

The Improved Biofuel Cell for Electrical Power Generation from Wastewaters

M. S. Kilic, S. Korkut, B. Hazer

Abstract—Newly synthesized Polypropylene-g-Polyethylene glycol polymer was first time used for a compartment-less enzymatic fuel cell. Working electrodes based on Polypropylene-g-Polyethylene glycol were operated as unmediated and mediated system (with ferrocene and gold/cobalt oxide nanoparticles). Glucose oxidase and bilirubin oxidase was selected as anodic and cathodic enzyme, respectively. Glucose was used as fuel in a single-compartment and membrane-less cell. Maximum power density was obtained as 0.65 nW cm⁻², 65 nW cm⁻² and 23500 nW cm⁻² from the unmediated, ferrocene and gold/cobalt oxide modified polymeric film, respectively. Power density was calculated to be ~16000 nW cm⁻² for undiluted wastewater sample with gold/cobalt oxide nanoparticles including system.

Keywords—Bilirubin oxidase, Enzymatic fuel cell, Glucose oxidase, Nanoparticles.

I. INTRODUCTION

AN enzymatic fuel cell (EFC) uses enzymes to convert chemical energy to electrical energy. The advantage of EFCs is to exploit the selectivity of enzymes in order to build a fuel cell that does not require the separation of the anode from the cathode. Anode and cathode together can be immersed in one compartment containing fuel. By this way, EFC is miniaturized in principle down to micrometer scale, in order to be used, e.g., for implantable devices and in vivo mediums [1]-[3].

Recently, conducting polymers, sol-gels, metal oxides, self-assembled monolayers and nanocomposites, along with different biomolecule immobilization strategies and polymer modifications have been used to achieve enhanced electron transfer rate [4]. To create a bridge between the enzyme and the electrode surface, metallic electrode surfaces have been modified by deposition of these kinds of polymers. To mediate the electron transfer between the enzyme and the electrode surface, low molecular weight redox compounds called mediators have been adsorbed or entrapped into the polymeric films [5]. Higher power generation and minimal interference effect at a lower cell potential is achieved by using mediator in fuel cell systems. A redox mediator of appropriate redox potential is required to shuttle electrons between enzyme and electrode surface, because direct electron transfer to buried redox sites within these enzymes is generally not possible

given the distance of the active site from the electrode surface [6].

In this work, unmediated, ferrocene and gold/cobalt oxide modified Polypropylene-g-Polyethylene glycol (PP-g-PEG) was newly synthesized and first time used for an EFC including anodic enzyme glucose oxidase and cathodic enzyme bilirubin oxidase and glucose as fuel. The prepared working electrodes were used in unmediated, ferrocene and gold/cobalt oxide modified fuel cell system, and performance results were compared with each other. Gold/cobalt oxide modified biofuel cell was operated in wastewater sample to generate electrical energy by using glucose content of the wastewater.

II. MATERIALS AND METHODS

A. Reagents

Chlorinated polypropylene (PP) (Mw 150000 Da, three repeating units have 1 Cl in average), polyethylene glycol with Mn = 8000 Da (PEG8000), Tetrahydrofuran (THF, ≥99% GC grade), Glucose oxidase from *Aspergillus niger* (GOx) (10 KU), Bilirubin oxidase from *Myrothecium verrucaria* (BOD) (25 U) and Laccase from *Trametes versicolor* were obtained from Sigma-Aldrich. Sodium hydroxide, hydrochloric acid, potassium di-hydrogen phosphate and di-potassium hydrogen phosphate were purchased from Merck. Glucose monohydrate was supplied from Riedel. Stock solutions of enzymes and glucose were daily prepared in 100 mM pH 7.4 phosphate buffers.

B. Apparatus

Fuel cell experiments were performed by using a CHI 1040B Model electrochemical analyzer. Glassy carbon (Ø=3 mm) working electrode, Platinum wire counter electrode, Ag/AgCl (3M NaCl) reference electrode, and a conventional electrochemical cell obtained from the same firm.

C. Synthesis of Modified Polypropylene-g-Polyethylene Glycol and Preparation of EFC Electrodes

The amphiphilic polymer was synthesized by modifying a previously reported procedure [7]-[9]. Typically, chlorinated polypropylene, 1.5 g (10 mmol Cl) was dissolved in 50 mL of freshly distilled THF. 20 mL of a THF solution containing Polyethylene glycol 8000 (20 g, 10 mmol) and 2 mL of an aqueous NaOH solution (50 wt%) was added drop wise to the chlorinated polypropylene solution within 20 min. The reaction mixture was poured into 500 mL of water containing 1 mL of concentrated HCl after stirring 3 days at room temperature. The polymer was filtered, washed with water and

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dried under vacuum overnight. For the purification, it was redissolved in THF and re-precipitated in 200 mL of distilled water and then dried under vacuum overnight at 50°C. H-NMR, FT-IR, FTIR-ATR and GPC characterizations of the final polymer were previously reported by our chemistry group [7]-[9]. Ferrocene modified polymer was prepared by adding of 1 mg of ferrocene into the 10 mg mL⁻¹ of polymer solution.

After the purification step gold and cobalt oxide nanoparticles were embedded into the polymer as following procedure: Solutions of metal salts 0.58 g of HAuCl₄ in 20 mL THF, 0.35 g of CoCl₂·6H₂O in 6.4 mL pure water and the reducing agent 65 mg of NaBH₄ in 3.2 g of pure water were prepared. PP-g-PEG (0.2 g) was dissolved in 10 mL of THF. 4 drops of metal salt solutions were added into the polymer solution and vigorously stirred at room temperature for an hour. Then, 2 drops of the reducing agent was added to this mixture and stirred again for 2 hours. Final solution was poured into a Petri dish (Ø=5 cm) to achieve film formation via solvent casting. Composite polymer film was peeled away from the Petri dish after 24 hours, washed with methanol and dried under vacuum at room temperature for one day. 10 mg mL⁻¹ of polymer solution was prepared in toluene for readily use in fuel cell system.

The electrode fabrication step was preceded by a cleaning phase of the glassy carbon electrode surface using gamma alumina powder then, rinsing with distilled water. 2 µL of the polymer solution (10 mg mL⁻¹) was directly spread onto the surface of the cleaned anode and cathode. The electrodes were then allowed to dry for solvent evaporation at room temperature. Working electrodes were washed with distilled water. 30 µL of GOx (10 mg mL⁻¹) and 30 µL of BOD (10 mg mL⁻¹) were dropped onto the polymer film coated anode and cathode, respectively. The electrodes were allowed to dry and wait for enzyme immobilization at room temperature for 2 hours. Then the electrodes were washed in 2 mL of 100 mM, pH 7.4 phosphate buffer solution to remove the unbound enzyme from the surface of the electrodes. Enzyme was physically adsorbed onto the long Polyethylene glycol side-chain of the polymer.

III. RESULTS AND DISCUSSIONS

A. Electrochemical Characterization

The electrochemical characteristic of the gold/cobalt oxide nanoparticles embedded PP-g-PEG, ferrocene modified PP-g-PEG and pure PP-g-PEG film coated electrodes were evaluated through cyclic voltammetry (CV) (Fig. 1). The CVs obtained in 10 mL of 100 mM pH 7.4 phosphate buffer at a potential scan between -600 and +600 mV at a scan rate of 100 mV s⁻¹. It was clearly understood that the pure polymer film coated electrode did not show any electroactivity (Fig. 1). In the case of gold/cobalt oxide nanoparticles presented in polymer, CV waves were shaped since the polymer showed electroactivity and did not block the electron transport between the electrode materials and the enzymes. A well-defined cathodic peak observed at +360 mV is attributed to the

reduction of ferrocene in the PP-g-PEG film. Presence of this peak confirmed the incorporation of ferrocene molecule into the polymeric film structure which made the fuel cell system mediator controlled. The formal reduction potential of ferrocene is around +300 and +400 mV which is similar with previously reported papers [10]-[13].

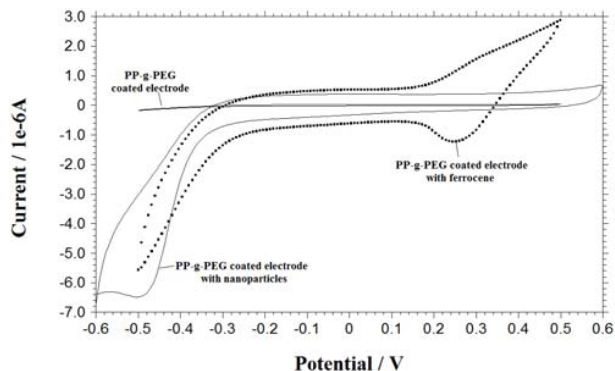
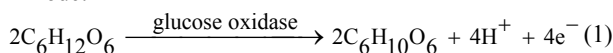


Fig. 1 Cyclic voltammogram of nanoparticle embedded PP-g-PEG/BOD, ferrocene including PP-g-PEG/BOD and pure PP-g-PEG coated electrode in 10 mL of 100 mM phosphate buffer solution (pH 7.4) at a potential scan ranging between -600 and +600 mV (vs Ag/AgCl) with a scan rate of 100 mV s⁻¹.

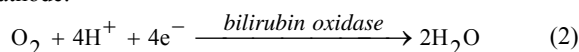
B. Power Generation of the Fuel Cell

The anodic enzyme glucose oxidase has been very commonly used due to its high stability at physiological pH of 7-7.5 and high turnover rates. Bilirubin oxidase has emerged as the best cathodic enzyme primarily because of its high stability at physiological pH. The redox reaction of glucose oxidase and bilirubin oxidase is stated below [14]:

Anode:

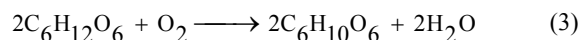


Cathode:



Resulting in the net bioelectrochemical power generation reaction;

Enzymatic fuel cell:



EFCs system is worked by applying a proper potential to anode and cathode to supply electron flow through the circuit. Glucose is oxidized to gluconolactone by the anodic enzyme GOx, and electrons are released (1). The released electrons are transferred to the polymer from the enzyme and then the anode electrode material. The electrons are flowed through to the cathode due to the driving force of the applied anodic potential. They are cathodized by cathode with the aid of cathodic potential, then by PP-g-PEG. BOD reduces oxygen to water by using these electrons (2) (Fig. 2). The electron flows through cathode is observed as electrical current with applied cell voltage ($E_{\text{cell}} = E_{\text{cathode}} - E_{\text{anode}}$). The electrical power

density, P of a biofuel cell is defined as the product of the cell potential and the generated current density: $P = I \times V_{\text{cell}}$. It can be calculated by normalizing the power with respect to the electrode cross-sectional area or the electrode volume [15].

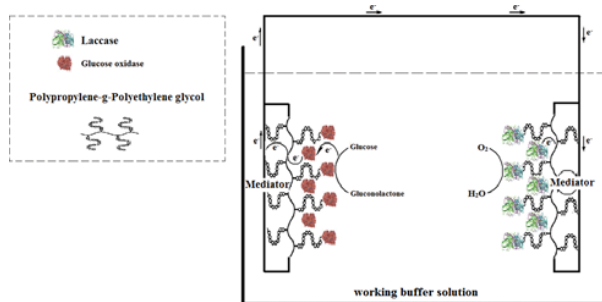


Fig. 2 Scheme of the bioelectrochemical reactions for the proposed enzymatic fuel cell

Fig. 3 B shows that cathode side of the gold/cobalt oxide including polymer presented excellent cathodic currents in comparison to ferrocene modified and unmediated cathode. Cathode side of the ferrocene mediated fuel cell system presented 100-fold higher power generation in comparison with the unmediated one (Fig. 3 A). Maximum power density was obtained as 0.65 nW cm^{-2} , 65 nW cm^{-2} and 23500 nW cm^{-2} with 20 mM glucose from the unmediated, ferrocene and gold/cobalt oxide modified polymeric film at pH 7.4, respectively. It was clearly seen from the Fig. 3 that gold/cobalt oxide embedded polymeric film was effective for cathodic enzyme bilirubin oxidase to produce higher currents owing to the fast electron transport towards to the cathode. It should be noted that the power density was within the range of those ($5000\text{--}10000 \text{ nW cm}^{-2}$) already described for EFCs combined with various mediators [16]–[18]. However, these systems were based on more expensive and complex configurations involving for instance carbon nanotubes and ionic liquid or multi-walled carbon nanotubes and electrogenerated polymer as immobilization matrices [18], [19].

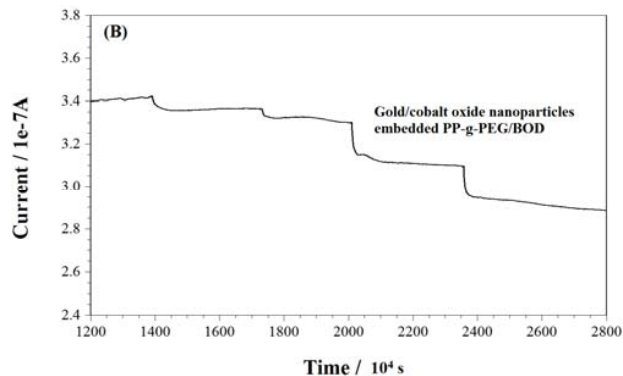
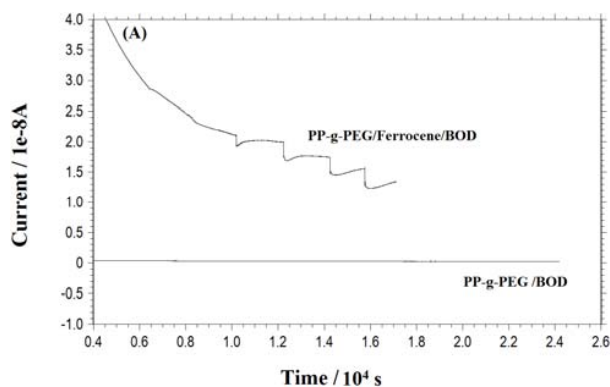


Fig. 3 Amperometric responses of PP-g-PEG /BOD (with a cell potential of +700 mV) and PP-g-PEG/Ferrocene/BOD (with a cell potential of +560 mV) (A) and gold/cobalt oxide nanoparticles embedded PP-g-PEG/BOD (with a cell potential of +560 mV) (B) electrodes to increasing glucose concentrations ranging between 30 and 300 mM in the 100 mM pH 7.4 phosphate buffer vs. Ag/AgCl, 3 M NaCl

C. Power Generation from Municipal Wastewater

The EFC was operated using glucose as renewable fuel found in wastewater to generate power. The wastewater was collected from the activated sludge reactor (near the inlet region) of the Wastewater Treatment Plant of Zonguldak City. Sample was filtered to remove bacteria and other particulate metabolites prior to experiments. EFC was immersed into 10 mL of phosphate buffer solution and operated at a cell potential of +560 mV. 500 μL of wastewater sample was added into the reaction medium. Fig. 4 presents cathodic currents of the system utilized glucose content of wastewater. Power density was calculated to be $\sim 16000 \text{ nW cm}^{-2}$ for undiluted wastewater sample. Power densities from the various microbial fuel cell studies operated with domestic wastewaters were reported as 2400, 2800 and 14600 nW cm^{-2} [20]. Other group examined the possibility of generating electricity from dairy-cow waste slurry using microbial fuel cell and reported a power density of 34 nW cm^{-2} [21].

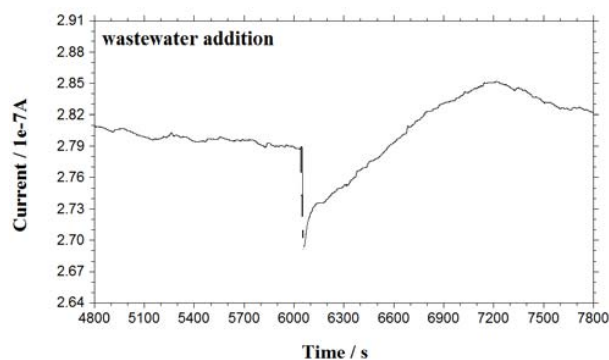


Fig. 4 Current signals of the EFC working electrodes obtained from the addition of municipal wastewater sample into the working buffer at the cell voltage of +560 mV vs Ag/AgCl

IV. CONCLUSION

In the present study a novel Polypropylene-g-Polyethylene glycol film based membrane-less biofuel cell used glucose as a fuel was prepared. Glucose oxidase with anode and Bilirubin oxidase with cathode enzymes were adsorbed onto the Polyethylene side-chain groups of the polymer. Gold/cobalt oxide nanoparticles and ferrocene were successfully incorporated into the polymer and acted as a mediator to promote electron transfer for the fuel cell system. Maximum power density was calculated to be 0.65 nW cm^{-2} , 65 nW cm^{-2} and 23500 nW cm^{-2} by the addition of 20 mM of glucose at $\text{pH } 7.4$ phosphate buffer for the unmediated, ferrocene and gold/cobalt oxide modified polymeric film, respectively. Power density was calculated to be $\sim 16000 \text{ nW cm}^{-2}$ for undiluted wastewater sample.

ACKNOWLEDGMENT

This work was financially supported by "The Scientific & Technological Research Council of Turkey" (TUBITAK) (Grant no. 112Y100) and the Bulent Ecevit University Research Fund (Grant no. BEU-2013-77047330-01).

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