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A novel poly(propylene-*co*-imidazole) based biofuel cell: System optimization and operation for energy generation

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ABSTRACT

This study describes the construction of an enzymatic fuel cell comprised of novel gold nanoparticles embedded poly(propylene-*co*-imidazole) coated anode and cathode. Working electrode fabrication steps and operational conditions for the fuel cell have been optimized to get enhanced power output. Electrical generation capacity of the optimized cell was tested by using the municipal wastewater sample. The enzymatic fuel cell system reached to maximum power density with 1 μg and 8 μg of polymer quantity and bilirubin oxidase on electrode surface, respectively. The maximum power output was calculated to be 5 μW cm^{-2} at +0.56 V (vs. Ag/AgCl) in phosphate buffer (pH 7.4, 100 mM, 20 °C) by the addition of 15 mM of glucose as a fuel source. The optimized enzymatic fuel cell generated a power density of 0.46 μW cm^{-2} for the municipal wastewater sample. Poly(propylene-*co*-imidazole) was easily used for a fuel cell system owing to its metallic nanoparticle content. The developed fuel cell will play a significant role for energy conversion by using glucose readily found in wastewater and in vivo mediums.

1. Introduction

Enzyme-based biofuel cells (EFCs) provide versatile means to generate electrical power from biomass or biofuel substrates, and use biological fluids as fuel-sources for the electrical activation of implantable electronic medical devices, or prosthetic aids [1–3]. In addition, the electrode reactions do not produce any harmful side products, and hence allow the direct and safe conversion of the chemical energy present within naturally available molecules into usable electrical power [4]. While the original integrated biofuel cells included electrically contacted biocatalysts in monolayer configurations that resulted in limited electrical power, the second and third generations of biofuel cells included the entrapment of the enzymes in redox-active polymer hydrogels, or the immobilization of the enzymes on nano-objects, such as carbon nanotubes or nanoparticles (gold, cobalt etc.). These approaches led to higher contents of the biocatalysts contacted with the electrodes, and to enhanced values of the power output [5]. Immobilization can achieve high surface utilization by locating mediators and biocatalysts within nanometers of polymer surfaces and allows for dense packing of enzymes within electrode volumes at the expense of long-distance electron mediation between the enzyme active center and electrode surface [6]. This concept represents an important advance, as it precludes the need to separate the anode and cathode half-cells from each other using a membrane, provided that no solution redox reaction between fuel and oxygen occurs (as is the case for glucose) [7]. Recently, nanomaterials, such as gold [8–11] and magnetic nanoparticles have attracted much interest in the construction of biosensors due to their unique chemical and physical properties. Gold nanoparticles, in particular, have been widely used to construct biosensors because of their excellent ability to immobilize biomolecules and at the same time retain the biocatalytic activities of those biomolecules [12].

Polypropylene (PP) is one of the most important polyolefines due to its wide industrial production, low cost, good mechanical properties, easy processing, and excellent recyclability [13–20]. Furthermore, it is a very versatile, hydrophobic polymer that has medical and industrial applications due to its good film and fiber properties [21]. Gold nanoparticles are applied in analytical chemistry and electrochemistry due to their novel optical, electrical and catalytic properties [22–24]. Combination of gold nanoparticles brings together their unique properties generating a new nanocomposite with superior characteristic [22,25]. However, this combination alone is not enough to obtain an efficient performance from a biosensor and an EFC system. It is necessary to optimize the system step-by-step by starting from the working electrode fabrication to improve the yield.

In this study, gold nanoparticles embedded Poly(propylene-*co*-imidazole) (PP-*co*-Im) was chemically synthesized and used as polymeric matrix for working electrodes of an EFC system. Glucose oxidase (GOx) and bilirubin oxidase (BOD) was selected as anodic and cathodic enzyme, respectively. Electrode fabrication steps and operational conditions were carefully optimized to enhance the system performance. EFC

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ability to generate electrical energy from a standard glucose and a real wastewater sample were investigated.

2. Experimental

2.1. Reagents

Chlorinated polypropylene (PP-Cl) (Mw 150,000 Da, three repeating units have 1 Cl in average), imidazole, toluene (99.9% HPLC grade), tetrahydrafurane (THF, 99.9% GC grade), glucose oxidase from *Aspergillus niger* (GOx) (10 KU), bilirubin oxidase from *Myrothecium verrucaria* (BOD) (25 U) and laccase from *Trametes versicolor* were provided from Sigma-Aldrich. Potassium di-hydrogen phosphate and di-potassium hydrogen phosphate were purchased from Merck. Glucose monohydrate, acetic acid and sodium acetate trihydrate were obtained from Riedel. Stock solutions of enzymes and glucose were daily prepared in 100 mM pH 7.4 phosphate buffers.

2.2. Apparatus and electrochemical measurements

EFC experiments were performed by using a CH Instruments 1040B Electrochemical Analyzer. Glassy carbon (Ø = 3 mm) and gold (Ø = 2 mm) working electrodes, platinum wire counter electrode, Ag/AgCl (3 M NaCl) reference electrode, and a conventional electrochemical cell were obtained from the same firm. Electrochemical measurements were carried out in aerated phosphate buffer (pH 7.4, 100 mM, 20 °C) with an applied potential of −0.2 V for anode and +0.36 V for cathode under continuous stirring at 100 rpm by using four-electrode compartment-less cell. Four-electrode system was immersed into the cell; an optimized operating potential was applied to anode and cathode side and waited for reaching to a steady-state background current. Then, various concentrations of glucose ranged between 10 and 300 mM were added successively into the reaction medium to produce amperometric current–time recordings.

2.3. Synthesis of gold nanoparticles embedded PP-co-Im

Synthesizing reaction was performed by dissolving 0.25 g of imidazole and 1.43 g of chlorinated polypropylene (PP-Cl) in 30 mL of purified THF separately. 0.20 g of NaH in oil (60 wt.%) was added to the imidazole solution and the reaction mixture was stirred at room temperature under argon atmosphere for 3 h. Sodium salt of imidazole solution was added to the PP-Cl solution and stirred for half an hour. The reaction mixture was stirred one more day and poured into 500 mL of 1 M HCl. The precipitated polymer was filtered, washed with distilled water and dried under vacuum at 50 °C for 24 h. For the purification, it was re-dissolved in chloroform and re-precipitated in 200 mL of methanol and dried under vacuum overnight at room temperature. 100 mg of synthesized PP-*co*-Im was dissolved in 10 mL of THF. 0.05 mL of 0.2 M $HAuCl_4$ aqueous solution was poured into this solution and stirred for 30 min. The color of Au was observed within 2–3 min. Following this step, the solution was stirred for 1 h and poured into a Petri dish (diameter, 7 cm) by means of film formation via solvent casting. After a day, gold nanoparticles embedded PP-*co*-Im film was peeled away from the Petri dish. The polymeric film was washed with methanol and dried under vacuum at room temperature for 24 h. 10 mg mL^{-1} of the gold nanoparticles embedded PP-*co*-Im solution was prepared in toluene for readily use in EFC experiments.

2.4. EFC working electrode fabrication

The electrode fabrication step was preceded by a cleaning phase of working electrode surface using gamma alumina powder then, rinsing with distilled water. 1 μL of the gold nanoparticles embedded PP-*co*-Im solution was directly spread onto the surface of the cleaned anode and cathode then dried for solvent evaporation at room temperature. After washing the electrodes with distilled water, 15 μL of GOx and BOD (20 mg mL^{-1}) was dropped onto the PP-*co*-Im coated anode and cathode, respectively, and then waited for enzyme adsorption at room temperature for 2 h. The electrodes were washed in 2.5 mL of phosphate buffer solution (pH 7.4, 100 mM, 20 °C) to remove the unbound enzyme.

3. Results and discussion

3.1. Characterization studies

FT-IR spectra measurement was conducted by using Perkin Elmer Spectrum 100 spectrometer. The spectrum of the pure PP-*co*-Im was presented in Fig. 1. The strong signals were observed at 1081 cm^{-1} (C–N stretching) and 1495 cm^{-1} (C═N stretching). The characteristic signals of C═C and C–H stretch were observed at 1604 cm^{-1} and 2920–3027 cm^{-1}, respectively [26]. These bonds indicated that imidazole was chemically bonded to the side chains of the PP.

Scanning electron microscope (SEM) images of the PP-*co*-Im (Fig. 2A), GOx immobilized PP-*co*-Im (Fig. 2B) and BOD immobilized PP-*co*-Im (Fig. 2C) coated electrode surface were taken with Quanta FEG 450 model device. The SEM of the PP-*co*-Im film presented a uniform polymeric layer. It was observed that the surface morphologies of enzyme immobilized PP-*co*-Im coated working electrodes were different from the PP-*co*-Im layer.

The electrochemical characteristic of the enzyme immobilized gold nanoparticles embedded PP-*co*-Im film coated electrodes was evaluated through cyclic voltammetry (CV) (Fig. 3). The CVs were obtained in 10 mL of phosphate buffer (pH 7.4, 100 mM, 20 °C) at a potential scan between −0.5 and +0.5 V at a scan rate of 100 mV s^{-1}. CV shapes suggested that the polymeric film did not block the electron transport between the electrode materials and the enzymes. This result might be attributed to the metallic nanoparticle content of the polymer. PP-*co*-Im (without nanoparticle) coated electrodes did not show an applicable CV shape and electroactivity (*not shown*).

3.2. Selection of the cathodic enzyme

The oxygen reduction reaction requires addition of catalysts, such as platinum, to increase electrode kinetics in traditional fuel cell cathodes. However such catalysts are non-selective, and reportedly less efficient in comparison to enzyme catalysts. The enzyme catalysts used for the application of oxygen reduction reaction in EFCs are the multiple copper oxidases such as laccasses, bilirubin oxidases and polyphenol oxidases since they can reduce oxygen directly to water [27]. The redox potential of the bilirubin oxidases is over 0.3 V more negative than laccase from *T. versicolor*, which reduces the maximum possible EMF of a glucose–O_2 biofuel cell [4]. Therefore, in most biofuel cells, laccase enzyme has been selected as the cathode although its performance and stability requires an acidic pH [4,28–33]. Laccase activity can be higher in comparison to BOD activity when operating conditions were optimized for both of them [34]. However, both BOD and GOx shows efficient activity at the same pH level around 7 [35]. This is an advantage for our EFC system operated in compartment-less reaction cell where anode and cathode was immersed into the same solution. From this viewpoint, laccase or BOD enzyme was tested for the cathode side of the EFC system, and energy generations were compared with each other. For this purpose, two fuel cell systems including GOx/laccase or GOx/BOD electrodes were fabricated with the same fabrication procedure as following: 1 μL of nanoparticles embedded PP-*co*-Im solution was directly spread onto the surface of the cleaned gold anode and cathode. The electrodes were then allowed to dry for solvent evaporation at room temperature. 15 μL of GOx (20 mg mL^{-1}) was dropped onto the anode, 15 μL of BOD (20 mg mL^{-1}) or 15 μL of laccase (20 mg mL^{-1}) were dropped onto the cathode, respectively. The working electrodes were allowed to dry and waited for enzyme immobilization at room temperature for 2 h.