<sub>2</sub> biofuel cell was constructed by assembling the prepared ADH/emimTf<sub>2</sub>N/HOOC-MWCNTs/ GC or Cfelt as bioanodes and the Pt/C as cathode. The cell voltage ( $V_{cell}$ ) was measured by potentiostate in V-I curve (polarization curve) and power output density ( $P_{cell}$ ) was calculated as a function of the cell current density,  $j_{cell}$  and  $V_{cell}$  given by  $j_{cell} \times V_{cell}$  was determined. The polarization curve and the relationship between  $P_{cell}$  and  $j_{cell}$  for the ethanol/O<sub>2</sub> biofuel cell (Figs. 5 A and B), in which the ADH/emimTf<sub>2</sub>N/HOOC-MWCNTs/GC or Cfelt bioanodes and the Pt/C cathode were immersed in O<sub>2</sub> saturated PBS (0.1M, pH 7.5) containing 1 mM NAD<sup>+</sup> and 1 mM ethanol. The open circuit voltage (OCV) was found to be 0.6 V and the maximum power density ( $P_{max\ cell}$ ) of BFC was found about 1.73  $\mu\text{Wcm}^{-2}$  for ADH/emimTf<sub>2</sub>N/HOOC-MWCNTs/GC bioanode but the OCV of ADH/emimTf<sub>2</sub>N/HOOC-MWCNT/Cfelt was 360 mV bioanode and maximum power density of BFC was about 10.02  $\mu\text{Wcm}^{-2}$ . The comparison of P-V and I-V curves (Fig.5) for BFCs using each of two bioanodes showed that power density and current density of

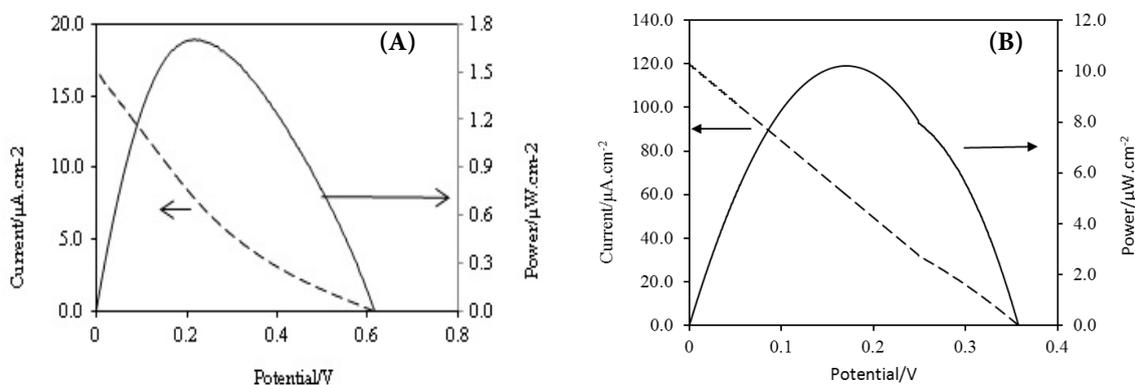


Fig. 5: Polarization curve (dotted line) and power curve (solid line) obtained by the ethanol/O<sub>2</sub> biofuel cell with ADH/emim Tf<sub>2</sub>N /HOOC-MWCNT/GC or Cfelt bioanodes (A and B). The experiments were measured in oxygen saturated PBS (0.1 M, pH 7.5) containing 1 mM ethanol and 1 mM NAD<sup>+</sup>.

Table 1: Comparison between two electrodes that used as bioanode

Bioanode Composition	Power density ( $\mu\text{W}\cdot\text{cm}^{-2}$ )	OCV (mV)	References
ADH/emimTf <sub>2</sub> N/HOOC-MWCNT/GC	1.71	600	-
ADH/emimTf <sub>2</sub> N/HOOC-MWCNT/Cfelt	10.02	360	-
GOx/MWCNTs/Nafion/Cfelt	58	353	46
CDH / Graphite	5	500	47
CDH/Os-redox polymer /Graphite	1.9	600	44

ADH/emimTf<sub>2</sub>N/HOOC-MWCNT/GC or Cfelt is bigger than BFC using other bioanodes [43-45]. The Cfelt is better electrode for construction bioanode in biofuel cell than GC electrode because of more adsorption capacity of ADH and ionic liquid, besides the good effect of environmental and electrical of CNTs and ionic liquid. We assume the larger power density is because of good electron transferring of sufficient contacts of ADH in nanocomposite ADH/emimTf<sub>2</sub>N/HOOC-MWCNT with electrode surface when Cfelt is used.

## CONCLUSION

Enzymatic biofuel cells based on single NAD<sup>+</sup> dependent dehydrogenase enzyme, emimTf<sub>2</sub>N and carboxylated carbon nanotube on different electrodes (GC and Cfelt) were constructed and evaluated in continuous operation. Some limitations in the anode performance that were reflected in maximum power densities approaching  $\sim 1.73 \mu\text{W}\cdot\text{cm}^{-2}$  (at 0.6 V) for GC bioanode in BFC. Improvements in design have been suggested including the utilization of electrode materials that will allow facilitated electron transferring in anode, thereby reducing overall ohmic losses in the biofuel cell. With this paper, we integrate the electrode material efforts in both enzymatic electrodes that are product of various projects and collaborations. The direction of our research is to move towards an active electron transport in anode that can be combined with custom cathode. The electrode materials in enzymatic electrodes have a significant effect on the performance of microfluidic fuel cells. In this work, two electrode materials (GC and Cfelt) were projected in which the enzyme was immobilized on two different electrode surfaces. The carbon felt electrode showed the higher power density ( $10.02 \mu\text{W}\cdot\text{cm}^{-2}$ ) herein reported. This performance, compared with the other electrode materials, was related to an enhancement in the electron transfer probably because of the short distance between them which allowed a better conduction of charge from both electrooxidation reactions. Also, the order of magnitude

(microwatts) of the cell performance was achieved with the use of three dimensional electrodes. In addition, direct adsorption of the enzyme on the surface of a GC or Cfelt electrode based on emim Tf<sub>2</sub>N, HOOC-MWCNT was used to enhance the stability of the BFC. As seen in Table 1, the nanocomposite emim Tf<sub>2</sub>N /HOOC-MWCNT can provide good environment for immobilizing ADH and in comparison with other BFCs can produce more power output. As shown in Table 1, the Cfelt electrode has better power density in comparison with GC electrode as bioanode in BFC.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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