ELSEVIER

Enzyme-based biofuel cells

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Enzyme-based biofuel cells possess several positive attributes for energy conversion, including renewable catalysts, flexibility of fuels (including renewables), and the ability to operate at room temperature. However, enzyme-based biofuel cells remain limited by short lifetimes, low power densities and inefficient oxidation of fuels. Recent advances in biofuel cell technology have addressed these deficiencies and include methods to increase lifetime and environmental stability.

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Current Opinion in Biotechnology 2007, **18**:1–7

This review comes from a themed issue on
Energy biotechnology
Edited by Lars Angenent

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2007.03.007

Introduction

Fuel cells are devices that convert chemical energy into electrical energy. Biofuel cells are a subset of fuel cells that employ biocatalysts. The main types of biofuel cells are defined by the type of biocatalyst. Microbial biofuel cells employ living cells to catalyze the oxidation of the fuel, whereas enzymatic biofuel cells use enzymes for this purpose. The current advantage to microbial biofuel cells is that they typically have long lifetimes (up to five years) [1,2] and are capable of completely oxidizing simple sugars to carbon dioxide [3]. They are limited, however, by low power densities (i.e. the power generated per unit electrode surface area, typically in Watts/cm^2) owing to slow transport across cellular membranes [4]. By contrast, enzymatic biofuel cells typically possess orders of magnitude higher power densities (although still lower than conventional fuel cells), but can only partially oxidize the fuel and have limited lifetimes (typically 7–10 days) owing to the fragile nature of the enzyme [5••,6•]. Enzymes have the added advantage of specificity, which can eliminate the need for a membrane separator. A conventional enzymatic fuel cell is shown in Figure 1 and it is important to note that the polymer electrolyte membrane (PEM) shown is standard, but if there are selective enzymes on both the cathode and the anode then the PEM is unnecessary.

Both microbial and enzymatic biofuel cells are also plagued by the lifetime and efficiency of their mediators. Mediators are those compounds that shuttle the electrons from the oxidized fuel to the electrode surface. These mediators are typically organic dyes or organometallic complexes, which are either in solution or immobilized at the electrode surface. Although originally overlooked, their lifetime and performance have proven to be as problematic as the enzymes themselves. In this review, we will detail the recent advances in biofuel cells that have provided possible solutions to the mediator and lifetime problems.

Recent advances in enzymatic biofuel cells

One of the most significant advances in biofuel cells has been the development of biocathodes and bioanodes that employ direct electron transfer (DET) instead of mediated electron transfer (MET). The importance of DET is that the electrons are transferred from the catalyst (enzyme) directly to the electrode and problems associated with the use of mediators are overcome. Although DET has been observed for several enzymes in electroanalytical applications, it was not employed in a biofuel cell until 2006 when researchers developed biocathodes for the reduction of oxygen using laccase [7,8] and bilirubin oxidase [9] and bioanodes employing glucose oxidase [10••].

A second key advance has been an increase in the active lifetime of the immobilized enzymes. Enzymes are proteins that typically have short lifetimes (8 h to 2 days) in buffer solution [6], although their active lifetimes can be extended to 7–20 days by immobilization on electrode surfaces via entrapment, chemical bonding, and cross-linking [6]. Recently, active lifetimes have even been extended beyond 1 year through encapsulation in micellar polymers. These polymers physically confine the enzyme and prevent it from denaturing by providing a biocompatible hydrophobic nest and buffered pH microchemical environment [11,12•,13].

Key issues in developing effective enzymatic biofuel cells

Fuel cells require porous anode and cathode structures that support fuel transport to the catalyst reaction sites. Enzymatic fuel cells are no different except that, if they are to compete against primary batteries as a power source, they will require anodes that maximize power density. This requirement demands a solution to three technical hurdles.

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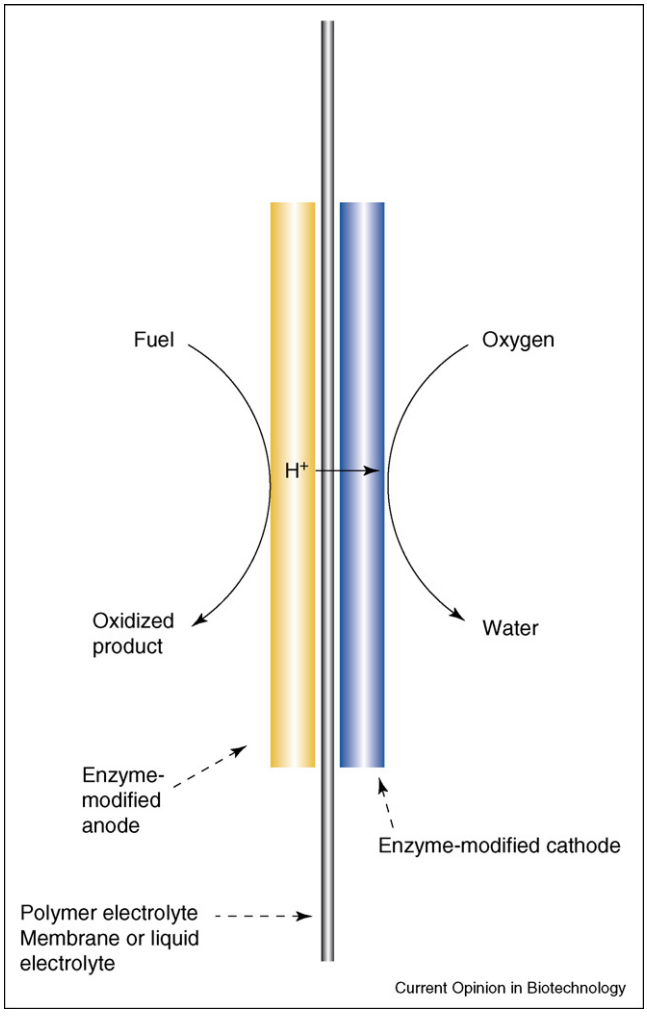
Figure 1



Schematic of an enzymatic biofuel cell.

Firstly, biofuel cell anodes should be three-dimensional, as opposed to biosensors which are less sensitive to this requirement. They should be able to optimize the need for surface area, which generally means smaller pores that increase the reactive surface area and thus the current generated, and the need for larger pores, which generally supports the mass transport of liquid phase fuel.

Secondly, the successful immobilization of multienzyme systems that can completely oxidize the fuel to carbon dioxide is needed. Current enzymatic biofuel cells have low efficiency, as only a single type of enzyme is employed and can only partially oxidize the fuel. This is in direct contrast to living cells that can completely oxidize biofuels (e.g. ethanol, lactate and glucose) to carbon dioxide and water. The first biofuel-based multistep oxidation was demonstrated by Palmore *et al.* [14] who employed alcohol dehydrogenase, aldehyde dehydrogenase and formate dehydrogenase to completely oxidize methanol to carbon dioxide. Since then, little has been done to expand this technique to more complex fuels, although Katz *et al.* [15•] demonstrated the oxidation of mixed fuels using glucose oxidase and lactate dehydrogenase. Akers *et al.* [12•] studied the two-step oxidation of ethanol to acetate using alcohol dehydrogenase and aldehyde dehydrogenase. Although successful, their system permitted only a 33% oxidation of the fuel, which represents a low fuel utilization and energy density.

Finally, the anode must support efficient charge transfer mechanisms, whether it be direct or mediated, and balance electron transfer with proton transfer. It is therefore crucial to understand this interplay between porosity (for fuel delivery and effluent flow), surface area (for catalytic rate), and electronic and proton conductivity (to minimize resistive losses). Although a thorough discussion of these issues is beyond the scope of this work, some commentary on solutions is provided below.

Possible solutions

Three-dimensional electrode architecture

In terms of maximizing power density, three-dimensional bioelectrocatalytic electrodes should possess multidimensional and multidirectional pore structures. Multidimensionality provides both small pores to support enzyme stabilization and high loading densities, and larger ones to support mass transport of liquid phase fuel. Multidirectionality provides greater surface area and permeability to liquid phase fuel transport, but will eventually decrease structural strength (and surface area) if too extensive. Multidirectionality is also important because it eliminates blind pores and reduces the volume of solid polymer that remains inaccessible and unused (i.e. it increases the porosity of the immobilization support). Although the advantages of high pore interconnectivity have been extensively discussed within the context of membrane technology [16], as well as in matrices for tissue scaffolds [17], its application to the development of enzyme-catalyzed three-dimensional electrodes as a means to improve power density remains relatively unexplored. Recently, however, we have begun to explore the use of chitosan polymer as a material that promises control over the degree of dimensionality and directionality. Chitosan is a biocompatible crystalline polysaccharide with carboxyl and amine side groups that can serve as protein-binding ligands for enzyme immobilization [18–20].

Porous chitosan scaffolds can be made through a thermally induced phase separation in which the solution temperature is lowered below the freezing point to introduce a phase separation from the homogenous polymer solution [21]. In this process the solvent (i.e. water) and solute (i.e. chitosan) separates as the mixture cools until the solvent freezes, thereby arresting the solid-liquid phase separation mechanism that concentrates the

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polymer solution. Evaporation of the water under vacuum and at temperatures below the melting point of the chitosan polymer leaves a three-dimensional pore structure. As the pore structure represents the shape of the ice crystals, the pore structure morphology can be manipulated by adjusting those variables that effect the process of phase separation: namely, the polymer concentration, altering the solvent or by controlling the cooling rate. As such, the pore structure (e.g. mean pore diameter, degree of interconnectivity and geometric shape) can be manipulated through careful control of the cooling rate and the directionality of applied thermal gradients [22,23]. An example is given in Figure 2, which presents typical chitosan gels after freezing at −20 °C and freeze drying.

The chitosan scaffolds discussed above can be made conductive when doped with carbon nanotubes at relatively low weight percents [24–28]. Such a composite material holds great promise to support either direct or mediator-based electron transfer systems [18,24,29–31]. Carbon nanotubes are conductive materials [32–35] that support direct electron transfer [10••,36,37•] from enzymes that are immobilized by either physio-adsorption [18,35,38–40] or covalent attachment [33,41]. They have also been shown to oxidize NADH at low potentials, a feature attractive for biofuel cells utilizing NADH-dependent dehydrogenase enzymes [42,43]. To date, however, these chitosan-carbon nanotube scaffolds have been exclusively applied to biosensors and not to the development of three-dimensional electrodes employing enzyme catalysts. In our laboratories we are currently evaluating the potential of such scaffolds with optimal pore structures.

Enzyme cascades (multienzyme systems)

In living systems multistep enzymatic cycles (i.e. Kreb's cycle) and pathways (i.e. glycolysis) are responsible for the complete oxidation of complex fuels (food) to highly reduced compounds such as carbon dioxide. They include both electron-producing enzymes (oxidoreductases) and enzymes for catalyzing chemical reactions (kinases, etc.). To completely oxidize biofuels (ethanol, lactate, pyruvate, glucose, etc.) to carbon dioxide one could immobilize cascades of enzymes taken from these pathways at an electrode surface [44,45] or within a three-dimensional entrapment polymer. Figure 3 shows the common enzyme cascades (pathways) for complete oxidation of several common biofuels, including ethanol, glucose, lactate, pyruvate and glycerol. Sections of these pathways could be duplicated in anodes to meet the needs of a specific biofuel. Currently, we are working on strategies for immobilizing a cascade of enzymes at an electrode surface in a particular spatial architecture to decrease transport limitations, using immobilization schemes that protect the enzyme from the environment.

Characterization of enzymatic biofuel cells

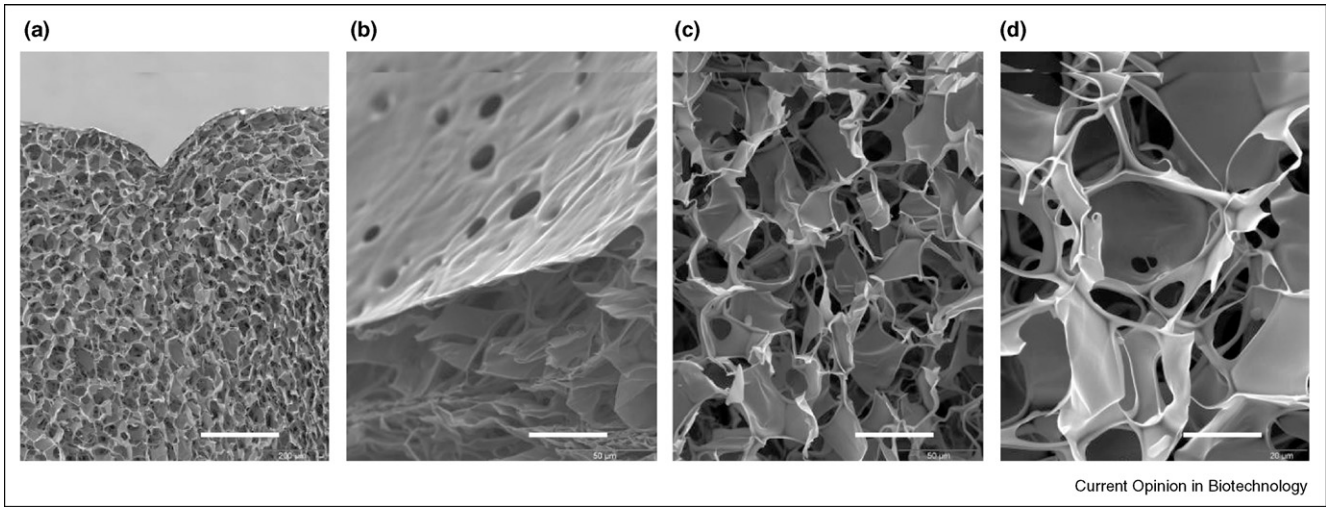
Testing platform

It can be argued that a critically overlooked parameter in the development of enzymatic fuel cells, and particularly with respect to increasing power density, is the use of a testing platform of common geometry. Its importance can be better explained by understanding how the power is measured and used to evaluate performance. The power density is described by the following equation

$$\bar{P} = \left(V^{\circ}_{cell} - \bar{I} \cdot R_{cell}\right) \cdot \bar{I} = V^{\circ}_{cell} \cdot \bar{I} - \bar{I}^{2} \cdot R_{cell} \quad (1)$$

This equation suggests that for any given rate of operation in the fuel cell, there are two clear paths to achieve improved power density. The first, which is not affected by geometry, is the thermodynamic consideration as represented in the first term of Equation 1, where the equilibrium potential of the cell V^{o}_{cell} is governed by the

Figure 2



Scanning electron micrographs of chitosan scaffolds. Scale bars: **(a)** 200 μm, **(b)** 50 μm, **(c)** 50 μm and **(d)** 20 μm.

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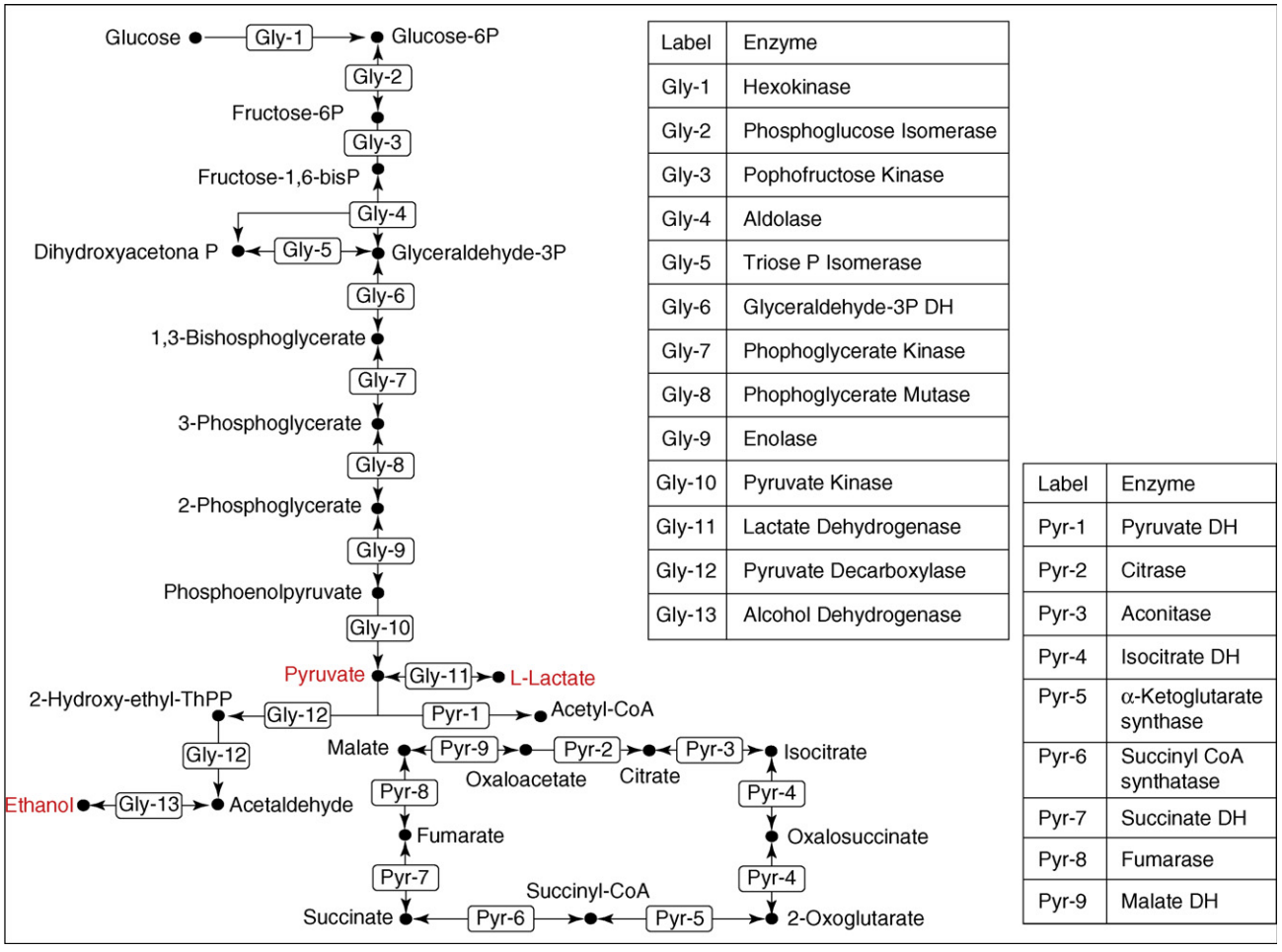
Figure 3



Label	Enzyme
Gly-1	Hexokinase
Gly-2	Phosphoglucose Isomerase
Gly-3	Pophofructose Kinase
Gly-4	Aldolase
Gly-5	Triose P Isomerase
Gly-6	Glyceraldehyde-3P DH
Gly-7	Phophoglycerate Kinase
Gly-8	Phophoglycerate Mutase
Gly-9	Enolase
Gly-10	Pyruvate Kinase
Gly-11	Lactate Dehydrogenase
Gly-12	Pyruvate Decarboxylase
Gly-13	Alcohol Dehydrogenase

Label	Enzyme
Pyr-1	Pyruvate DH
Pyr-2	Citrase
Pyr-3	Aconitase
Pyr-4	Isocitrate DH
Pyr-5	α-Ketoglutarate synthase
Pyr-6	Succinyl CoA synthatase
Pyr-7	Succinate DH
Pyr-8	Fumarase
Pyr-9	Malate DH

Schematic of enzyme cascades used for the oxidation of common biofuels.

cell chemistry through a judicious choice of the oxidation and reduction reactions at the anode and cathode, respectively. The second term, however, emphasizes the importance of kinetics where the cell internal resistance R_{cell} is a crucial parameter in determining the power density in a cell reaction. R_{cell} is a lump sum of various attributes, including charge transfer resistance of the electrode, electrode connections, membrane conductance, and the electrolyte conductance. It is important to note that these terms are highly affected by the geometry of the test cell. As such, when developing power curves, it is not sufficient to report the results per electrode surface area (cm^2). Rather, it is important to report the geometry of the cell in terms of electrode placement, cell volume, etc. and, if possible, to maintain a fixed geometry across comparisons. This is particularly crucial when comparing the performance of various anodes and cathodes across laboratories. It is also most useful in modeling studies. One of the more promising geometries is the stack cell design and a typical example is presented in Figure 4.

Fluorescence

The entrapment of enzymes within polymer networks has found numerous applications in the design of bioelectrochemical devices [16,21], primarily owing to the simplicity and mild conditions of the procedure and its ability to preserve the catalytic activity of biomolecules. In the context of biosensor and biofuel cell development, electrochemistry has generally been the method of choice when characterizing the performance of polymer-immobilized enzymes. Other methods, however, such as scanning electron microscopy [22,23], infrared spectroscopy, X-ray diffraction [24–28], atomic force microscopy [18,24,29–31], and small angle neutron scattering [32–35] have also been employed to further characterize enzyme–polymer systems. Nevertheless, none of these methods is able to provide insight into the chemical interaction between the enzyme and the polymer on both micro- and macro-scales, something that affects the spatial distribution, activity and lifetime of the enzyme.

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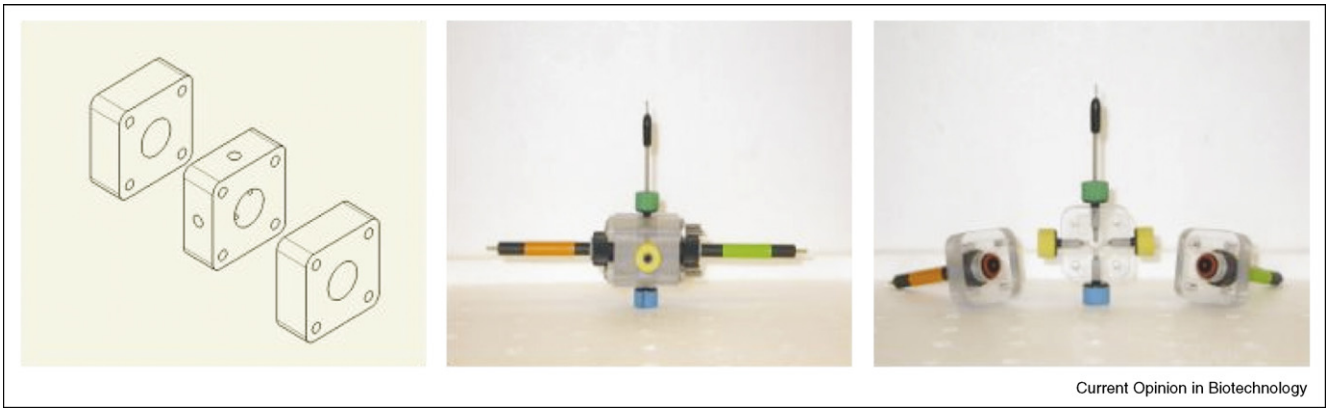
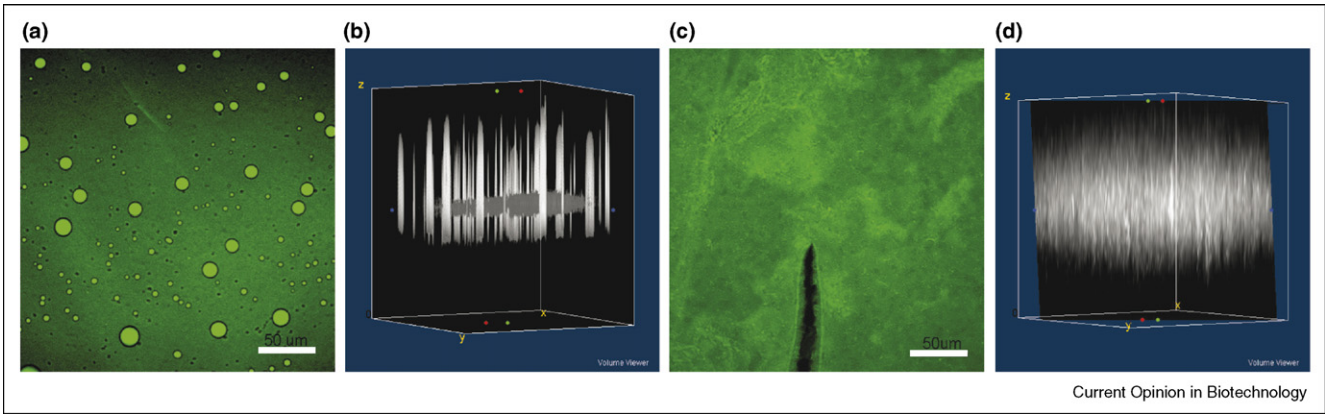
Figure 4



A stack design for a standard configuration biofuel cell with glassy carbon anode and cathode.

Figure 5



Fluorescently tagged enzymes in polymer films. Alcohol dehydogenase in an Eastman AQ membrane as shown **(a)** in a single plane and **(b)** in three-dimensions. Alcohol dehydogenase in a Nafion membrane shown **(c)** in a single plane and **(d)** in three dimensions.

Fluorescence, with its inherent sensitivity and selectivity, is a promising tool to probe enzyme–polymer interactions. This is because the intensity of the emission spectra, which is a function of the excitation wavelength and surrounding microchemical environment, can be both qualitatively visualized with laser scanning confocal microscopy and quantitatively measured with photon detectors. In view of this, fluorescence can be used to probe the nature of the interaction between the incorporated species and the polymer, whether it be in solution or the solid matrix [46–49], and to locate enzymes in varied supports and to characterize their movement and activity [46–52,53•]. In our laboratories we have already demonstrated the potential to characterize the distribution of alcohol dehydrogenase within Eastman AQ and Nafion films [53•]. An example of this is given in Figure 5. We are currently extending this work to chitosan films and the use of fluorescence to investigate the interactions between enzymes and polymers (as they are both charged species) and how this affects the enzyme's spatial distribution within the film.

Conclusions

Although enzyme-based biofuel cells have many advantages over traditional fuel cells and primary batteries, they remain limited by short lifetimes, catalytic inefficiencies, low fuel utilization, and low power densities. Recently, working solutions to short lifetimes and catalytic inefficiencies have been introduced, but similar advances in improved fuel utilization and power density are needed. Improvements in these areas will require electrochemical characterization in standardized test geometries, and the use of additional spectroscopic procedures that can be coupled to classic electrochemical measurements.

Acknowledgements

The authors gratefully acknowledge support from the Office of Naval Research (contract number N0014-01-1-0928) through the Hawaii

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Environmental and Energy Technology (HEET) initiative (Richard Rocheleau, PI) and from the Air Force (contract number FA9550-06-1-0264) through the Fundamentals and Bioengineering of Enzyme Fuel Cells (P Atanassov, PI). The authors also thank Mona Windmeisser and Anastassija Konash for their contributions to the SEM and fluorescence images, respectively. The authors would also like to thank Michael Beilke for figure development.

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