INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Development of Small Scale Alcohol Biofuel Cells using Enzymes as Catalyst

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SHORT ABSTRACT

The major objective of the present study is to develop small-scale alcohol fuel-based enzymatic biofuel cells (EFCs) for power generation and alcohol biosensing applications using alcohol oxidase (AOx) as anodic catalyst. One of the key approaches we propose to develop the small-scale EFCs is to exclude external pumping system from fabricating the devices. To make the idea effective, we introduce natural cellulosic materials (cotton or papers) in the device fabrications to deliver the fuel to the bioanode through passive diffusion activity. An additional important objective of the present work is to develop a biocompatible conductive ink with high aqueous stability for fabrication of the bioelectrodes, as these are vital properties of an ink to harvest stable bioelectrocatalytic function of the redox enzymes on the electrode surfaces. We studied some biopolymers and eventually identified silk-sericin and polyethylene glycol (PEG) as suitable materials for developing a graphite-based conductive ink in a ratio of 0.03:2.0:1.0 for sericin:PEG:graphite. Interestingly, sericin facilitates transformation of amorphous graphite powder to a crystalline form in PEG environment that improved the conductivity of the ink (11.2 $mS.cm^{-1}$) by 5.6-folds from the ink devoid of sericin. The viscosity and shear rate values of the ink were calculated as 0.11 Pa.s and $100s^{-1}$, respectively, thermos-stability up to 100 ^0C, and heat of formation (ΔH_f) -4.204 KJ/g. In addition, the ink coated over paper surface retains high aqueous stability and the reason being recognized as the enhancement of β–sheets content of sericin by 2.8% upon mixing it with PEG. Then we moved towards the selection of a cathodic enzyme and used laccase for the same. The laccase enzyme, which is extracted from *Tramates versicolor* fungi, can oxidize phenolic compounds with concomitant reduction of molecular oxygen to water. Laccase was immobilized on a glassy carbon electrode using a nanocomposite matrix comprising of osmium tetroxide on poly 4-vinylpyridine, multiwalled carbon nanotubes, nafion and carbon black. SEM images revealed that the nanocomposite matrix provides a porous structure for easy immobilization of the enzyme. Whereas cyclic voltammetry studies explained that, the nanocomposite matrix offers a highly electroactive surface for facile diffusion-free electron transfer kinetics. The response of the bioelectrode for oxygen substrate at the determined formal potential of laccase was established. The heterogeneous electron transfer rate constant (k_s) and surface concentration of the ionic species (Γ) of the bioelectrode were discerned as $0.67s^{-1}$ and 1.32×10^{-8} $mol.cm^{-2}$, respectively. The results infer the potential

application of the constructed bioelectrode as oxygen breathed biocathode for EFC application. This bioelectrode also offers a reliable electrochemical response towards pyrocatechol in a biocatalytic mode. The response of the fabricated biosensor was generated at a potential of 0.14 V from the electrocatalyzed reduction of 1, 2-benzoquinone formed from the biocatalyzed oxidation of pyrocatechol. The bioelectrode showed a linear range of output current against pyrocatechol in the concentration range of 3.98 nM-16.71 nM with a minimum detection limit of 2.82 nM and a sensitivity of 3.82 ± 0.31 nA nM^{-1}. After working on cathodic enzyme, we focus on developing a small sized EFC utilizing paper (pEFC) as support material for methanol biosensing application. We used the as prepared Graphite-PEG-Sericin conductive ink for making support electrode for enzymes on chromatography paper surface. To this end, we immobilized AOx on anode, and bilirubin oxidase (BOx) instead of laccase on cathode. It may be mentioned that due to incompatibility of laccase in physiological pH, we later replaced this enzyme with BOx, which exhibits high activity in the pH value. The sensor showed a linear range of output current of 0.03125μM -0.5μM (R^2 = 0.9988), sensitivity of 0.66245 $\mu A \mu M^{-1}$ and a detection limit of 0.022 μM for methanol validating the potential of the pEFC for methanol biosensing application. Next, we report a methanol-fueled pure EFC fabricated by using AOx and BOx as anodic and cathodic catalysts, respectively with a focus on power generation. Here, we have fabricated EFC with a new design strategy comprising a passive fuel pumping facility to the anode, efficient anoxic conditions in the anodic chamber and adequate airflow to the cathode for enhancing oxygen reduction reactions, and a connected storage tank for fuel. A bio-nanocomposite paste comprising of the as prepared Graphite-PEG-Sericin ink with magnetic nanoparticle over the supporting carbon cloth electrode was used as the biocompatible enzyme immobilization matrix for harvesting electron in the EFC through direct electron transfer mechanism. The open circuit potential of the device increased to 4.3-fold (3.1V) upon stacking six units of the EFCs in series. The device also rested at a stable state under a light emitting diode as load with a half-life of 372 days (w.r.t voltage) and a coulombic efficiency of 60%. This exceptional high operational stability has been accredited to the efficient anoxic setup in the anodic chamber that supported the stability of AOx, the activity of which was intact even after 49 days of the operation. This work also validated that the prolonged interaction of molecular oxygen with AOx significantly inactivates it without affecting the structural integrity of the enzyme protein. This EFC with improved design and functions is a step forward for achieving practical application as a standalone power supply to small-scale devices.