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Microbiofuel cell powered by glucose/O_2 based on electrodeposition of enzyme, conducting polymer and redox mediators. Part II: Influence of the electropolymerized monomer on the output power density and stability

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ABSTRACT

In this study, we investigated the influence of nature of the electropolymerized monomer on the resulting power output and stability of a glucose/O_2 powered biofuel cells (BFCs). The bioanode was prepared from a mixture of glucose oxidase-polymeric monomer-ferrocenium hexafluorophosphate-pyrroloquinoline quinone (abbreviated as, GOx-monomer-FHFP-PQQ) and the biocathode from laccase enzyme-polymeric monomer-4,4-sulfonyldiphenol-Bis-(bipyridine)-(5-aminophenanthroline) ruthenium bis (hexafluorophosphate) (abbreviated as, LAc-monomer-SDP-RuPy) electrodeposited from low conductivity solutions using pulsed square wave potentials (10 s at 4 V, then 3 s at 0.5 V) for 180 cycles. Three different monomers were investigated: aniline, phenol and pyridine. The power output of the aniline based BFCs reached 5.97 $\mu W.mm^{-2}$ which is higher than the pyrrole based BFCs reported previously (3.17 $\mu W.mm^{-2}$). With phenol monomer, the estimated maximum power density was only 0.276 $\mu W.mm^{-2}$. The pyridine based BFCs showed the lowest power density (0.046 $\mu W.mm^{-2}$) of all, even lower than the monomer-free BFC (0.124 $\mu W.mm^{-2}$). The evaluation of the BFCs in buffer solution pH 7.4 under air at 37 °C for 3 days of continuous operation showed that pyrrole and aniline based BFCs are the most stable followed by phenol based BFCs. Pyridine and monomer-free BFCs undergo significant deterioration with up to 75% loss in power density.

1. Introduction

Enzyme based biofuel cells (BFCs) are electrochemical devices able to directly convert chemicals into electrical energy via electrochemical reactions involving biochemical pathway [1]. Although the power outputs generated by enzymatic BFCs are thousand times smaller than the energy delivered by conventional fuel cells, they are high enough to supply micro and mini-scale electronic devices such as miniaturized sensors transmitters, micropumps and pacemakers that require relatively low power [2–5]. Recently, considerable attention has been paid to enzymatic BFCs because of the many interesting properties that this technology offers including ambient or physiological working temperature, neutral pH, and most importantly the possibility to be used for *in vivo* implantation in animals or humans as a power source for e.g., micro-pumps, pacemakers and, artificial organs [2–5]. In view of this, glucose BFCs look very promising as power sources for especially implantable devices because they can exploit glucose and O_2 from the biological fluid of tissues or bloodstream as a power source.

In enzymatic BFCs based glucose/O_2, glucose is electrooxidized at the anode by glucose oxidase (GOx), and oxygen is reduced at the cathode by laccase (LAc) or bilirubin oxidase enzymes. However, since enzymes have a complex 3-Dimentional structure where the electron transferring unit is deeply buried inside the structure, this result in a poor electrical communication between the redox active centers of the enzyme and the electrode. To this end, several approaches have been considered in order to improve the electrical communication between the enzyme and the electrode. Among these, different categories of electron mediators like small organic redox molecules, conducting polymers, carbon nanotubes and metal nanoparticles or combination of some of these have been explored [6–18].

A second problem to solve with enzymatic BFCs deals with oxygen in non-compartmentalized BFCs that is reduced at the biocathode but likewise at the bioanode. The latter leads to a decrease in the power output of BFCs [19,20]. To solve the

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problem, several avenues have been taken. The first deals with the use of reconstructed GOx that is less sensitive to oxygen [21]. The second approach is based on the use of efficient mediators like osmium containing redox polymers which yields efficient electron mediation with GOx than oxygen. With respect to this, numerous membranes based osmium redox polymers have been explored for GOx at the anode and laccase [22,23] or bilirubin oxidase [15,24] at the cathode. The power outputs of these enzymatic BFCs varied between 0.4 and 4.4 $\mu W.mm^{-2}$, depending on the structure of the osmium polymer and the operating conditions.

Previously, we reported a new, easy, rapid and inexpensive procedure to manufacture a micro-biofuel cell powered by glucose/O_2 [25]. Enzyme, mediators and pyrrole monomer have been electrochemically deposited from very low ionic strength solution (ultrapure water) at relatively high applied potential of 4 V. The maximum power density of the resulting pyrrole biofuel cell reached 3.1 $\mu W.mm^{-2}$ at a cell voltage of 0.28 V in phosphate buffer solution pH 7.4 under air containing 10 mM glucose at 37 °C. In the course of this study, we wanted to investigate the effect of nature of the electropolymerized monomer on the power output of the BFC, which after electropolymerization forms the encapsulating matrix in which the enzymes and redox mediators are immobilized. The latter might be of great interest for fuel cells and biofuel cells technology.

2. Experimental

2.1. Materials

Ultrapure water milliQ grade with a resistivity of 18.2 MΩ cm was used for all the experiments. Glucose oxidase (GOx) crude from *Aspergillus Niger* (5.6 units/mg) and laccase (LAc) from *Trametes Versicolor* (1 units/mg) were purchased from Sigma and used without further purification. Pyrrole, aniline, phenol and pyridine were obtained from Acros or Sigma-Aldrich. Ferrocenium hexafluorophosphate (FHFP), 4,4-sulfonyldiphenol (SDP), pyrroloquinoline quinone (PQQ) 99% from Sigma–Aldrich. Bis-(bipyridine)-(5-aminophenanthroline) ruthenium bis (hexafluorophosphate) (RuPy) from Fluka. Glassy carbon (GC) rods 1 mm in diameter purchased from Alfa Aesar were connected electrically to a copper wire sealed in a resin then cut and polished with emery paper. D-Glucose (Glu) 99% from Fisher Scientific was prepared 24 h before use. Phosphate salts (NaH_2PO_4 and Na_2HPO_4) and sodium chloride analytical grade were purchased from Acros Organic. The buffered saline pH 7.4 was prepared from phosphate salts (0.1 M) and sodium chloride (0.15 M) is used for the testing of the BFCs. For the fabrication of the BFCs electrodes, ultrapure water was used as a dissolving media. However, because the enzymes have not been purified, hence they still contain large amount of salts to ensure electroneutrality. This results in the increase of conductivity of the enzyme solutions.

2.2. Equipment

An EG&G 273 potentiostat from Princeton Applied Research was used for the preparation and testing of the bioanodes and biocathodes. To avoid shifts in potentials due to eventual deposits from the enzyme-monomer solution on the reference electrode, separates Ag/AgCl (+0.238 V versus NHE) was used as a reference electrode for both the fabrication and testing. The deposition rate was investigated using a microbalance with a precision of 1 μg (Mettler Toledo, AT 21). Scanning Electron Microscopy (SEM) was carried out using an XL 30 FEG from FEI. Voltage and current measurements of the BFCs were carried out with two multimeters from Fluke 87 True RMS. The bioanode and biocathode electrodes were placed in small cell with a capacity of 7 mL containing 5 mL of buffer solution with 10 mM glucose. The two electrodes were connected to two multimeters, one for potential recording and the other for current recording. The voltage and current were varied using 1 MΩ potentiometer and the data were fitted using polynomial orders of 2 to 8 depending of the regression.

2.3. Methodology

Glassy carbon (GC) disk electrodes (1 mm in diameter, surface area ~0.78 mm^2) are used as a substrate for the GOx bioanode and LAc biocathode. The manufacturing procedure has been previously reported [25]. In brief, for the bioanode, one of the GC electrodes was immersed in a small glass tube containing GOx (50 mg/0.4 mL ultrapure water), 10 μL of PQQ (1 mg/200 μL water), 10 μL of FHFP (5 mg/200 μL water), then 2 μL pyrrole, phenol or pyridine monomer was added to the GOx solution. For aniline, due to its low solubility in the solution, it was first dissolved in a diluted sulfuric acid (pH 3), then equivalent concentration was added to the enzyme-mediator mixture. The final pH of the solution is around 6.7. Platinum and Ag/AgCl wires were immersed in the cell as a counter and reference electrodes. The electrodeposition of the GOx-monomer-FHFP-PQQ mixture is carried out using the pulsed square wave potential (10 s at 4 V, then 3 s at 0.5 V) for 180 cycles shown in Fig. 1. This deposition procedure was applied a second time to the electrode by switching the polarity of the square wave. It is observed that during this second step, no supplementary material is deposited on the substrate because the monomers cannot be electropolymerized at negative potentials. However, an increase in the anode voltage was noticed. The electrode was then removed from the cell and washed with ultrapure water then stored in the fridge at 4 °C until use.

The biocathode was prepared by immersing the second GC electrode in LAc solution (50 mg/0.4 mL ultrapure water) then 10 μL of RuPy (1 mg/200 μL water), 10 μL of SDP (5 mg/200 μL water) and 2 μL of pyrrole, phenol or pyridine were added to the enzyme solution. For aniline monomer, as stated for the bioanode, due to its low solubility in the solution, it was first dissolved in sulfuric acid (pH 3). Then equivalent concentration of monomer was added to the enzyme-mediators mixture. A platinum and Ag/AgCl wire were immersed in the same cell. LAc-monomer-SDP-RuPy were then electrodeposited using the pulsed square wave potential shown in Fig. 1. The electrode was then removed from the cell and washed with ultrapure water then stored in the fridge at 4 °C until use.

For mass measurements, the initial mass of a glassy carbon rod (1 mm in diameter and 7 mm in length) was determined with the microbalance. The electrode was subsequently immersed in the enzyme–monomer–redox mediators mixture and modified as previously described. The electrode was washed with ultrapure water, dried at 40 °C for 1 h and weighted a second time. The amount of the deposited material was obtained from the difference between the initial mass and the mass after deposition.

3. Results and discussion

3.1. Effect of the electropolymerized monomer on the electrodeposited film

Previously, we reported a BFC powered by glucose/O_2 based on electrodeposition of enzymes, mediators and pyrrole monomer from low conductivity solutions and found that the BFC generates a maximum power density of 3.1 $\mu W.mm^{-2}$ in phosphate buffer solution of pH 7.4 and physiological temperature of 37 °C [25]. This power output is substantially higher if compared to most of the glucose/O_2 BFCs reported in literature, even those using