Enzymatic Biofuel Cells for Micro-Power Source Applications

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Abstract

Enzymatic bio-fuel cells present a viable candidate for ultimate miniaturization because it is a class of energy conversion devices that employ molecular electrocatalysts – enzymes and hierarchical surface architectures developed in the course of the advancement of bio-nano interface technology. Many of the fabrication technologies and 3D structural motifs can be achieved with today's MEMS fabrication tools. In general, enzymatic bio-fuel cells can be successfully employed in devices where "scavenging" for environmentally available fuel sources is feasible and diversity of fuels is desirable.

Keywords: Enzymatic Bio-Fuel Cells, Micro-Fuel Cells, Power-MEMS, Biosensors and Autonomous Microsystems

1 - INTRODUCTION

Enzymatic fuel cells use enzymatic bio-catalysis to convert chemical energy directly to electrical energy. [1] As the technologies required for these devices are advancing, they will be available to serve as portable, flexible, compact, and sustainable micro-power sources. The tremendous catalytic abilities of enzymes will allow for the use of either single or multiple reactions that can utilize a wide range of fuel sources, including ambient carbohydrates and macromolecules. This will allow a bio-fuel cell powered device to refuel itself from the environment, which will enable flexible, efficient, and distributed power generation. The extreme selectivity of enzymes also dramatically simplifies of the design of fuel cells, as separators, seals, and casings can be eliminated from their fabrication.

This paper will address the approaches to overcome the three main scientific challenges that hamper the development of bio-fuel cells. First, the interface between the enzyme and the electrode must be characterized and optimized. An ideal enzymatic electrode should be limited by catalyst loading, and not by the transport of electrons between the enzyme and the electrode. The second key challenge is the stability of the bio-electrodes used in the bio-fuel cell. There is often a tradeoff between enzymatic stability and activity, but optimal bio-fuel cells will exhibit both high stability and activity.

And the final challenge is the spatial control of catalyst and substrate distribution at the electrode. Mastery of this will be required for bio-fuel cell practical applications and mass fabrication. The paper will present a discussion of the power source design envelope in terms of limitations of power and energy densities of enzymatic bio-fuel cells placed in the context of MEMS-based micro-power sources. Figure 1 is aiming to illustrate our initial hypothesis in terms of volumetric and gravimetric energy densities of bio-fuel cells as compared to the current secondary battery technologies.
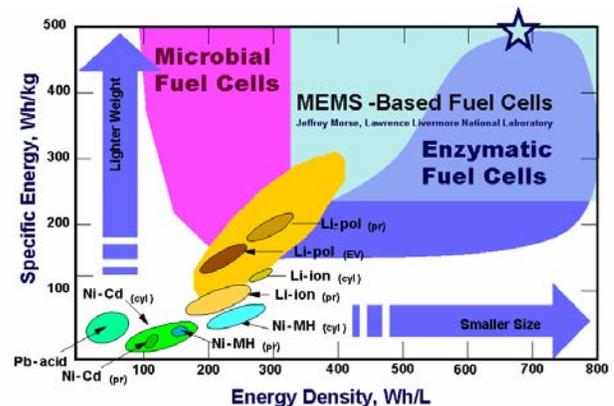
Figure 1: Volumetric and gravimetric energy densities of bio-fuel cells as compared to the secondary battery technologies.

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Use of enzymes as a catalyst for biologically derived fuels represents an opportunity to tap into natural fuel sources, such as mammals and plants, rather than having to depend on more logistic power sources such as batteries for field operations. An enzyme that is fully functional in the laboratory, however, may not perform well in service in an actual power application, due to the multitude of additional factors that are imposed on the catalyst during actual operations. Some of these factors do not even require field conditions to become relevant, such as the stability of the enzymes against electrodes during the process of assembling the power system, and their compatibility with other components in the power system. University of New Mexico and Sandia National Laboratories has been developing technologies that are critical for micro-fabrication of fuel cells and integration them into a MEMS device. They represent an integration of *bottom-up* techniques such as self-assembly of organic and inorganic materials and *top-down* technologies such as lithography and micro-machining. An overview of some of the issues faced between the development and understanding of enzymatic catalysis, integration in micro-devices and applications in a field environment, as is the ultimate goal, will be discussed, with emphasis on assembly and test of the systems under "real world" conditions.

2 – BIOINTERFACE & SURFASE ARCHITECTURES

One of the major fuel cell technology paradigms, that should be considered conservative in the case of the scale down to MEMS-size devices with expected foot-print in the order of 1 mm^2 is the high surface-to-volume ratio for the fuel oxidizing anode and the related tri-phase interface design of the air (oxygen) gas-diffusion cathodes. The challenge in applying this paradigm to enzyme-catalyzed electrodes is in the fact that those architectures should also serve as a immobilization matrix for the corresponding enzymes. In case of direct electron transfer (DET) they should facilitate favorable enzyme protein globule orientation and interaction with the enzyme active site in the process of catalysis. [2] In the case of mediator-assisted electron transfer (MET) the electrode should serve as a suitable substrate not only for enzyme immobilization but also for integration of the low-molecular weight redox mediator into the surface architecture. This could be accomplished by electro-deposition or electro-polymerization or by coating technologies among which, layer-by-layer (LBL) deposition has been attracting considerable attention. Among the materials based technologies for such electrode structures that are inheritably scalable down carbon nanotubes present several substantial advantages.

Carbon nanotubes (CNT) allow for engineering a broad range of electrode structures useful in biosensor and bio-fuel cell devices. CNT allow for building surface architectures with distinct levels of hierarchical organization of pore structures. Nano-structured supports derived from such nanotubes demonstrate pore structures that provide unique capabilities due to large surface-to-volume ratio combined with substantial porosity. This is of particular importance in designing flow-through anodes for bio-fuel cells.
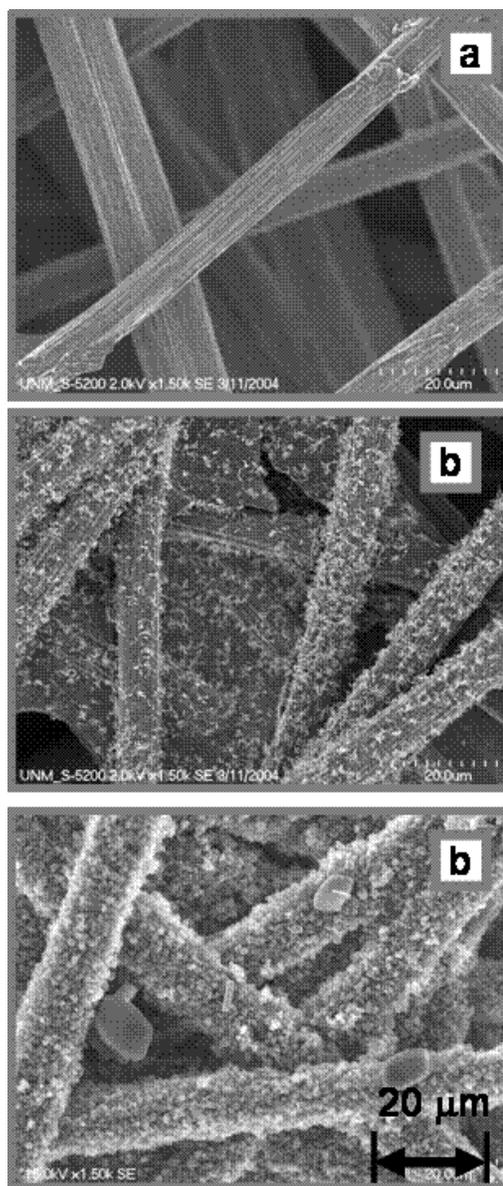

Figure 2: Toray carbon paper (a) with CVD deposited multi-walled CNT (b) and the final glucose electrode structure after PEI coating and Glucose Oxidase immobilization in modified Nafion matrix (c).

We have developed the synthesis of a hierarchically porous conductive membranes derived from carbon papers with consecutively grown multi-walled carbon nanotubes (MWCNT). Carbon paper is a synthetic lightweight matrix with pore spaces on the micro-meter scale (see Fig. 2.a). The MWCNT's are grown on this matrix by chemical vapor deposition (CVD) from acetylene precursor at an elevated temperature. This process is preceded by electrodeposition of cobalt nano-particles as a catalyst for CNT growth.

As a result a decorative phase of CNT is grown on the filament substrate of the carbon paper (see Fig. 2.b). The length of the individual CNT filaments and the spacing between them is at the nano-meter scale. The density of the carbon nanotubes is evidently proportional to the amount of cobalt deposited and the volume fraction is proportional to the time of CVD. Being able to control the cobalt deposition and the time of carbon vapor deposition allows building surface architectures with distinct levels of hierarchical organization of pore structures.

We have demonstrated successful functionalization of the CNT carbon paper matrix with enzyme Glucose Oxidase as an avenue for engineering anode for miniature bio-fuel cell applications. [3] This bio-fuel architecture is viewed as a platform for a flexible design allowing fabrication of both mediated enzyme electrodes and electrodes based on direct electron transfer oxidation of the substrates. Similar design concept utilizing in contrast on hydrophobized carbon paper substrate is being developed for the oxygen reduction air-breathing cathode of the bio-fuel cells.

3 – DESIGN OF GLUCOSE OXIDATION ANODES

3.1 Mediated Glucose Oxidase Electrodes

The goal of this part of the project was to design glucose oxidation anode and demonstrate its utility in flow-through fuel cell electrode design. For mediated electron transfer electrode design the CNT-carbon paper composite is first dip-coated with a layer poly-ethyleninmine (PEI). This positively-charged polymer serves as an surface modifier for the layer-by-layer (LBL) immobilization of the negatively-charged charged Glucose Oxidase. Mediators from benzo-quinone family are then electrochemically grafted in the PEI matrix to prevent washing off during electrode operation in a flow-through mode. Glucose Oxidase is immobilized in a modified Nafion matrix (courtesy of Prof. S. Minteer, Saint Louis University) applied as a coating over the mediator-PEI layer (see Fig. 2.c). The Nafion is modified to sustain the optimal pH 7 for the Glucose Oxidase function as anode catalysts.

The electrodes were tested in a tri-electrode half-cell set-up to reveal their glucose-dependent polarization. The glucose (fuel) solution was with a concentration in the range from 5 to 50 mM corresponding to that of potentially environmentally available sources (plant sap or blood/tissue supplies from the animals). In galvanostatic experiments the electrodes have demonstrated limited current density up to 2 mA/cm^2. Those current densities were sustained at potentials lower (more cathodic) than – 200 mV vs. Ag/AgCl reference electrode. Combining such anode with an enzymatic air-breathing oxygen cathode will allow a bio-fuel cell with an operational voltage close to 1 V in 1 mW/cm^2 power density (see Figure 3).
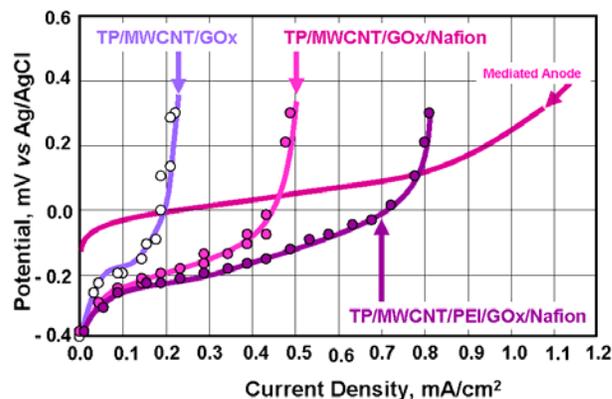

Figure 3: Galvanostatic polarization curves of the GOx anode obtained in half-cell set-up in 0.1 M phosphate buffer, pH 6.9, 20 mM glucose: 1 - TP/MWCNT/PEI/GOx/Nafion; 2 and 3 - TP/MWCNT/GOx/Nafion; and the polarization curve of the mediated GOx anode as described.

3.2 Direct Enzymatic Oxidation of Glucose

Glucose oxidase (GOx) is commonly used as an anode catalyst in enzyme bio-fuel cells. This enzyme is stable, readily available and active in glucose oxidation. Glucose as a fuel is widely spread in the environment. However, critical challenge in the successful development of a practically valuable GOx anode for bio-fuel cells is in effective electrical communication between enzyme molecule and electrode surface. As known, the FAD/FADH$_2$ redox centers are located deeply in the apoenzyme. Depth of the redox center for GOx is about 13 Å. Therefore, direct electron transfer (DET) rate between active site of GOx and electrode surface is usually slow. Last years numerous efforts have been made to achieve efficient DET between GOx and electrode surface by using different promoters. One of the strategies is to use nano-structured electrodes.

Carbon nanotubes (CNT), with their size, chemical stability and electrical conductivity, can be used as "nano-wires" for fast DET between enzyme and electrode surface. We have exploited unique properties of multi-walled carbon nanotubes (MWCNTs) for the fabrication of glucose oxidase electrode as an anode for biofuel cell based on mediatorless DET communication between the active site of enzyme and the electrode surface. Toray carbon paper (TP), which is a highly porous three-dimensional network of carbon fibers, was used as a basis for growth of MWCNTs followed by GOx immobilization.

Cyclic voltammograms of GOx modified electrodes show well defined redox peaks after immobilized of GOx on the TP/MWNTs and TP/MWNTs modified with polyethylene imine (PEI)). There are no peaks observed with just plain TP electrode with immobilized GOx. The redox peaks can be considered as a result of DET between the prosthetic (FAD/FADH$_2$) group bound to the apoenzyme and electrode surface. This fact has been confirmed by bio-electrocatalytic

oxidation of glucose on such electrodes. We found that there is linear proportional between the anodic and cathodic peak currents at the scan rate ranging from 40 to 220 mV/s. It is typical characteristics of a thin layer electrochemical behavior, suggesting that the reaction is not a diffusion-limited process and the redox peaks are from surface bound prosthetic group FAD of the GOx. The peak separations between 24-50 mV at scan rate of 10–80 mV/s indicate that the heterogeneous pseudo-reversible electron-transfer process was fast.

Typical galvanostatic polarization curves of glucose oxidation on such bio-fuel cell anodes are presented in Fig. 3. The GOx electrodes operate at 20°C in 0.1 M phosphate buffer solution, pH 6.9, 0.1 M KCl in the presence of 20 mM glucose. The observed open circuit potentials of GOx-modiied electrodes are close to the redox potential of the FAD/FADH$_2$ cofactor in the native enzyme: between -0.35 V and -0.4 V vs. Ag/AgCl. Depending on electrode architecture, limiting current densities for electro-oxidation of glucose from 0.2 to 0.8 mA/cm^2 were observed all being reached at potentials more anodic than 50 mV vs. Ag/AgCl (see Fig. 3). The GOx electrode based on DET allows operating at quite negative potentials closer to the redox potential of the FAD/FADH$_2$ cofactor in enzyme and provides excellent opportunity to simplify and miniaturize membrane-less bio-fuel cell construction.

4 – DESIGN OF AIR-BREATHING CATHODES

Development of bio-fuel cells in various design definitions frequently involves the need of a selective air cathode. [1] During the last two years research in our laboratory has been focused on gas-diffusion laccase- and bilirubin oxidase-catalyzed cathodes, operating under "air-breathing" conditions, which can utilize oxygen directly from atmospheric air. In this paper, we report on the development of such gas-diffusion enzyme-catalyzed cathode for integration with a corresponding glucose oxidase anode. Bio-fuel cell characteristics, such as, power density, cell voltage, cell design parameters all rely on compatibility of the two electrodes. Direct electron transfer (DET) offers the opportunity to design cells with close to maximal open circuit voltage since the electrode processes will occur with minimal overvoltage. [2] We have shown that the electrode surface chemistry is of particular importance for creating favorable conditions for DET of oxygen reduction reaction catalyzed by laccase. [4]

Our design of oxygen (air) gas-diffusion electrodes is based on a hierarchically porous conductive membranes derived from Toray carbon paper (TP) with consecutively grown multi-walled carbon nanotubes (MWCNT). TP is a synthetic lightweight matrix with pore spaces on the micro-meter scale. The MWCNTs are grown on this matrix by chemical vapor deposition (CVD) from acetylene precursor at an elevated temperature. As a result a decorative phase of MWCNT is grown on the filament substrate of TP. The

length of the individual MWCNT filaments and the spacing between them is at the nano-meter scale (see Fig. 1). In this work bilirubin oxidase (BOD) from *Myrothecium verrucaria* was used for bio-electroreduction of oxygen based on direct electron transfer. BOD was immobilized on the hydrophilic site of an anisotropic hydrophilic/hydrophobic composite membrane: gas-diffusion electrode.

Cyclic voltammograms of such electrodes show an on-set potential for electroreduction of oxygen at +0.60 V vs. Ag/AgCl with a decrease in the overpotential by 0.8 V compared to the blank electrode without the enzyme. Polarization curve was measured using BOD-catalyzed gas-diffusion electrode in the galvanostatic mode, with an open circuit voltage (OCV) of 0.60 V vs. Ag/AgCl (pH 7) demonstrate limiting current density that depends on the density of MWCNT used as a base for this electrode design (see Fig. 4). In this paper we will report on the characteristics of the BOD-catalyzed oxygen reduction. This paper will present in detail the structural analysis of the electrodes and electrochemical characterization of the interfacial electron-transfer rates, analysis of bio-catalytic rate constants and will provide a hypothesis on the mechanism of DET between BOD and composite MWCNT electrode surface. We will discuss the application of BOD-catalyzed air-breathing cathode in miniature enzymatic bio-fuel cell design.
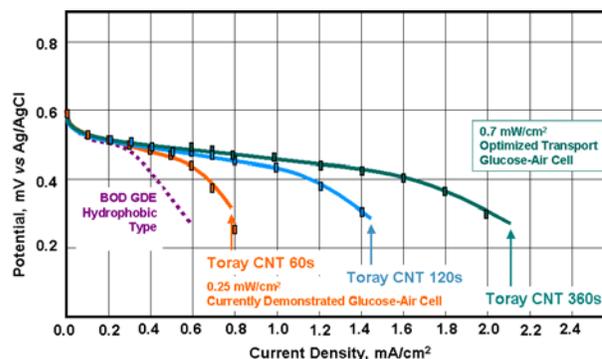

Figure 2: Galvanostatic polarization curves of the bio-fuel cell gas-diffusion air cathode with BOD as oxygen reduction electrocatalyst designed on the TP/MWCNT from Fig. 2 and compared with previously reported results.

References:

1. S.Calabrese Barton, J. Gallaway, and P. Atanasov, Enzymatic Biofuel Cells for Implantable and Microscale Devices, Chem. Rev., 104 (2004) 4867-4886.
2. A.L. Ghindilis, P. Atanasov and E. Wilkins, Enzyme Catalyzed Direct Electron Transfer: Fundamentals and Analytical Applications, *Electroanalysis*, 9 (1997) 661-674
3. D. Ivnitski, B. Branch, P. Atanassov, C. Apblett, GlucoseOxidase Anode for Biofuel Cell Based on Direct Electron Transfer, *Electrochemistry Communications* 2006, (2006) 1204-1210.
4. G. Gupta, V. Rajendran and P. Atanassov, Bioelectrocatalysis of Oxygen Reduction Reaction by Laccase on Gold Electrodes, *Electroanalysis*, 16 (2004) 1182-1185