Microfluidic paper based membraneless biofuel cell to harvest energy from various beverages

Prakash Rewatkar, Sanket Goel

MEMS and Microfluidics Lab, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science (BITS) Pilani, Hyderabad Campus, Hyderabad, 500078, India

Corresponding author: msanketgoel@gmail.com; Tel.: + 91-40-66303686

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Abstract
The present work establishes the cost-effective and miniature microfluidic self-pumping paper based enzymatic biofuel cell (P-EBFC). The developed Y-shaped P-EBFC consists of buckeye composite multiwall carbon nanotube (MWCNT) buckypaper (BP) based bio-anode and bio-cathode that were immobilized with electro-biocatalytic enzymes glucose oxidase (GOx) and laccase, respectively. The electrocatalytic activity of enzymes on electrode surface is confirmed using cyclic voltammetry (CV) technique. Such immobilized bio-anode and bio-cathode show exquisite electrocatalytic activity towards glucose and O₂, respectively. Most appealingly, P-EBFC can directly harvest energy from widely available beverages containing glucose such as Mountain Dew, Pepsi, 7up and fresh watermelon juice. This could provide potential application of P-EBFC as a portable power device.

Keywords
Paper based enzymatic biofuel cell (P-EBFC); beverages; carbon nanotubes (CNT); buckypaper (BP); cyclic voltammetry

Introduction
In recent decades, research towards enzymatic biofuel cell (EBFC) has drawn remarkable attention and is generally considered as an efficient energy conversion device. Energy conversion has 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 oxidant such as O₂ is reduced at the cathode side, producing a potential difference between bio-electrodes. Due to biocompatibility, normal operating conditions (neutral pH and room temperature) and high enzyme selectivity, EBFC devices have widen the range of their application toward implantable devices, security applications, and harvest energy by using supercapacitors [4,5]. Previously reported EBFCs are usually fabricated from a polymer material used for cell and carbon-based material used for
electrodes with the purpose of increasing the surface to volume ratio (SVR) [6]. Such devices are, however, bulky, complex and rigid in structure.

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

In earlier studies, our group made some attempts to overcome these challenges by establishing redox co-factor free biochemistry for enzyme immobilization and fabricating bio-electrodes [18]. To continue our work, a buckypaper (BP) is used as bio-electrodes by covalent immobilization with glucose oxidase (GOx) and laccase without any redox co-factor. These immobilized BP exhibited excellent performance towards electrochemical redox reactions and provided potential application as portable and low-cost power device [19]. In order to test the developed P-EBFC for its potential to deliver the power from commercially available beverages, various commercial beverages are specifically utilized as substitutes for glucose. The obtained results realized cost-effective, handheld, green, renewable and easily accessible features of developed P-EBFC.

Experimental

Materials and reagents

Multiwall carbon nanotube (MWCNT) based buckeye composite based 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-Benzooquinone, 2, 2-azino-bis (3-ethyl-benzothiazoline-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 were purchased from Sigma-Aldrich. Commercial beverages (Mountain Dew, Pepsi, 7up and fresh water-melon juice) were purchased from a local market. Throughout experimentation, 18.2 MΩ cm ultra-pure MilliQ water (Millipore) was used. 0.1 M phosphate buffer solutions (PBS) (pH 7.0 and 5.0) were used for bio-anode and bio-cathode electrochemical analysis.

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

Figure 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 Grahtec cutter and plotter (Grahtec CE-2000, Japan). This 50 mm long and 5 mm wide Y-shape design comprised of two inlets for forwarding fuel (glucose) and oxidant (O\textsubscript{2}) due to the capillary action mechanism and harvest energy up to long duration. A small jig to hold BFC was designed using 3D printer (FlashForge USA). This mini-platform consisted of two reservoirs to store electrolytes (anolyte and catholyte) and to transport electrolyte towards electrodes. This platform was also harnessed to hold P-EBFC which was adhered to glass side using a double-sided tissue tape. As shown in Figure 1, external connections were established using alligator clips put at the ends of bio-electrodes.

![Figure 1. Digital image of paper based enzymatic biofuel cell (P-EBFC) operating under different beverages](image)

Fabrication and immobilization of BP based bioelectrodes

The buckeye composite MWCNT based BP was used to fabricate bio-electrodes. The fabrication and enzymes immobilization process were carried out on the basis of the previously published work by our group [18,20]. In brief, for the modification of bio-anode, BP was firstly cut to 15 x 8 mm dimensions and cleaned with isopropyl alcohol (IPA) to remove the contamination and then preserved in hot air oven (90 °C) for 2 hours. For the activation of carboxylic group, the fabricated BP was submerged in EDC (30 mM)/NHS (90 mM) in 10 ml of MilliQ water (37 °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 x 8 mm) was immobilized into GOx enzymes solution and left at room temperature for two hours.

For bio-cathode, a similar procedure was carried out till the stage of immersion of BP in EDC (30 mM) / NHS (90 mM) solution for 2 hours for the activation of the carboxyl group. Thereafter, the bio-cathode 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 Figure 1, these modified bio-electrodes were assembled in parallel on both sides of the Y-shaped microchannel, keeping 1 mm between them.

Electrochemical measurements

The electrochemical analysis was carried out with a conventional 3-electrode cell and a computer controlled potentiostat/galvanostat SP-150 (Bio-Logic Science Instruments, France). Ag/AgCl

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(3 M NaCl) and platinum were used as reference and counter electrodes, respectively. In electrochemical measurements, cyclic voltammetry (CV) and open circuit potential (OCP) techniques were explored for fabricated bio-anode and bio-cathode. Subsequently, the polarization performance of the P-EBFC was carried out. All the experiments were conducted at 10 mV/s scan rate at room temperature.

Results and discussion

In the electrochemical studies, the cyclic voltammograms (CV) of immobilized bio-electrodes, measured at the scan rate of 10 mV/s and room temperature were carried out. In brief, for the bio-anode analysis, 40 mM glucose and 1 mM p-benzoquinone was added into 5 ml PBS (0.1 M pH 7.0). As shown in Figure 2 (a), a very small catalytic oxidation current peak can be noticed in the absence of glucose (red line), while a prominent catalytic oxidation current peak is noticed in presence of glucose (blue line). This peak corresponds to the electrocatalytic oxidation reaction of GOx with a redox mediator and reveals the direct electrocatalytic activity of GOx enzymes on the surface of BP. From the CV in Fig. 2a, the maximum current density is estimated as 9.32 mA/cm² at 0.33 V.

![Figure 2](image-url)

**Figure 2.** Cyclic voltammetry (scan rate = 10 mV/s) of enzymes immobilized bio-electrodes at room temperature: (a) GOx modified bio-anode in presence and absence of 40 mM glucose in 5 ml PBS (0.1 M, pH 7.0), (b) laccase modified bio-cathode in presence (air purged) and absence of oxygen (nitrogen purged) in 5 ml PBS (0.1 M, pH 5.0).

Similar CV measurements of BP based bio-cathode were evaluated in 5 ml PBS (0.1 M, pH 5.0), containing laccase with 1 mM ABTS as a cathodic mediator, in presence and absence of oxygen. As shown in Figure 2(b), the small electrocatalytic reduction current peak can be noticed in the presence of laccase saturated with nitrogen gas (red line). More prominent electrocatalytic reduction current peak can clearly be observed in the presence of laccase saturated with air (blue line), confirming the successful immobilization of laccase enzymes on BP surface. From the CV in Figure 2b, the maximum current density is estimated to be 2.25 mA/cm² at 0.43 V.

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, the polarization study was carried out using potentiostat. To characterize and analyze the harvested power from P-EBFC, widely available soft-beverages (Mountain Dew, Pepsi, and 7 Up) and fruit juice (fresh watermelon) containing glucose were used as a fuel feedstock. According to the tables of ingredients of different beverages, glucose contained in soft-drinks extracted less power compared...
to the fresh watermelon juice. This observation may be attributed to the higher glucose concentration and additional complex additive components [21]. 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 summarized in Table 1, proving that beverages with additional glucose have strong potential to harvest more energy.

Figure 3. Polarization performances 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, V</th>
<th>Current density, µA/cm²</th>
<th>Power density, µW/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh watermelon</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, simple structured and miniature microfluidic Y-shaped P-EBFC is demonstrated using buckypaper (BP) bio-electrodes. The capillary flow mechanism and self-pumping features of P-EBFC eliminated the need for a membrane between the anolyte and catholyte regions. In addition to the power outputs from glucose, P-EBFC are shown to have the potential to directly harvest energy from commercially available beverages containing glucose. This
simple, miniaturize and cost-effective P-EBFC has utilization potential for portable bioenergy device, which is the main subject of ongoing work in our lab.

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**References**


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