Microfluidic Paper based Membraneless Biofuel Cell to Harvest Energy from various Beverages

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Abstract

The present work establishes a cost-effective, miniature, microfluidic self-pumping paper based enzymatic biofuel cell (P-EBFC). The developed Y-shaped P-EBFC consist of buckeye composite Multiwall carbon nanotube (MWCNT) Buckypaper (BP) for the fabrication of bioanode and biocathode immobilized with electro-biocatalytic enzymes glucose oxidase (GOx) and laccase respectively. The electrocatalytic activity of enzymes on electrode surface was confirmed using cyclic voltammetry (CV) and Scanning Electron Microscopy (SEM). Such immobilized bioanode and biocathode show exquisite electrocatalytic activity towards glucose and O2 respectively. Most appealingly, the P-EBFC can directly harvest energy from the widely available beverages containing glucose, such as Mountain Dew, Pepsi, 7up and Fresh Watermelon Juice which could offer potential applications of the P-EBFC as a portable power device.

Keywords

Paper based enzymatic biofuel cell (P-EBFC); beverages; Carbon nanotube (CNT); Buckypaper (BP); Cyclic Voltammetry (CV)

Introduction

In recent decades, research towards enzymatic biofuel cell (EBFC) has drawn remarkable attention and been considered as an efficient energy conversion device. These conversions have been carried out by utilizing naturally procurable enzymes as bio-electrocatalyst to catalyze the fuel oxidation [1-3]. In EBFC, fuel (glucose) is oxidized at the anode side and an oxidant, such as O2, is reduced at the cathode side, leading to produce the potential difference between the bioelectrodes.
Due to its biocompatibility, normal operating conditions (neutral pH and room temperature) and high enzyme selectivity has widen the range of their application such as implantable devices, security application, and harvest energy by using supercapacitors [4],[5]. However, previously reported EBFC’s are usually fabricated from a polymer material for cell and carbon-based material for electrode fabrication with the purpose of increasing the surface to volume ratio (SVR) [6]. Such devices are bulky, complex and rigid in structure.

To overcome these drawbacks, recently, light-weight, low-cost, flexible and biocompatible paper based enzymatic biofuel cell (P-EBEC) are gaining more popularity in the scientific community. In addition, the self-pumping via capillary transport mechanism eliminates the need of external pressure pump and system for moving fluid towards the bioelectrodes. Even though enormous development has been carried out towards the study of P-EBFC; several challenges still need to be fixed before it can be used in practical applications including EBFC. From this perspective, extensive research has been carried out to miniaturize BFC without compromising on the performance and sustainability. In previously reported paper based platforms, carbon-based electrode materials, expensive equipment, and time-consuming and complex redox co-factor based electrochemistry for the immobilization of bioelectrodes, was used for the development of the miniaturized power device [7-11]. In addition, there are reports about EBFC to harvest energy from human physiological fluidics such as blood, saliva, serum, urine and some commercial beverages [12-17]. However, the utilization of such fuels usually needs high cost or complicated extraction methods.

Hence, there is a requirement to achieve miniaturization, simplified structure, and time-efficient and cost-effective redox co-factor free electrochemistry to fabricate bioelectrodes, and P-EBFC. Further, the developed P-EBFC should have the potential to extract the power from commercially available beverages.

In earlier studies, our group made attempts to overcome these challenges by established redox cofactor free biochemistry for enzyme immobilization and fabricating bioelectrodes [18]. To continue our work, Buckypaper (BP) are used as bioelectrodes by covalently immobilizing with glucose oxidase (GOx) and laccase without any redox co-factor. These immobilized BP exhibited excellent performance towards electrochemical redox reactions and provides a potential application for portable and low-cost power device. Here, various commercial beverages are specifically utilized in the EBFC, as a substitute for glucose, to deliver electric power, which realize cost-effective, handheld, green, renewable and easily accessible features of such P-EBFC.

**Experimental**

**Materials and Reagents**

Multiwall carbon nanotube (MWCNT) based Buckeye Composites Buckypaper (BP) was purchased from (NanoTechLabs, Inc.), USA. Whatman #1 filter paper was purchased from (Whatman). Glucose oxidase (GOx) from Aspergillus niger, Laccase from Trametes Versicolor and other analytical grade chemicals like D- (+)-glucose, P-Benzquinone, 2, 2- azino-bis (3-ethylbenothiazoline-6-sulphonic acid) (ABTS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxy succinimide (NHS), Monosodium phosphate (NaH₂PO₄), Disodium phosphate (Na₂HPO₄), Hydrochloric acid (HCl), Sodium Hydroxide (NaOH), Isopropyl alcohol (IPA) and acetone was purchased from Sigma-Aldrich. Commercial beverages (Mountain Dew, Pepsi, 7up and fresh watermelon juice) were purchased from a local market. Throughout experimentation, 18.2 MΩ cm
ultra-pure MilliQ water (Millipore) was used. 0.1M pH 7.0 and 5.0 phosphate buffer solution (PBS) was used for bioanode and biocathode electrochemical analysis.

The P-EBFC construction is described in two sections: The fuel cell design and fabrication with mini platform, and the fabrication and immobilization of BP based bioanode and biocathode

**Microfluidics fuel cell design and fabrication**

Fig. 1 illustrates a digital photo of the paper based enzymatic biofuel cell (P-EBFC). The Y-shaped microchannel design (Whatman paper) was cut using a commercially available computer controlled Graphtec cutter and plotter (Graphtec CE-2000, Japan). This 50 mm long and 5 mm wide Y-shape design comprised of two inlets for forwarding fuel (glucose) and oxidant (O2) due to the capillary action mechanism and harvest the energy up to long duration. A small jig to hold BFC was designed using a 3D printer (Flash forge creator pro, USA). This mini-platform consisted of two reservoirs to store electrolytes (anolyte and catholyte) and to transport electrolyte towards electrodes side. This platform was also harnessed to hold the P-EBFC which was adhered to glass side using a double-sided tissue tape. As shown in Fig. 1, external connections were established using alligator clips at the end of the bioelectrodes.

**Fabrication and immobilization of BP based bioelectrodes**

The Buckeye composite MWCNT based BP was used to fabricate the bioelectrodes. The fabrication and enzymes immobilization process was carried out on the basis of the previously published work by our group [18, 19]. In brief, for the modification of bioanode, first the BP was cut in 15 x 8 mm in dimensions and cleaned with isopropyl alcohol (IPA) to remove the contamination and preserved in hot air oven (90º C for 2 hours). For the activation of carboxylic group, the fabricated BP was submerged in the EDC (30mM) / NHS (90 mM) in 10 ml of MilliQ water (37º C for 2 hours). Thereafter, GOx enzyme solution was prepared by dissolving 5 mg/ml PBS (0.1 M pH 7.0) solution. Developed BP based anode (15 mm x 8 mm) was immobilized into GOx enzymes solution and letting it remain 2 hours at room temperature.

For biocathode, a similar procedure was carried out till the immersion of BP in EDC (30mM) / NHS (90 mM) solution for 2 hours for the activation of the carboxyl group. Thereafter, the biocathode was fabricated by dipping BP in the laccase enzyme solution (5 mg/ml PBS (0.1 M pH 5.0)) and kept under proper ventilation at room temperature for 2 hours. As shown in Fig. 1, these modified
bioelectrodes were parallelly assembled on both sides of the Y-shaped microchannel keeping 1 mm between them.

**Electrochemical measurement**

The electrochemical analysis was carried out with a conventional 3 electrode computer controlled Potentiostat / Galvanostat SP-150 (Bio-Logic Science Instruments, France). Ag/AgCl (3 M NaCl) and platinum was used as a reference and counter electrodes respectively. In the electrochemical measurement, Cyclic voltammetry (CV) and Open circuit potential (OCP) were explored for the fabricated bioanode and biocathode. Subsequently, the polarization performance of the P-EBFC was carried out. All the experiments were conducted at 10 mV/s scan rate at the room temperature. The polarization responses of P-EBFC for different beverages are summarized in Table 1.

**Results and Discussion**

In the electrochemical studies, the cyclic voltammograms (CV) of immobilized bioelectrode was carried out. In brief, for the bioanode analysis, 40 mM glucose and 1 mM P-Benzoquinone was used in presence of 5 ml PBS (0.1 M pH 7.0) with 10 mV/s. scan rate at room temperature. As shown in Figure 2 (a), a very small catalytic oxidation current peak was noticed in the absence of glucose (red line). Whereas the decent catalytic oxidation current peak was noticed in presence of glucose which corresponded to the electrocatalytic oxidation reaction of GOx with a redox mediator (blue line) and revealing the direct electrocatalytic activity of GOX enzymes on the surface of BP. From the CV study, the maximum current density was noticed as 9.32 mA/cm² at 0.33 V.

**Figure. 2** Cyclic Voltammetry of enzymes immobilized bioelectrodes (a) GOx modified bioanode in the presence and absence of 40 mM glucose in 5 ml PBS (0.1 M, pH 7.0), (b) Laccase modified biocathode in the presence (Air purged) and absence of oxygen (Nitrogen purged) in 5 ml PBS (0.1 M, pH 5.0) with 10 mV/s. scan rate at room temperature

Similarly, the CV analysis of BP based biocathode was evaluated presence and absence of the corresponding oxygen, laccase, with 1 mM ABTS as a cathodic mediator in 5 ml PBS (0.1 M, pH 5.0). As shown in Figure. 2(b), the small electrocatalytic reduction current peak was noticed in presence of laccase saturated with nitrogen gas (red line) and a decent electrocatalytic reduction current peak was clearly observed in the presence of laccase saturated with air (blue line) confirming the
successful immobilization of laccase enzymes on BP surface. From this CV analysis, the maximum current density was measured to be 2.25 mA/cm² at 0.43 V with 10 mV/sec scan rate at room temperature.

A 3D printed mini-platform was realized to integrate the modified bioelectrodes on Y-shaped paper microchannel and assemble the complete P-EBFC. For analyzing power performance, polarization study was carried out using a potentiostat. To characterize and analyze the harvested power from the P-EBFC, widely available soft-beverages (Mountain Dew, Pepsi, and 7 Up) and fruit juice (fresh watermelon) consisting of glucose were used as a fuel feedstock. According to the ingredients table of different beverages, this glucose contained in the soft-drinks extracted less power compared to the fresh watermelon juice. This observation may be attributed due to the higher glucose concentration and additional complex additive components [20]. Therefore, the output power was enhanced by diluting these beverages at a certain ratio. The polarization performance of all beverages is shown in Figure 3, and data are shown in Table 1 proves that the beverages with additional glucose has strong potential to harvest more energy.

![Polarization performance of different beverages](image)

Figure 3. Polarization performance of different beverages (a) Fresh watermelon juice, (b) 7 up, (c) Mountain Dew, and (d) Pepsi
Table 1. Summary of the polarization performance of different beverages

<table>
<thead>
<tr>
<th>Beverages</th>
<th>Open circuit potential (OCP) V</th>
<th>Current density (CD) µA/cm²</th>
<th>Power density (PD) µW/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh watermelon juice</td>
<td>0.6</td>
<td>47</td>
<td>14.5</td>
</tr>
<tr>
<td>7 Up</td>
<td>0.31</td>
<td>90</td>
<td>13.5</td>
</tr>
<tr>
<td>Mountain Dew</td>
<td>0.39</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>Pepsi</td>
<td>0.32</td>
<td>41</td>
<td>6.15</td>
</tr>
</tbody>
</table>

Conclusions

Herein, a portable, low-cost and simple in structure, miniature microfluidic Y-shaped P-EBFC is demonstrated using buckypaper (BP) bioelectrodes. The capillary flow mechanism and self-pumping features of the P-EBFC eliminate the need of a membrane between the anolyte and catholyte regions. In addition to the power outputs from glucose, the P-EBFC are shown to have the potential to directly harvest energy from commercially available beverages containing glucose. This simple, miniaturized and cost-effective P-EBFC has the potential to be used for portable bioenergy devices, which is an ongoing work in our lab.

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References