Glucose oxidase as a biocatalytic enzyme-based bio-fuel cell using Nafion membrane limiting crossover

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Abstract. A novel combination for an Enzyme-based Biofuel cell included a Nafion membrane as an ion transporter that maintained a working cell charge and inhibited membrane degradation. The prototype cell chamber used oxygen (O₂) in the cathode cell and glucose in the anode. The Nafion membrane stability studied here was evidently in the region of 0% loss of conductivity as the charge was constant and increased after the addition of glucose. The prototype cell chamber used NaCl in the cathode cell and glucose oxidase (GOx) in the anodic chamber was successfully studied for membrane stability showed in this study no evidence of poisoning from membrane leakage in a controlled pH environment. There was no crossover at the anaerobic operating ambient temperatures and under physiological pH 5 - 7 conditions. In this research we have successfully used a Nafion membrane together with GOx and under controlled conditions produced respectable power densities.

Introduction

Enzyme-based biofuel cells (EBBFC’s) were first reported in 1964 using glucose oxidase (GOx) as the anodic catalyst and glucose as the fuel [1]. Developments to improve the enzyme based fuel cell have significantly advanced the technology since [2-5]. Biological fuel cells (BFC’s) use Bio-catalysis where naturally occurring enzymes in a living organism that is able to transform organic compounds into sources of energy. The specialized function and applications include implantable devices, sensors, drug delivery, micro-chips, and portable power supplies [6-8]. Enzymes are isolated by laboratory validated techniques to perform these catalytic functions. Glucose oxidase alternatively known as Beta-D-glucose:oxygen 1-oxido-reductase, D-glucose-1-oxidase, Glucose aerodehydrogenase, Glucose oxyhydrase or GOD was used in this study. GOx catalyses the oxidation of glucose as follows:

\[ \text{Beta-D-glucose + O}_2 \rightarrow \text{D-glucono-1,5-lactone + H}_2\text{O}_2 \]  (1)

GOx functioning depends on a cofactor, flavin adenine dinucleotide (FAD). FAD is commonly found as a component in biological oxidation-reduction (redox) reactions. Redox reactions involve a reduction and oxidation process where gain or loss of electrons from a molecule occurs. In the GOx-catalysed redox reaction, FAD works as the initial electron acceptor and is reduced to FADH₂. The process involves the further processing of where FADH₂ is oxidized by the final electron acceptor, molecular oxygen (O₂), which can do so because it has a higher reduction potential. O₂ is then reduced to hydrogen peroxide (H₂O₂). Enzyme-based fuel cells feasibility has encouraged researchers to explore its potential further due to the increased production rates associated with enzymes that in turn provides an increased bio catalysis rate. BFC’s can readily produce a source of electrons however there is a critical deficiency in transporting the electrons to the electrode for the active site. Advances...
involving the immobilization of enzymes at electrode surfaces using many different methods have significantly facilitated the electron transfer rate to the electrode making EBBFC’s an even more attractive prospect. The immobilization, of the enzyme, Electrode Surface Enzyme Immobilization (ESEI) supported by electron carriers shuttling the electron to the electrodes was evidently in progress throughout the working period of the EBBFC. However previously direct electron transfer (DET) [9] between an enzyme and electrode has only been observed with several enzymes, such as cytochrome c, laccase, hydrogenase, and several peroxidases, including microperoxidases [10-13].

Although much of the early attention was devoted to the study of reactions occurring at anodes, biological molecules can also be utilised to catalyse the reduction of oxygen at the cathode as an alternative to the classical use of platinum. Microperoxidase [14] can be immobilised onto a gold cathode and along with a quinone modified cathode be utilized in a biofuel cell fuelled by NADH and H$_2$O$_2$. Similar cathodes were used along with apoglucose oxidase/quinone/flavin adenine dinucleotide phosphate modified anode [15] to construct a glucose/H$_2$O$_2$ fuel cell capable of generating 310 mV with a power output of 32 µW. The same anodes and cathodes could also be utilised in a biofuel cell containing two immiscible solvents with the anode being immersed in aqueous glucose and the cathode being immersed in cumene peroxide/dichloromethane [16]. Chemical and physical methods of ESEI do exist and most of the EBBFC’s reported thus far have been configured employed physically immobilized enzymes. It has become common practice to absorb the enzymes onto conductive particles such as carbon black or graphite powder. GOx was first adsorbed on synthetic graphite particles, and then the enzyme-adsorbed particles were suspended with 2-hexadecanone and ferrocene in a solvent of chloroform.

1. Experimental
The anode chamber contained Glucose Oxidase (Medical Grade) and glucose, 0.01% (m/v). The anode chamber contained neither facilitator nor mediator. The cathode chamber contained 10% (m/v) NaCl (Kimix). The electrodes used were carbon graphite rods (40mm length, 4mm diameter) 1 per chamber. Circuit connected using copper wire and chrome plated electrically conductive crocodile clip connections. The clip connections were not in contact with the solutions in either chamber. The experiments were conducted under ambient room temperature conditions and ranged from 20-25°C.

IR spectra were taken using Paragon 500 Infrared Spectrometer. pH influence on open circuit voltage (vs. E): 0.001 M HCl (Kimix batch no. 264) was monitored using Metrohm pH meter. Drop wise addition of NH4OH (Kimix) to maintain the optimum pH neutralizing the acidic properties of Nafion was employed. Electrochemical measurements have been performed using Metrohm Auto lab system.

2. Results and discussion
In this study a simplistic EBBFC construction of a glucose/O$_2$ biofuel cell and use of a of proton exchange membrane for adequate separation of anode and cathode compartments was tested. Obtained current density ranged from 0.6-0.5 µAcm$^{-2}$ at -0.30 V minimum. The OCV also varied and a minimum was recorded at -0.30 V. This was at physiological pH.

Enzyme based biofuel cell using GOx and glucose as a fuel source was conducted at pH as figure 1 represents the optimum functioning pH was approximately 5.5-6.0. The potential was found to be dependent on the pH. Using a combination of Graphite (Anode), Graphite (Cathode), membrane including Nafion use as the research focus area the following power output of 30-250 µW/cm$^2$ was obtained.
The acidic properties of Nafion are not conducive for biofuel cell performance due to enzyme inactivation at low pH’s; however, the use of NH\textsubscript{4}OH to neutralize the sulphonic acids while maintaining the optimum pH of approximately 6.5 was able to ensure longer periods of operation.

The initial catalysis of glucose reactions were slow for the first 2 days, thereafter activity increased steadily. A peak output 250 µW/cm\textsuperscript{2} was reached 72 hours after the glucose addition (figure 2-3). As this research study did not look at cathode modifications future enzyme based Biofuel cell use of Microperoxidase at cathode along with gold where the biofuel cell will be fueled by NADH and H\textsubscript{2}O\textsubscript{2}. Identification and continued pH sensitivity of anode and cathode reaction conditions will be tested also using acid/polyvinyl alcohol copolymers. A Biliruben Oxidase cathode will also be attempted in future projects. Other enzymes currently synthesized and sourced include alcohol dehydrogenase (ADH), amyloglucosidase (AGS), invertase (INV) and glucose dehydrogenase (GDH). These sources of oxidation reaction catalysis will be considered in future experiments to obtain the optimum combination of electrode type and absorbed immobilized enzyme.
3. Conclusions
Research communities are constantly seeking new sustainable energy sources and have identified BFC’s and more specifically enzyme-based biofuel cells as a source of renewable and sustainable power. They are highly attractive for special miniaturized applications, such as implantable devices, sensors, physiological applications including drug delivery, microchips, and other portable power supplies. Several drawbacks, such as short lifetime and low power density, have limited enzyme-based biofuel cells from being used for practical applications. Recent developments in the newly emerging nano-biocatalysis appear to be promising because they provide some solutions in overcoming the present problems facing researches attempting to find cost effective alternative energy sources. Better understanding and further developments of nano-biocatalysis will fast track the improvement of biofuel cells, and once these variables have been identified and optimized to produce high performance biofuel cells the future role of BFC’s will be significant in this dynamic energy market. In this research we have successfully used a Nafion membrane together with GOx and under controlled conditions produced respectable power densities.

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