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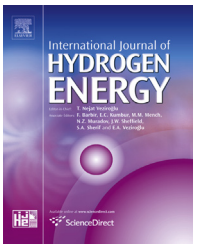

Paper based biofuel cells: Incorporating enzymatic cascades for ethanol and methanol oxidation

Carolin Lau [a], *Michael J. Moehlenbrock* [c], *Robert L. Arechederra* [c], *Akinbayowa Falase* [a], *Kristen Garcia* [a], *Rosalba Rincon* [a], *Shelley D. Minteer* [b], *Scott Banta* [d], *Gautam Gupta* [e], *Sofia Babanova* [a], *Plamen Atanassov* [a,*]

[a] *Department of Chemical and Biological Engineering, Center for Micro-Engineered Materials, University of New Mexico, Advanced Materials Laboratory, Albuquerque, NM 87131, USA*

[b] *Department of Chemistry and Materials Science, University of Utah, 50 Central Campus Dr, Salt Lake City, UT 84112, USA*

[c] *Department of Chemistry, Saint Louis University, One North Grand, St. Louis, MO 63103, USA*

[d] *Department of Chemical Engineering, Columbia University, Rm. 801 Mudd, 500 West 120th Street, New York, NY 10027, USA*

[e] *Center for Integrated Nanoechnologies and MPA-11, Los Alamos National Laboratory, Los Alamos, NM, USA*

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ABSTRACT

Here we developed a flow-based system resulting in improved performance of enzyme cascade-based biofuel cells. A paper-based biofuel cell with passive laminar flow was build to show the impact of flow on the performance of two different enzyme cascades – methanol and ethanol cascade. Both cascades demonstrated enhanced electrochemical output as a consequence of the decreased diffusion path of reaction intermediates identifying the intermediates diffusion in between enzymatic active sites as the rate limiting step in cascade operating systems.

Introduction

The need for alternative sources of energy has led to the exploration of non-traditional fuel sources, unique inorganic and biological catalysts, and alternative power generating devices. Enzymes derived from bacteria and other sources have been widely reported in the literature as potential catalysts for fuel cells [1–4]. These enzymatic fuel cells are fast becoming an important research focus due to the ubiquity of the catalysts, the simplicity of the design, and the availability of hydrocarbon fuels such as sugars (e.g. glucose) and alcohols

* *Corresponding author*. Tel.: +1 505 277 2640; fax: +1 505 277 1421.
E-mail address: plamen@unm.edu (P. Atanassov).

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(e.g. methanol, ethanol, glycerol). Most biofuel cell devices in the literature are actually operated more like battery devices than fuel cell, because they contain separate catholyte and anolyte solution containers and there is no flow of anolyte or catholyte solutions. This means that transport in the biofuel cell is due to diffusion, which is relatively slow. This approach can be advantageous as the reactions take place in a single phase, and soluble cofactors and mediators will stay within the system. The downsides to this approach include product accumulation and lack of scalability for large-scale power production.

In biological organisms, groups of enzymes are used in cascades to oxidize complex fuels completely to CO_2. Recent research has shown that incorporating these enzyme cascades can improve the extent of oxidation, power densities, and energy densities of biobatteries and biofuel cells [5–8]. However, most enzymatic cascades become transport limited, because the product of the first enzyme must diffuse over long length scales to find the active site of the second enzyme for the second step of electrocatalysis. Adding flow to these systems can potentially improve transport between active sites in the cascade, but this can introduce new challenges such as the removal of intermediate species from the cascade before complete oxidation. And, flow can lead to the removal of essential cofactors and mediators.

Recently, a paper-based biofuel cell has been developed that utilizes a filter paper fan platform to induce passive flow via wicking of the solution up the paper while evaporation is occurring at the top of the paper fan [9]. This configuration decreases the distance between the anode and the cathode to minimize resistance, as well as providing a steady laminar flow of fuel across the bioanodes.

A detailed study of the flow in porous membranes of complex shape such as the paper-based design has been provided by Mendez et al. [10]. A simple mathematical model (Eq. (1)) of the flow of a liquid driven by a capillary action within pores of a porous membrane has been developed.

$$\nabla \bullet \mathbf{v} = -Q,\ \mathbf{v} = -\frac{k}{\mu}\nabla P \tag{1}$$

where $\mathbf{v}$ is the flow velocity, Q is an evaporation term, k is permeability, μ is liquid viscosity and P is the atmospheric pressure. This model shows that both geometric and evaporation effects significantly influence the liquid imbibition and give a steady state flux into the medium. Over time, the liquid–air interface within the porous body migrates toward the dry regions as a consequence of the surface tension induced pressure differential at the interface [10].

The paper-based device has been already demonstrated via the incorporation of air-breathing bilirubin oxidase biocathodes for reducing oxygen from the air to water [9,11]. We hypothesized that this configuration could also improve the overall utilization of the fuel by improving transport between enzymes within enzymatic cascades. In this paper, we have incorporated two different NAD(H)-dependent enzyme cascades for the multi-step oxidation of fuels in the paper-based biofuel cell platform. We first explored a two enzyme cascade that can oxidize ethanol to acetate and then we explored a three enzyme cascade for complete methanol oxidation to CO_2 (Scheme 1).

Materials and methods

Air-breathing bilirubin oxidase cathode

Approximately 100 mg of teflonized carbon blacks were hydraulically pressed onto a perforated piece of Toray® Paper (1.8 cm^2) which acts as a gas diffusion layer. These were pressed against a multi-walled carbon nanotube (MWCNT) paper that acts as the hydrophilic conductive electrode for enzyme immobilization. This electrode was incubated overnight with 15 mg bilirubin oxidase (BOD, Amano enzymes 1.2 U/mg) dissolved in 5 mL phosphate buffer (0.1 M, pH 7.5).

Ethanol cascade anode with enzymes immobilized in (polyethylene imide) PEI

The PEI procedure consisted of making the casting solution which contained 10 mg/mL linear PEI (LPEI) dissolved in purified water. To 500 μL of this solution, 100 units of each enzyme (AlDH A9770 and ADH A3263, Sigma) were added and the LPEI was cross-linked using a 0.3–1 mol ratio of ethylene glycol diglycidyl ether. Immediately after adding the ethylene glycol diglycidyl ether, 500 μL of this cross-linked suspension was cast onto a PMG coated MWCNT paper and allowed to dry under convective airflow. The PMG coated MWCNT paper was fabricated as previously described [12]. The fuel used in the study was 0.1 M ethanol in phosphate buffer (pH 7.5), with 100 mM KCl and 50 mM NAD^+.

Methanol hydrogel cascade anode

The methanol cascade enzymes were expressed and purified as previously described [13]. 10 mg of lyophilized mixed enzymes (1 U/mg per enzyme) were introduced to 50 μL of 100 mM Tris buffer (pH 8.5) and casted onto a 1 cm^2 PMG coated piece of MWCNT paper, where the PMG coated MWCNT paper was fabricated as previously described [12]. The mixture was allowed to dry for about 30 min before being included in the biofuel cell. Fuel used in the study was 0.1 M formic acid, 0.1 M formaldehyde and 0.1 M methanol individually or with all three fuels (each 0.1 M) together in phosphate buffer (pH 7.5), 100 mM KCl and 50 mM NAD^+.

Assembly of full paper based biofuel cell and electrochemical measurements

The electrochemical cell was assembled by placing bio-anode and bio-cathode on opposite sides of a piece of filter paper (Whatman) and keeping them in place with a piece of tape (3 M), see Scheme 1. The Toray® Paper backbone for the cathode was created with an additional flap that serves as contact. A painted contact made from carbon fibre and highly conductive graphite paint was painted on the backside of the MWCNT-paper cathode for electrical connection with alligator clips. Electrochemical performance data were obtained with a Gamry Ref600 potentiostat. In case of half-cell measurements, an Ag/AgCl reference electrode was placed in the same feeding solution as the fuel delivery layer and a Pt-counter electrode was used instead of the cathode. One hour

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