

## Design and Fabrication of Glucose/O<sub>2</sub> Enzymatic Biofuel Cell

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(Received 6 September 2016, Accepted 29 December 2016)

### ABSTRACT

Enzyme-based biofuel cells (EBFCs) are systems that use a variety of organic compounds to produce electricity through oxidoreductase enzymes, such as oxidase or dehydrogenase as biocatalysts immobilized on electrodes. In this study, a single-chamber EBFC consisting of carbon electrodes that operating at ambient temperature in phosphate buffer, pH 7 is reported. The EBFC anode was based on glucose oxidase (GOx) enzyme from *Aspergillus niger* immobilized on carbon fiber electrode based on carbon nanotube (CNT) and Nafion. The cathode was air-breathing based on Pt/C. The GOx/CNT/Nafion-based bioanode and air-breathing cathode were combined into a functional EBFC. The operation of EBFC was carefully investigated by use of glucose/O<sub>2</sub> as substrate and oxidant, respectively. The maximum power delivered by the assembled biofuel cell in stationary state, reached 106.4  $\mu\text{W cm}^{-2}$  at 0.45 V with 0.2 M glucose at 25 °C. Also the best Results of the fabricated cell show that present biofuel cell could be used as a power source to generate electricity for small power bioelectronic devices.

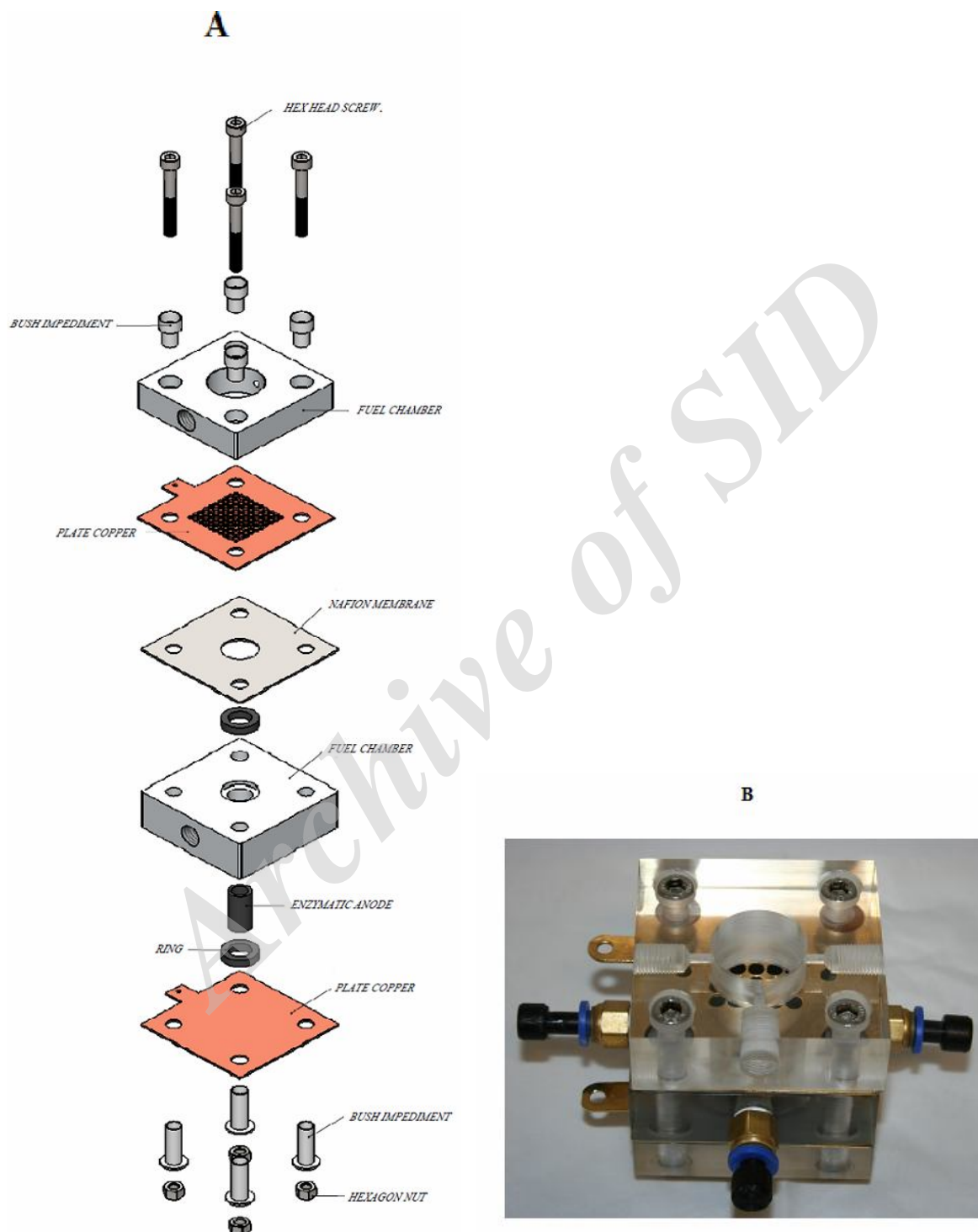
**Keywords:** Enzymatic biofuel cell, Glucose oxidase, Glucose/O<sub>2</sub>, Carbon nanotube, Nafion

### INTRODUCTION

Biofuel cell is a bio-electronic device which converts biochemical energy into electrical energy using biocatalysts such as enzymes, microorganisms, and organelles. Enzymatic biofuel cells (EBFCs) can employ enzymes to catalyze chemical reactions instead of inorganic catalysts in conventional fuel cells. In recent years, research interests in energy harvesting device like EBFC have been increased. Reasons for EBFC interest are based on unique conditions of operation under moderate condition (20-40 °C, near physiological pH) without environmental pollution [1,2]. Due to good operation properties, EBFC can be an attractive prospect for use in applications that need high temperatures or harsh reaction conditions. Furthermore, enzymes have considerable advantages rather than conventional catalysts, such as biocompatibility, higher performance, higher activity and higher specific selectivity [3,4]. In EBFC, enzymes are used as a catalyst at the cathode, anode or at both electrodes. They are traditionally sugar or alcohol

oxidizing enzymes at the anode such as glucose oxidase (GOx), alcohol dehydrogenase, fructose dehydrogenase or cellobiose dehydrogenase and oxidases such as laccase or bilirubin oxidase (BOx) at the cathode. An example of a conventional biofuel cell is based on GOx ( $\beta$ -D-glucose: oxygen 1-oxidoreductase; EC 1.1.3.4, 160 kDa molecular weight, *Aspergillus niger*) at the anode that has been widely surveyed as one of the best applicants for glucose-based biofuel cells. There are many specific attentions in the development of glucose EBFCs for use in implantable power sources and other power generating devices [5,6]. Also, considerable progresses in EBFC applications have been made. In the case of EBFCs, we should improve the bioelectrode operations with regard to function and stability of enzymes and electrode materials. One of the EBFC problems is electron transfer at the electrode surfaces. By improving the electron transferring, long-term operation and power output of EBFCs could be improved [7]. Commonly, electron transferring in EBFCs can be categorized into two mechanisms: mediated electron transfer (MET) and direct electron transfer (DET). Recently, researches have been paid attention on carrying out direct electrical communication between redox centers and electrodes,

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**Fig. 1.** (A) Schematic of EBFC assembly. (B) Photograph of a single-chamber EBFC that used in this project.

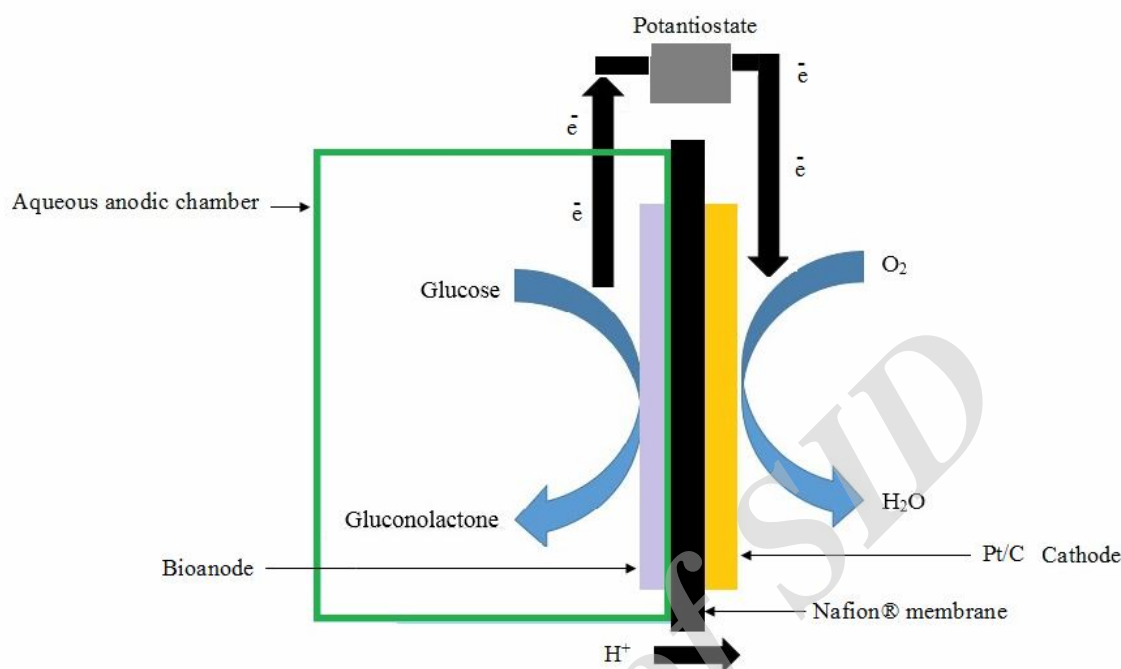


Fig. 2. Schematic of electron transfer in this enzymatic biofuel cell.

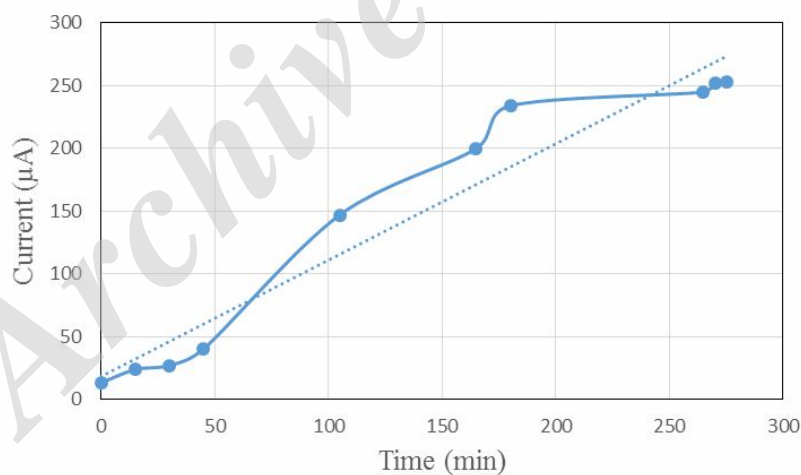


Fig. 3. The current-time curves of EBFC as a function of operating time at 0.2 M glucose, pH 7, 25 °C.

because mediators also have some stability and toxicity issues [1,8]. Some important advancement in DET has been performed by use of carbon nanomaterials including carbon nanotubes (CNTs) with large surface area. The usage of carbon nanotubes for extending the special area of the electrode will increase current density of EBFC and cause

considerable progress in this field. These materials also make easy the effective association of biological and electronic ingredients. Recent studies show the application of carbon nanotube in design of enzymatic fuel cells like glucose oxidase based biofuel cell [2,9]. Nafion with good physical characteristics also can be used to immobilize

enzyme and participate in EBFC by three different methods: In first method, enzymes could be connected to the electrode surface that is modified by nafion. The problem of this approach usually is decrease in enzyme activity and exposing of enzyme to the chemical environment. The second approach for enzyme immobilization is trapping of enzyme between nafion and electrode surface. This method will be done by placing enzyme on the electrode surface prior to deposition of nafion. This is a good way to enzyme immobilization, but enzyme can't distribute uniformly within nafion. The third method is trapping enzyme in nafion membrane. This method has been shown the most effective enzyme immobilization method [10,11]. This paper describes the design and fabrication of one assembled EBFC for first time in Iran. Also, it presents the development of a DET biofuel cell with GOx as the anodic enzyme that is immobilized on the carbon electrode by using CNT and nafion with an air-breathing cathode for the direct reduction of oxygen.

## MATERIALS AND METHODS

### Materials

Glucose oxidase (GOx) from *Aspergillus niger* (EC 1.1.3.4), ( $\beta$ -D) glucose, H<sub>2</sub>SO<sub>4</sub> 98%, H<sub>2</sub>O<sub>2</sub> 30%, DMF (dimethylformamide), Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, nafion 5% (Nafion®), were purchased from Sigma-Aldrich (U.S.A). MWCNT was purchased from nanotimes (China). Stock solutions of glucose were prepared in water and refrigerated at 4 °C when not in use. All solutions were prepared with deionized water passed through a purification system.

### Preparation of Enzymatic Anode

Carbon fiber electrode was polished successively on alumina grades of 0.3  $\mu$ m, and washed thoroughly with deionized water after each polishing step. MWCNT solution was prepared by dissolving in DMF (dimethylformamide) solution until the final concentration of the MWCNT was 10 mg ml<sup>-1</sup>. Anodic electrode was prepared by a two-step procedure: first, 200  $\mu$ l of a mixing nanotube/GOx (1:1 ratio) was cast on top of a polished carbon fiber electrode and allowed to dry at room temperature. The GOx

solution was prepared with concentration of 1 mg ml<sup>-1</sup> in 0.1 M phosphate buffer (pH 7). Second, 100  $\mu$ l of Nafion solution was deposited on top of a nanotube/GOx coated carbon fiber electrode and allowed to dry at room temperature [12].

### Preparation of Air-breathing Cathode

The Pt/C air cathode consisting of platinum 20% on carbon black (Vulcan XC-72) hot pressed to the Nafion® membrane. The cathodic side of air-breathing cathode was in direct contact with air [13,14].

### EBFC Construction and Operation

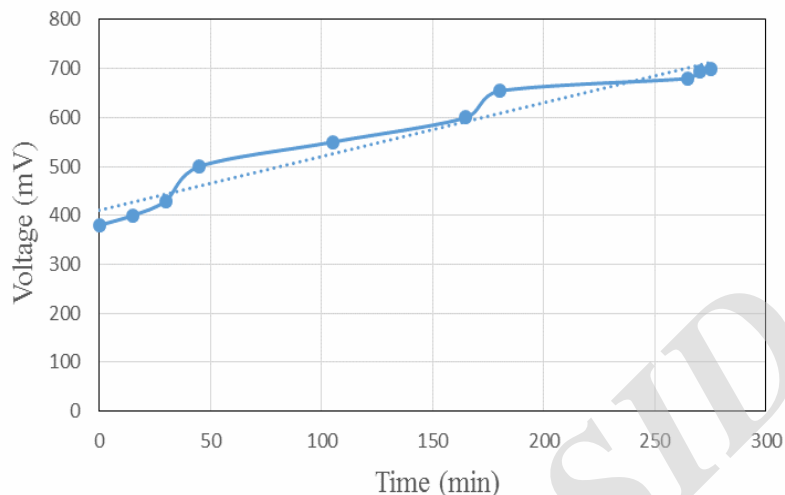
A one-compartment EBFC with anode spacing and volumes of 6 ml was constructed using plastic (Plexiglas) cylindrical chamber as the main body. In non-compartmentalized cell a Nafion® 212 proton exchange membrane was employed to separate the aqueous anodic chamber and the air-breathing cathode. The cell compartment (6 ml) was filled with 0.1 M phosphate buffer solution and 0.2 M glucose. The constructed EBFC is shown in Fig. 1. The electrons from the oxidation of glucose are passed from GOx to the MWCNTs until they reach the electrode surface. Then, through external circuit, the electrons transfer to air-breathing cathode, where the reduction of oxygen to water occurs. Figure 2 shows a schematic diagram of the electron transferring path in this biofuel cell.

### Analysis Methods

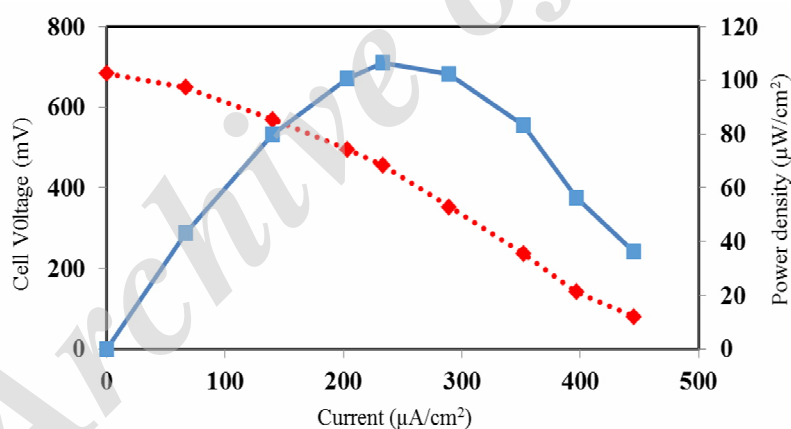
The performance of mentioned EBFC was evaluated at room temperature in pH 7 aqueous solution. The background electrolyte was 0.1 M phosphate buffered saline (PBS) and the temperature was maintained at 25  $\pm$  1 °C. Glucose (0.2 M) was used as the substrate. Current of EBFC was measured at various operation times by using Hioki 3256-50 Digital Multimeter. Voltage (V) of cell was recorded using Multimeter mentioned above, and used to calculate the power of biofuel cell too.

## RESULTS AND DISCUSSION

Generally, in biofuel cell experiments, evaluation of device is considered in respect to power output of system



**Fig. 4.** The voltage-time curves of EBFC as a function of operating time at 0.2 M glucose, pH 7, 25 °C.



**Fig. 5.** Power output (dotted line) and polarization curve (solid line) of cell in aqueous solution (pH 7) containing 0.2 M glucose at room temperature (25 °C).

[1]. Therefore, the voltage output and current of the complete single-chamber air-cathode EBFC were evaluated. Figure 3 shows the current-time curves of EBFC as a function of operating time. Figure 4 shows the voltage-time curves of EBFC as a function of operating time. Figure 5 shows power output and polarization curve of cell in aqueous solution. In all of them the pH and temperature are 7 and 25 °C, respectively and glucose concentration is 0.2 M.

## CONCLUSIONS

In this study, a single-chamber glucose oxidase-based biofuel cell was designed and fabricated. Basic enzyme-immobilization method is applied for the construction of cell. The biofuel cell is able to produce an electrical current in the presence of glucose as a substrate. Then, the performance of the air-cathode EBFC was investigated successfully. EBFC exhibited long term-stability for 4 days

without adding substrate. The maximum power delivered by the assembled biofuel cell in stationary state, reached  $106.4 \mu\text{W cm}^{-2}$  at 0.45 V with 0.2 M glucose at 25 °C. The result is comparable with similar researches [15-17]. The single-chamber air-cathode glucose/O<sub>2</sub> EBFC in this study provide insight into the conditions needed to develop an EBFC successfully.

## ACKNOWLEDGMENTS

The authors thank the "Kavosh Seal Tehran co" Company of Iran for support in cell configuration. The authors also thank to Dr. Kheirmand from Shiraz University of Iran for his help in air-breathing cathode preparation.

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