

## Chapter 14

# Microbial and Enzymatic Biofuel Cells

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This review summarizes the literature published after 1985 relevant to microbial and enzymatic biofuel cells. It tabulates the experimental conditions used in operation, the characteristics, and the performance of the reported biofuel cells. The present state of research in biofuel cells is analyzed and suggestions for future research are included.

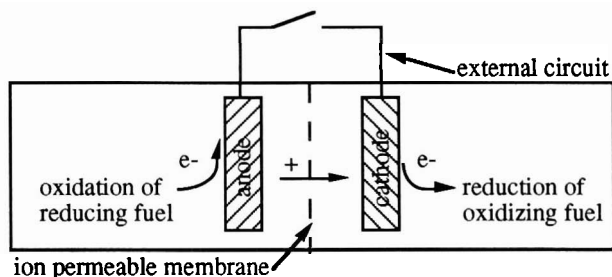
The purpose of this chapter is to review recent work in biofuel cells -- that is, fuel cells relying at least in part on enzymatic catalysis for their activity. The chapter is divided into three sections: (1) a brief review of the principles common to fuel cells (both biological and non-biological), supported with a bibliography of more comprehensive treatments, (2) an overview of the literature directly relevant to biofuel cells that encompasses both microbial and enzymatic cells, and (3) a summary of the obstacles that remain along the path to a practical biofuel cell, with suggestions for future research.

### Fundamentals of Fuel Cells and the Distinction between Biofuel Cells and Non-Biological Fuel Cells

A fuel cell is an electrochemical device that manages the flow of electrons and charge-compensating positive ions (typically protons or alkali metal cations such as  $\text{Li}^+$  or  $\text{Na}^+$ ) in a redox reaction in such a way that the electrons move through an external circuit where they can do electrochemical work (Scheme 1). The only fuels now used in practical fuel cells are  $\text{H}_2$  and  $\text{O}_2$ ; other reducing fuels are converted to  $\text{H}_2$  before they enter the fuel cell.

A biological fuel cell (abbreviated as *biofuel cell*) is the offspring of two parent technologies: fuel cells and biotechnology. Like conventional fuel cells, biofuel cells comprise an anode and a cathode separated by a barrier that is selective for the passage of positively charged ions; they require the addition of fuel to generate power (1-9). Unlike conventional fuel cells, which usually use precious metals as catalysts, biofuel cells utilize enzymatic catalysts, either as they occur in microorganisms, or as isolated proteins (10). There are two types of biofuel cells: direct and indirect (11). The *direct* biofuel cell is a configuration in which fuel is oxidized *at* the surface of the electrode. The role of the biological catalysts in this type of fuel cell is to catalyze these surface reactions. The *indirect* biofuel cell is a

configuration in which fuel reacts not at the electrode, but in solution or in a separate compartment, and a redox active *mediator* is added to shuttle electrons between the site of reaction and the electrode. Biological catalysts have been used in both direct and indirect configurations: typically, enzymes have been used as catalysts in direct biofuel cells, while microorganisms have often been used in indirect biofuel cells.



**Scheme 1.** Direction of electron and ion flow in fuel cell: external circuit open, no electrons or ions flow; external circuit closed, electrons move from the anode to the cathode and positively charged ions move from the anodic compartment to the cathodic compartment.

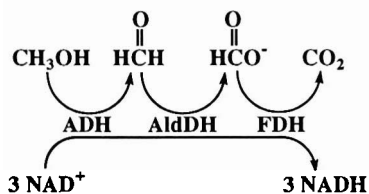
Power (i.e., electrochemical work,  $W$ ) is abstracted from fuel cells via an external electrical circuit connecting the anode and the cathode. The power available from a fuel cell is the product of cell voltage ( $V_{\text{cell}}$ ) and cell current ( $I_{\text{cell}}$ ). Ideally,  $V_{\text{cell}}$  for a direct fuel cell is the difference between the formal potentials of the *fuels* in the anode and cathode compartments;  $V_{\text{cell}}$  for an indirect fuel cell is the difference between the formal potentials of the *mediators* in the anode and cathode compartments. Irreversible losses in voltage (overpotentials) reflect the influence of slow kinetics of heterogeneous electron transfer, ohmic resistances, and concentration gradients (12).  $I_{\text{cell}}$  is the rate at which charge moves through the fuel cell and is dependent on electrode size, the rates of the processes occurring in the fuel cell, and the rate of movement of ions across the membrane separating the fuel cell compartments. A range of different processes -- catalytic reactions in solution and at interfaces, mass transport, electron transfer, ion permeation, and leakage of fuels between compartments -- can limit the power extractable from a particular fuel cell, either individually or collectively. Power lost to irreversible processes usually appears as heat, and the management of heat is an issue in biofuel cells in which the enzymes are temperature sensitive. Maximizing both  $V_{\text{cell}}$  and  $I_{\text{cell}}$  are two important challenges in developing practical biofuel cells (13).

Dioxygen, either pure or as a component of air, is the most commonly used cathodic fuel: it is readily available; thermodynamically a good oxidant (although often kinetically poor); its reduction product ( $\text{H}_2\text{O}$ ) is benign. The choice of the anodic fuel is more complicated. Dihydrogen has been used almost universally for its excellent electrode kinetics and potential. Because of issues of cost, engineering and safety associated with the generation, transport and storage of dihydrogen, methanol is now being considered seriously as an anodic fuel. Other fuels -- especially hydrocarbons or hydrocarbon mixtures such as methane, natural gas, gasoline and diesel fuel -- would be enormously interesting, but work on developing redox systems capable of using these fuels (other than by converting them to dihydrogen) is in its infancy.

Methanol is a non-explosive, water-soluble fuel that can be produced industrially in large quantities from coal, natural gas, or renewable resources. Ease in transport, storage, and safety are the advantages of methanol over dihydrogen as fuel.

Moreover, the theoretical cell voltage for a methanol/dioxygen fuel cell (1.19 V) is approximately the same as that of a dihydrogen/dioxygen fuel cell (1.23 V). Consequently, the development of catalysts that will oxidize methanol at low temperatures ( $< 200^{\circ}\text{C}$ ) is a high priority in fuel cell research (14,15).

One approach to the challenge of developing catalysts for the oxidation of methanol is to utilize biotechnology. Living organisms contain a range of enzymatic catalysts that oxidize methanol and other potential fuels (ethanol, glucose, glycerol, sucrose) to carbon dioxide under mild conditions (room temperature, atmospheric pressure, neutral pH). For example, the enzymatic oxidation of methanol to carbon dioxide has been studied using a combination of alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AldDH), and formate dehydrogenase (FDH) with nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) as cofactor (Scheme 2) (16,17). This method reduces three mol of  $\text{NAD}^+$  to NADH for every mol of methanol oxidized. NADH must subsequently be reconverted to  $\text{NAD}^+$  to continue the enzymatic reaction or to generate power.



**Scheme 2.** Dehydrogenase/NAD<sup>+</sup> catalyzed oxidation of methanol to CO<sub>2</sub>.

Both biochemical and electrochemical regeneration of  $\text{NAD}^+$  have been the topic of many reports (18-27). The formal potential ( $E^\circ$ ) of  $\text{NAD}^+$  is -0.32 V vs. NHE, pH 7.0 (-0.62 V vs. SCE at pH 7.5) (28). The standard potential,  $E^\circ$ , refers to the reduction potential of a given specie under standard conditions and unit activity for all reactants and products in the cell. Because it is difficult to construct a cell in which all activities are unity, one utilizes the Nernst equation to extract the value of  $E^\circ$  from the formal potential,  $E^\circ$ , which is the potential *measured* under non-standard conditions). At bare platinum or glassy carbon electrodes, however, the potential at which NADH is oxidized is ~1 V positive to  $E^\circ$  (~0.4 V vs. SCE at pH 7.5) (29,30). Minimizing this 1 V overpotential is crucial if  $\text{NAD}^+$  dependent catalysts are to be used in biofuel cells. The goal is to find a system (i.e. a combination of mediators and catalysts) that rapidly oxidizes NADH at its formal potential for a given pH. All approaches explored thus far have exhibited substantial voltage losses. Alternatively, enzymes that oxidize methanol in the absence of  $\text{NAD}^+/\text{NADH}$  have been tried in fuel cells. This approach however, has only been demonstrated using unstable dye mediators with high formal potentials (see the section on enzymatic biofuel cells).

Enzymes can be efficient catalysts, although the hallmark of most enzymatic catalysts in biological systems is in their *selectivity* in converting substrate to product, and their susceptibility to metabolic control, rather than their catalytic rate acceleration. There are enzymes (e.g. triose phosphate isomerase, superoxide dismutase) however, that operate at or near mass-transport limited rates (31). To estimate a qualitative upper limit for the current that might plausibly be generated in a biofuel cell using an enzyme as catalyst, one can assume a specific activity of  $10^3$  U  $\text{mg}^{-1}$  of enzyme (1 U =  $1 \mu\text{mol}$  of product produced per min). For a reaction producing one electron per molecule of substrate, 1 mg of enzyme, operating at concentrations of substrates at which the active site is saturated, would produce 1 mmol of electrons per min, or 96.5 coulombs per min. (Faraday constant = 96,487 coulombs per mol of electrons; 1 ampere = 1 coulomb  $\text{sec}^{-1}$ ; 1 watt = 1 volt ampere).

Since 1 ampere = 1 coulomb  $\text{sec}^{-1}$ , 1 mg of enzyme with an activity of  $10^3 \text{ U mg}^{-1}$  would be capable of catalyzing sufficient reaction to generate a current of  $\sim 1.6$  amps, provided there are no other losses in the system.

In fact, most enzymes have much lower specific activities (values of 0.1 to  $10 \text{ U mg}^{-1}$  are common), and enzyme-catalyzed reactions usually operate at less than their theoretical rates. Thus, in practice, the catalytic activity of an enzymatic system might plausibly be such that it would generate 1  $\mu\text{A}$  to 1 mA of current per mg of enzyme. By contrast, the current generated by a platinum catalyst in a fuel cell operating with dihydrogen is in the order of  $\sim 5\text{--}15 \text{ mA mg}^{-1}$  of platinum. (This value is based on typical current densities of  $100\text{--}300 \text{ mA cm}^{-2}$  for a dihydrogen/dioxygen fuel cell (see reference 2) using Pt-gauze electrodes;  $2.12 \text{ mg Pt cm}^{-2}$  gauze, 100 mesh woven from 0.762 mm diameter platinum wire).

The attractions of biofuel cells are several, despite their possible kinetic disadvantages relative to a dihydrogen/dioxygen fuel cell using platinum anodic and cathodic catalysts. First, they may be able to use fuels such as glucose, wastes containing paper or other biologically derived materials that cannot be handled by any existing transition metal-based fuel cell. Neither non-biological nor biological fuel cells have been operated successfully with unfunctionalized hydrocarbons but there are microorganisms that can use such hydrocarbons as a carbon source; the enzymes involved in these processes are potential candidates for use in fuel cells. Second, biofuel cells are intrinsically non-polluting and their enzymes operate at low temperatures in water close to pH 7. Third, enzymes can, in principle, be produced inexpensively by fermentation using genetic engineering. Fourth, enzymes may be able to contribute to the solution of problems such as the overpotential at the dioxygen cathode that have resisted solution by conventional fuel cell technology: oxidative metabolism using dioxygen is highly optimized in biological systems. Fifth, if it were possible to find a system using enzymes that operated on fuels with specific activities close to  $10^3 \text{ U mg}^{-1}$ , these enzymes probably *would* offer advantages in rate over platinum. Sixth, biofuel cells may be applicable to certain speculative but important applications such as a fuel cell that could be implanted in humans and provide power for other implanted prosthetic devices.

### Overview of the Literature of Biofuel Cells

Several comprehensive reviews on the development of biofuel cells appeared prior to 1985 (10,11,13,32-34). The literature subsequent to 1985 will be the focus of this section, with key studies prior to 1985 included only for historical perspective. The literature described in this section is divided into three categories of subject: (1) microbial biofuel cells, (2) photomicrobial biofuel cells, and (3) enzymatic biofuel cells. The experimental conditions used with, and the characteristics of, the biofuel cells in each category are tabulated in Tables I-III, respectively. Throughout this section, we refer to chemical mediators by their acronyms. The chemical names or structures of these mediators can be found at the end of this chapter under the heading "Legend of Symbols and Selected Chemical Structures".

In the 1960's, the United States space program became interested in developing biofuel cells as an energy-saving waste disposal system for extended space flights. Initially, biofuel cells were operated using microorganisms and were classified according to how the microorganism was utilized; either as a manufacturer of an electroactive product (i.e. dihydrogen) that was fed directly into a conventional fuel cell, or in conjunction with a redox mediator that served to transfer electrons to the electrode from an intercepted metabolic pathway of the microorganism. The advantages of microbial biofuel cells that generate dihydrogen (or, in principle, other conventional fuels) are: (1) a variety of complex organic substances such as carbohydrates, fats, and proteins can be utilized as fuels, and (2) known non-

Table I. Summary of Microbial Biofuel Cells

Reference	Experimental Conditions and Results
(35)	(glucose oxidase), <i>E. Coli</i> , <i>Nocardia</i> oxidizing glucose and using MB as mediator: Pt-sheet anode and cathode (60 in <sup>2</sup> each), GOD: 0.28 V at open-circuit; no current given, <i>E. Coli</i> : 0.625 V at open-circuit; no current given, <i>Nocardia</i> : 0.3 V at open-circuit; 2 mA at 1 k $\Omega$ .
(36)	<i>Pseudomonas methanica</i> producing H <sub>2</sub> (from methane) using NSIDCP as mediator: platinized-Pt anode and cathode (12.6 cm <sup>2</sup> each), 0.5-0.6 V at open-circuit; 2.8 $\mu$ A/cm <sup>2</sup> at 0.35 V.
(38)	agar gel-immobilized <i>C. butyricum</i> producing H <sub>2</sub> (from molasses): 2 Pt-blackened Ni anodes (100 mesh, 10.4 cm diameter), 2 Pd-blackened Ni cathodes (250 mesh, 10.4 cm diameter), 0.55 V (fuel cell I) and 0.66 V (fuel cell II) at 1 $\Omega$ , 0.95 V at 100 $\Omega$ for fuel cells I and II connected in series, 40 mA/cm <sup>2</sup> at 1 $\Omega$ ; 550 mA at 2 $\Omega$ and 1.3 V for 7 days.
(39)	<i>C. butyricum</i> producing H <sub>2</sub> (from waste water): 5 Pt-blackened Ni anodes (100 mesh, 14.5 cm diam.), 5 Pd-blackened Ni cathodes (100 mesh, 14.5 cm diam.), 0.62 V at 1 $\Omega$ ; 0.8 A at 2.2 V for 7 days, 5 fuel cells connected in series.
(40)	<i>C. butyricum</i> producing H <sub>2</sub> (from molasses): 2 carbon fiber (impregnated with Pt-blackened carbon pellets) anodes and cathodes, 200 $^{\circ}$ C, 0.9-0.93 V at open-circuit (two fuel cells connected in parallel); 10-14 A.
(41)	<i>Proteus vulgaris</i> , <i>E. coli</i> , <i>Bacillus subtilis</i> , or <i>Alcaligenes eutrophus</i> oxidizing glucose or succinate using ABB, BCB, BV <sup>2+</sup> , DCPIP, DMST-2, GCN, NMB, PES, PTZ, RS, SF, TB, or TH <sup>+</sup> as mediator: RVC anode (35 x 50 x 7 mm, 800 cm <sup>2</sup> surface area), Pt-foil cathode poised at 0.43 V vs. SCE (10 x 40 mm), 0.2 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, <i>Proteus vulgaris</i> : 0.64 V at open-circuit; 0.8 mA at 560 $\Omega$ , <i>E. coli</i> : open-circuit voltage not given; $\sim$ 0.8 mA at 560 $\Omega$ , <i>Bacillus subtilis</i> : open-circuit voltage not given; $\sim$ 0.8 mA at 560 $\Omega$ , <i>Alcaligenes eutrophus</i> : 0.55 V at open-circuit; current not given.
(43)	<i>Proteus vulgaris</i> oxidizing glucose using TH <sup>+</sup> as mediator: RVC anode (35 x 50 x 7 mm, 800 cm <sup>2</sup> surface area), Pt-foil cathode (10 x 40 mm), 0.2 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, open-circuit voltage not given; 3.5 mA at 100 $\Omega$ (calc. from V/I).
(44)	<i>Proteus vulgaris</i> oxidizing sucrose using TH <sup>+</sup> as mediator: carbon anode and cathode, 0.1 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, open-circuit voltage not given; 3.5 mA at 100 $\Omega$ and 0.35 V (calc.).
(45)	<i>E. coli</i> oxidizing glucose using TH <sup>+</sup> , DST-1, DST-2, TST-1, or FeCyDTA as mediator: materials and dimensions of anode and cathode not given, open-circuit voltage not given; < 0.7 mA at 560 $\Omega$ .

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Table I. Continued

Reference	Experimental Conditions and Results
(46)	<i>Lactobacillus plantarum</i> , <i>Streptococcus lactis</i> , or <i>Erwinia dissolvens</i> oxidizing glucose using FeEDTA, FeCyDTA, FeDTPA, FeTTHA, or FeEDADPA as mediator: materials and dimensions of anode and cathode not given, <i>L. plantarum</i> and <i>S. lactis</i> : 0.2 V at open-circuit, 0.05 V at 560 $\Omega$ , <i>E. dissolvens</i> : 0.5 V at open-circuit, 0.4 V at 560 $\Omega$ .
(47)	<i>Bacillus W1</i> oxidizing glucose using MV <sup>2+</sup> , BCB, HNQ, or FeEDTA as mediator: materials and dimensions of anode and cathode not given, open-circuit voltage not given; 10 mA/g dry wt. at 500 $\Omega$ .
(48)	<i>Enterobacter aerogenes</i> producing H <sub>2</sub> (from glucose): Pt-blackened stainless steel net anode (25 cm <sup>2</sup> ), Pt cathode, 0.01 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, 1.04 V at open-circuit, 0.79 V at 500 $\Omega$ ; 60 $\mu$ A/cm <sup>2</sup> .
(49,50)	<i>E. coli</i> oxidizing glucose using HNQ as mediator: GC anode (12.5 cm <sup>2</sup> ) or anode comprised of packed bed of graphite particles (surface of packed bed was ~200 cm <sup>2</sup> ), O <sub>2</sub> gas-diffusion cathode, 0.53 V at 10 k $\Omega$ ; GC anode: 0.18 mA/cm <sup>2</sup> , packed bed anode: 1.3 mA/cm <sup>2</sup> cross-sectional area of the bed.
(51)	<i>Desulfovibrio desulfuricans</i> oxidizing sulfide: 3 porous graphite anodes impregnated with Co(OH) <sub>2</sub> (100 cm <sup>2</sup> ), 3 porous graphite cathodes impregnated with Fe(phthal) (100 cm <sup>2</sup> ), 2.8 V at open-circuit for 3 fuel cells connected in series; 2 days, < 1 A after 1 day.

Table II. Summary of Photomicrobial Biofuel Cells

Reference	Experimental Conditions and Results
(52)	<i>Anabaena variabilis</i> oxidizing glycogen and using HNQ as mediator: materials and dimensions of anode and cathode not given, 0.1 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, 0.4 V at 400 $\Omega$ ; 1-2 mA at 200 $\Omega$ .
(53)	<i>Anabaena variabilis</i> oxidizing glycogen and using HNQ as mediator: materials and dimensions of anode and cathode not given, 0.1 M K <sub>3</sub> [Fe(CN) <sub>6</sub> ] catholyte, open-circuit voltage not given; 400 $\mu$ A at 200 $\Omega$ for 19 h.
(54)	immobilized <i>Rhodospirillum rubrum</i> producing H <sub>2</sub> : carbon-fiber (impregnated with Pt-blackened carbon pellets) anodes and cathodes (2 each) operated at 200 $^{\circ}$ C, dimensions not given, 0.2-0.35 V at open-circuit for 2 fuel cells connected in parallel; 0.5-0.6 A for 6 h.

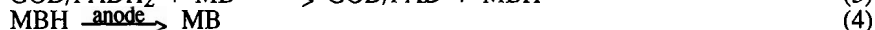
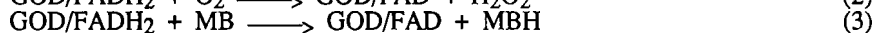
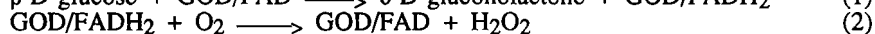
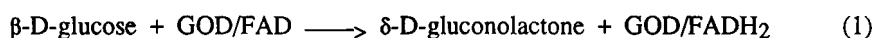
**Table III. Summary of Enzymatic Biofuel Cells**

<i>Reference</i>	<i>Experimental Conditions and Results</i>
(55)	<i>methanol dehydrogenase</i> oxidizing methanol and formaldehyde using PMS or PES as mediator: Pt-gauze anode (50 mesh, 7 cm <sup>2</sup> ), Pt-gauze cathode (80 mesh, 4.4 cm <sup>2</sup> ), 0.3 V at open-circuit; 3.7 mA at 10 $\Omega$ .
(56)	PQQ-linked <i>alcohol dehydrogenase</i> oxidizing methanol using TMPD as mediator: Pt-gauze anode (50 mesh, 7 cm <sup>2</sup> ), Pt-gauze cathode (80 mesh, 4.4 cm <sup>2</sup> ), 0.16 V at 133 k $\Omega$ ; 20 $\mu$ A/cm <sup>2</sup> at 10 $\Omega$ and 20 °C.
(57)	<i>methanol</i> and <i>formate dehydrogenase</i> oxidizing methanol using PMS or PES as mediator: carbon fabric anode and cathode (40 cm <sup>2</sup> each), open-circuit voltage not given; 0.9 mA at 10 $\Omega$ and 0.065 V.
(58)	<i>glucose oxidase</i> oxidizing $\beta$ -D-glucopyranose in the presence of elemental Fe: Pt-sheet anode and cathode (64 cm <sup>2</sup> each), at open circuit: 0.35 V without Fe, 0.75 V with Fe; 0.21 $\mu$ A/cm <sup>2</sup> without Fe, 17 $\mu$ A/cm <sup>2</sup> with Fe.
(59)	immobilized <i>D-glucose dehydrogenase</i> oxidizing glucose using MB as mediator: graphite disk anode (16 cm <sup>2</sup> , 740 rev/min), Pt-gauze cathode poised at 0.56 V vs. SCE, open-circuit voltage not given; 0.2 mA cm <sup>-2</sup> at $\sim$ 0.8 V.
(60)	<i>glucose oxidase</i> oxidizing glucose in anode using DCPIP as mediator; <i>chloroperoxidase</i> chlorinating barbituric acid in cathode: Pt-gauze anode (50 mesh, 7.5 cm <sup>2</sup> ), Au-sheet cathode (22.8 cm <sup>2</sup> ), 0.6 V at open-circuit; 350 $\mu$ A.

biological fuel cell technology can be utilized. Unfortunately, microbial biofuel cells have uniformly generated current densities too low to be of practical value as power sources.

The availability of isolated enzymes has made it possible to eliminate the microorganism from the biofuel cell and to substitute with individual enzymes, the result being a substantial gain in volumetric catalytic activity. Individual enzymes offer the advantage of much higher catalytic capacity (in terms of catalytic activity per unit weight or volume), but at substantially higher cost than enzymes present in microorganisms. Genetic engineering has not yet been applied to the area of biofuel cells, either to lower the cost or to improve the characteristics of the enzymes used. Enzymatic biofuel cells are typically more efficient than microbial biofuel cells in converting fuel to electricity for two reasons: the cellular membrane that interferes with electron transfer is absent and fuel-consuming microbial growth is eliminated. For practical reasons however, enzymatic biofuel cells are limited to fuels that are oxidized to CO<sub>2</sub> with a minimal number of enzymatic transformations. Moreover, unsupported enzymes are often more vulnerable to denaturation than enzymes encapsulated within a microbial membrane, and enzymatic biofuel cells are typically less stable than those using living organisms.

**Microbial Biofuel Cells.** One of the earliest examples of electrical energy derived from both microorganisms and enzymes was demonstrated by Davis and Yarbrough in 1962 (35). The microorganisms used in the fuel cell were *Nocardia* and *Escherichia coli* and the enzyme used was glucose oxidase (GOD). The authors began their study using a fuel cell configured with GOD, presumably containing bound flavin adenine dinucleotide (FAD), glucose, buffer, and a Pt-sheet electrode in the dinitrogen-purged anodic compartment. The cathodic compartment, containing a Pt-sheet electrode and buffer, was saturated with dioxygen. GOD catalyzes the oxidation of  $\beta$ -D-glucose to  $\delta$ -D-gluconolactone with the concomitant reduction of FAD to FADH<sub>2</sub> (equation 1). Under biological conditions, FADH<sub>2</sub> is reoxidized by dioxygen (equation 2), but because the anodic chamber had been purged to remove dioxygen, this reaction did not occur. Methylene blue (MB) was added to the anode compartment both to reoxidize FADH<sub>2</sub> and to shuttle the reducing equivalents made available by the enzymatic reaction to the anode (equations 3 and 4).



In the absence of MB, the fuel cell yielded no electricity.  $V_{\text{cell}}$  at open-circuit was 0.28 V and voltages between 0.075 to 0.09 V could be maintained under a load of 1 k $\Omega$ . Because the glucose-GOD-MB system under a dinitrogen atmosphere generated reduced MB that produced electricity, the authors reasoned that a facultative anaerobe would also produce measurable current. *Escherichia coli* was added to the anode compartment in place of GOD, and a  $V_{\text{cell}}$  of 0.625 V at open-circuit was obtained. Under a 1 k $\Omega$  load,  $V_{\text{cell}}$  could be maintained at 0.52 V for over 1 h. The use of ethane as fuel was attempted using an ethane-oxidizing *Nocardia* as catalyst. No current was produced even in the presence of MB. When glucose was substituted for ethane, metabolic activity increased. An open-circuit voltage of 0.3 V and a maximum current of 2 mA under a 1 k $\Omega$  load was reported. This value of maximum current seems unlikely, given the upper limit of 0.3 mA imposed by Ohms law ( $V = IR$ ).

A unique microbial biofuel cell that utilized methane as fuel was demonstrated by van Hees (36). To our knowledge, no other methane-fed biofuel cell has been



reported in the literature. The anodic compartment, containing *Pseudomonas methanica*, 1-naphthol-2-sulfonate indo-2,6-dichlorophenol (NSIDCP), and a platinum anode, was purged of dioxygen and supplied with methane. The cathode compartment housed a platinum cathode in a sterile medium and was supplied with dioxygen in the form of air. Inclusion of NSIDCP in the anode compartment had no effect on  $V_{\text{cell}}$  at open-circuit, suggesting that this dye did not function as an electrochemical mediator. This conclusion was further supported by enclosing the microorganisms in dye-permeable dialysis tubing and observing that the value for  $V_{\text{cell}}$  at open-circuit was established only very slowly even in the presence of NSIDCP; this observation suggests that microbial contact with the anode was necessary for electron transfer. The power density was low ( $0.98 \mu\text{W cm}^{-2}$ ) and prolonged open-circuit conditions or high current drain were observed to be detrimental to the obligate aerobe used in this fuel cell. The explanation given for these observations was elaborate and the reader is referred to the original text. In summary, the authors concluded that biofuel cells utilizing obligate aerobes are best suited to functioning under moderate current drain.

Karube, Suzuki *et al* studied several microorganisms for their production of dihydrogen from glucose (37-40). Of the organisms studied, *Clostridium butyricum* IFO3847 produced dihydrogen at the highest rate and was therefore used to supply dihydrogen to the anode compartment of a conventional fuel cell. Immobilization of the microorganism in a gel of agar-acetylcellulose both stabilized the dihydrogen-producing system and increased the rate of dihydrogen production. For every mol of glucose consumed, 0.6 mol of dihydrogen and 0.2 mol of formic acid were produced. Two kg of wet gel containing ~200 g of wet microorganism produced 40 ml (1.7 mmol) of dihydrogen per min, equivalent to ~5 A.

Four different fuel cell configurations were studied; the two most recent are described here. One configuration, a fuel cell stack of five fuel cells using Pt-blackened Ni-gauze anodes and Pd-blackened Ni-gauze cathodes, was operated at room temperature. Dihydrogen was passed from a 5 L reservoir containing ~2 kg of immobilized whole cells to the anode compartments of the fuel cell stack at a rate of  $40 \text{ ml min}^{-1}$  while dioxygen was passed through the cathode compartments. The fuel cell system (dihydrogen producing microbial reservoir and five fuel cells) was operated for 7 days and maintained a current of 0.8 A at 2.2 V. The poor current efficiency was not explained; a high temperature phosphoric acid fuel cell stack was, however, used in the subsequent report.

A second configuration used ~3 kg of immobilized microorganisms in a 200 L fermentation vessel containing 150 L of molasses. The biogas produced, containing both dihydrogen and carbon dioxide, was passed into the anode compartment of a phosphoric acid fuel cell stack (two fuel cells in parallel operated at  $200^\circ\text{C}$ ) at a rate of  $400\text{--}800 \text{ ml min}^{-1}$  while dioxygen was passed into the cathode compartment at a rate of  $1\text{--}1.5 \text{ L min}^{-1}$ . The open-circuit voltage was between 0.9-0.93 V and a stable current of 10-14 A was maintained for four hours with a power output of 9-13 W. Current density, power density, and electrode size were not given. The authors analyzed the electrical energy balance for the microbial/phosphoric acid fuel cell configuration and found that only 1% of the energy consumed to operate the system (pumps and heaters) was generated by the microbial fuel cell.

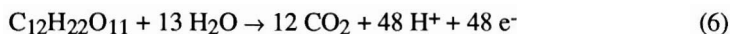
Delaney, *et al*., studied fuel cell performance using different microorganism-mediator-substrate combinations (41,42).  $V_{\text{cell}}$  at open-circuit was predicted to range from 0.9-1.2 V based on the difference between  $E^\circ$  of the different mediators used in the anode compartment and  $E^\circ$  of the potassium ferricyanide used in the cathode compartment.  $V_{\text{cell}}$ , however, was found to be 0.2-0.4 V lower than predicted, indicating activation overpotentials at the electrodes. The fuel cell that gave the best performance contained *Proteus vulgaris*, thionine ( $\text{TH}^+$ ), and glucose in the anodic compartment. Typical current-voltage curves were obtained by discharging the fuel

cell through a range of loads. The coulombic yield of the fuel cell was determined by integration of the area under the current-time curve. (Coulombic yield is the total number of coulombs passed through the fuel cell, as determined by integration of the current-time curve in units of amp·s = coulombs, relative to the theoretical number that might be obtained by complete conversion of the reactants to products). Coulombic yields of 32-62% were obtained based on the theoretical maximum coulombic equivalent for complete oxidation of glucose in accordance with equation 5. (The theoretical maximum coulombic equivalent of glucose is 24 mol electron mol<sup>-1</sup> glucose x 96,487 C mol<sup>-1</sup> electron = 2.3 x 10<sup>6</sup> C mol<sup>-1</sup> glucose):



Thurston *et al* argue that if fuels such as carbohydrates are to be used, microorganisms will be more economical catalysts than individual enzymes because the multi-step oxidations involved with complex fuels would require multiple, expensive enzyme purifications (43). *Proteus vulgaris* was chosen as the biocatalyst for glucose oxidation and <sup>14</sup>C-labeled glucose was used as fuel to establish the distribution of products. The amount of CO<sub>2</sub> evolved from the oxidation of glucose was determined by scintillation methods and a sodium hydroxide trap. Conversion of glucose to CO<sub>2</sub> was 40-50%, and coulombic yields indicated near unit current efficiency (based on 50% conversion of glucose). The most significant loss in energy was from the conversion of 30% of glucose to acetate; it is possible that this side reaction might be avoided by metabolic or genetic modification of the microorganism.

Bennetto *et al* used sucrose as fuel in a TH<sup>+</sup> mediated microbial biofuel cell containing *Proteus vulgaris* as biocatalyst (44). Equation 6 gives the stoichiometry of complete oxidation of sucrose.



The biofuel cell was discharged through a resistance of 100 Ω after the addition of various amounts of sucrose. The amount of CO<sub>2</sub> evolved from the anode compartment confirmed near complete oxidation of sucrose. The cell current peaked at 3.5 mA and the production of electricity from the oxidative conversion of sucrose was >95%, determined from both coulombic yield and CO<sub>2</sub> evolution. At higher concentrations of sucrose, the buffering capacity of the anodic medium was exceeded due to acid formation. Preliminary tests using molasses as fuel suggested that >70% of the carbohydrate was oxidized. Neither the open-circuit voltage nor electrode size were given.

Since TH<sup>+</sup> has frequently been used as a mediator in biofuel cells, Lithgow *et al* prepared sulfonated derivatives of thionine to determine the effect of ring substitution on mediation of electron transfer from *E. Coli* to the anode (45). Changing from TH<sup>+</sup> to 2-sulfonated thionine (DST-1) and 2,6-disulfonated thionine (DST-2) resulted in an increase in the efficiency of mediated electron transfer. This increase was reflected by changes in cell current under a 560 Ω load: 0.35 mA for TH<sup>+</sup>, 0.45 mA for DST-1, and 0.6 mA for DST-2. The low efficiencies of the fuel cells operating with TH<sup>+</sup> and DST-1 were attributed to interference with electron transfer by adsorption of mediator to the microbial membrane, and a resulting decrease of the rate of oxidation of glucose at that membrane.

The authors also compared the thionine derivatives to ferric cyclohexane-1,2-diamine-N,N,N',N'-tetraacetic acid (FeCyDTA) and found that lower concentrations of both TH<sup>+</sup> and the sulfonated derivatives gave better mediation than the iron chelate. The significant inference from this report was that the power output of

microbial biofuel cells could be improved by introducing hydrophilic groups into several mediators.

Vega and Fernandez studied mediation using several ferric chelates -- FeCyDTA, ferric diethylenetriamine pentaacetic acid (FeDTPA), ferric ethylenediamine diacetic acid dipropionic acid (FeEDADPA), ferric ethylenediamine tetraacetic acid (FeEDTA), and ferric triethylenetetramine hexaacetic acid (FeTTHA) -- with microbial biofuel cells containing *Lactobacillus plantarum*, *Streptococcus lactis*, and *Erwinia dissolvans* (46). *Lactobacillus plantarum* and *Streptococcus lactis* grow on dairy products and *Erwinia dissolvans* grows on coffee wastes. The objective of this study was to determine which microorganism/mediator combination would be the most efficient in a microbial biofuel cell. Fuel cells using *Erwinia dissolvans* mediated with FeCyDTA ran for five consecutive days, with coulombic yields between 80 and 90%. *Lactobacillus plantarum* and *Streptococcus lactis* did not produce any significant electrical output with any of the ferric chelates. The main results from this study were: 1) different microorganisms, using the same mediators and the same amount of fuel, gave different coulombic yields, 2) biofuel cells using *E. dissolvans* as the biocatalyst gave higher coulombic yields than those using *E. Coli*. The interaction of the microbial membrane with the mediator was suggested to be the source of these effects.

Akiba *et al* studied a microbial biofuel cell that operated at basic pH (47). Operating a fuel cell at basic pH circumvents problems associated with fuel cells operated at neutral pH, such as low ionic conductivity and inefficient operation of the O<sub>2</sub> cathode. Problems associated with CO<sub>2</sub> rejection are significant however, in fuel cells operating at basic pH. Methyl viologen (MV<sup>2+</sup>), brilliant cresyl blue (BCB), and 2-hydroxy-1,4-naphthoquinone (HNQ) were employed as mediators, either alone or in combination with FeEDTA. The anode compartment was loaded with 35-50 mg of *Bacillus W1*, an unstated amount of mediator, 10  $\mu$ mol of glucose, and 0.1 M KH<sub>2</sub>PO<sub>4</sub> buffer (pH ranging between 10.0-11.0). The fuel cell was discharged through resistors of 300 or 500  $\Omega$  at 35  $^{\circ}$ C. The coulombic yields were highest at pH 10.5 and were > 93.5% with the different mediators. The rate of mediator reduction was however, found to be an order of magnitude slower than that at neutral pH. Because the reduced mediators are less stable at higher operating temperatures, the cells were not examined at such temperatures. No open-circuit voltages were given.

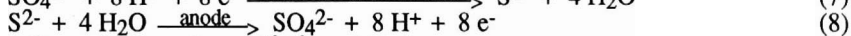
*Clostridia* are known for their production of dihydrogen, and they have frequently been used in microbial fuel cells. Because *Clostridia* are obligate anaerobes, however, both dihydrogen production and microbial growth are oxygen sensitive. Tanisho *et al* isolated a strain of the facultative anaerobe, *Enterobacter aerogenes*, and found it to produce dihydrogen from glucose (48). This microorganism, grown under aerobic conditions, consumes dioxygen to oxidize organic substrates; under anaerobic conditions, it degrades substrate into dihydrogen.

During operation of the fuel cell, the anolyte was circulated between the microbial reservoir and the anodic chamber of the fuel cell with a peristaltic pump. The reservoir contained microorganisms, phosphate buffer, ammonium sulfate, sodium citrate, magnesium sulfate, and glucose. Peptone, typically included in culture broth, was not included as it often causes microorganisms to adhere to the electrode. The fuel cell was operated with circulating microorganisms for 35 h; nutrients were added after 25 h. Whenever the anode compartment was opened to the atmosphere, the operating voltage would temporarily decrease, but would recover within ten minutes. The microorganisms were reported to produce 11 mol of dihydrogen/L culture/h (equivalent to 269 L dihydrogen/h at 1 atm and 25 $^{\circ}$ C). The authors assert that if all dihydrogen produced by the 330 ml of culture contained in the reservoir were utilized by the fuel cell (we calculate this to be 3.63 mol dihydrogen/h based on 11 mol of dihydrogen/L culture/h), then 330 mA would be generated. In fact, complete oxidation of 3.63 mol of dihydrogen/h would generate a current of 195 A; some

elements of the report are thus internally incompatible. Experimentally, an average current of only 1.4 mA was observed. The authors suggested switching to a molten carbonate fuel cell (MCFC) working on a gas mixture of dihydrogen and carbon dioxide to obtain higher current densities. Operating a MCFC at 650°C has, of course, a substantial cost in energy relative to cells operating close to room temperature.

Sell *et al* employed HNQ as a redox mediator in a fuel cell using *E. coli* as the biocatalyst for the oxidation of glucose (49,50). The fuel cell was operated for 10 h under a 250  $\Omega$  load, during which time  $V_{\text{cell}}$  decayed from 250 mV to 75 mV, presumably due to glucose consumption. The mechanism for HNQ reduction was not identified, but it was determined that HNQ is not reduced by isolated ADH. The significant finding in this report was that higher voltage and current density could be obtained by switching from a glassy carbon (GC) disc anode (0.18 mA/cm<sup>2</sup>) to a packed bed of graphite particles (1.3 mA/cm<sup>2</sup> of cross-sectional area of bed); thus the rate of electron transfer at the electrode seemed to limit current.

Habermann and Pommer demonstrated a microbial fuel cell utilizing the sulfate-reducing microorganism, *Desulfovibrio desulfuricans* (51). Sulfide, produced from the metabolic reduction of sulfate, was oxidized at the anode using air as the terminal oxidant according to equations 7-9:



The authors listed glucose, fructose, cane sugar, starch, and hydrocarbons as the substances selected to provide the reducing equivalents required to convert  $\text{SO}_4^{2-}$  to  $\text{S}^{2-}$ , and to meet the nutrient requirements of the microorganism.

The fuel cell consisted of an anode fabricated from porous graphite impregnated with cobalt hydroxide. In the presence of sulfate-reducing microorganisms, cobalt hydroxide was converted into a cobalt oxide/cobalt sulfide mixture. As a result, reducing equivalents in the form of sulfide were concentrated within the porous network of the graphite anode during conditions of open-circuit. The cathode was fabricated from graphite activated with iron-phthalocyanine [Fe(phthal)]. The electrolyte used was a solution containing between 0.1 and 5% by weight sodium sulfate, 0.1% by weight urea and dextrose, and trace elements. The highest percentage of sulfate reduction by *Desulfovibrio desulfuricans* occurred when using 0.5 % by weight sodium sulfate (pH 8.5) electrolyte. Three fuel cells were connected in series and gave an open-circuit voltage of 2.8 V. The current level after one day of operation was < 1 mA.

**Photomicrobial Biofuel Cells.** Electric current is generated by microbial fuel cells when electrons from the oxidation of endogenous or exogenous substrates are transferred to an electrode. The use of photosynthetic microorganisms in fuel cells permits incident light to be converted into current. Photosynthetic microorganisms are envisaged to function in a fuel cell in two ways. First, energy can be stored as products of the photosynthetic apparatus during illumination and subsequently released and processed as in non-photosynthetic microbial biofuel cells. (A photomicrobial biofuel cell operated in this mode is different from a conventional biofuel cell only in that the energy ultimately converted to reduced mediator is derived from light rather than from a chemical fuel added separately). Second, energy might be abstracted directly from the photosynthetic apparatus by a mediator that transports electrons to the anode.

Tanaka *et al* assembled a biofuel cell using the photosynthetic microorganisms, *Anabaena variabilis* (52,53). Charge was mediated by the light-stable mediator, HNQ. In the dark, electrons from the oxidation of endogenous glycogen powered this biofuel cell. The fuel cell was discharged under a 400  $\Omega$  load and maintained  $V_{\text{cell}}$  at

0.4 V for >12 h. Illumination of the microorganism-containing anode compartment increased the coulombic yield by 60% over the dark anode due to the additional source of electrons provided by the photosynthetic oxidation of water.

Karube and Suzuki utilized a purple photosynthetic microorganism, *Rhodospirillum rubrum*, in a fuel cell (54). Wastewater containing acetate, propionate, and butyrate was used as the medium from which dihydrogen was produced by the immobilized microorganism. The organic acids in the wastewater are claimed to be the hydrogen source for the microorganisms. The authors present the difference in organic acid concentration between the inlet and outlet streams of the bioreactor as evidence to support this claim. Whether or not hydrogen from the organic acids was incorporated into the dihydrogen produced by the microorganism cannot be determined from the results of this study. The conditions employed to optimize microbial production of dihydrogen were pH 7.0, 30°C, and a light intensity of 5000 lux. Dihydrogen, produced by two reactors (20 L each) containing immobilized microorganisms, was transferred to the anode compartment of a phosphoric acid fuel cell at 19–31 ml min<sup>-1</sup> while dioxygen was fed to the cathode compartment at 100 ml min<sup>-1</sup>. The fuel cell operated between 0.2 and 0.35 V and delivered 0.5–0.6 A for 6 h.

### Enzymatic Biofuel Cells

**C<sub>1</sub> Compounds as Anodic Fuel.** Plotkin, *et al* studied the oxidation of methanol to formate using a NAD<sup>+</sup>-independent methanol dehydrogenase (MDH) (55). The redox mediators investigated were phenazine methosulfate (PMS) and phenazine ethosulfate (PES). At pH 9.5, the optimal pH for enzymatic activity, PES was more stable than PMS. The authors claim that a current less than 3 mA would represent 90% of the maximum current possible based on the specific activity of the enzyme (0.42 μmol of methanol oxidized/min/mg MDH). Our calculations indicate however, that 4.6 mg of MDH with the stated specific activity should produce 12.4 mA of current. Consequently, < 25% of the current available from the enzymatic reaction was observed. Aside from poor current efficiency, the main drawback to this fuel cell was the accumulation of formate in the anode compartment.

Davis *et al* studied the oxidation of methanol in a biofuel cell using a quinoprotein-linked ADH as catalyst (56). This ADH catalyzes the oxidation of methanol, other primary alcohols and formaldehyde. The authors evaluated several mediators (BCB; PMS; PES; safranin-O; phenolsafranin; brilliant blue-B; malachite green; 1-methoxyphenazine methosulfate; methylviolet-6B; N,N,N',N'-tetramethyl-4-phenylenediamine (TMPD)) and found TMPD to be the best. The biofuel cell, using TMPD as mediator, operated at 20°C and pH 10.5 with a current fluctuation of less than 10% over a 24 h period. Maximum output of power (12 μW) was obtained at 0.2 mA and 60 mV.

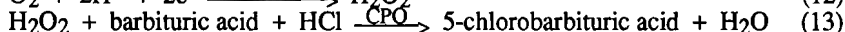
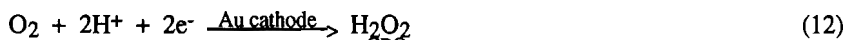
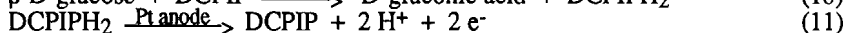
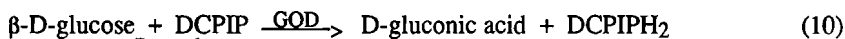
To eliminate the accumulation of formate in the anode compartment, Yue and Lowther used both MDH and FDH in their biofuel cell (57). Both PMS and PES were used as mediators for electron transfer. Because PMS and PES are unstable, current efficiencies were poor (36%) and decreased with time (27% in one hour). Immobilization of the enzymes onto the anode increased the average current by a factor of two. The power density was calculated to be 1.5 μW/cm<sup>2</sup> at 10 Ω based on the surface area of the anode; no open-circuit voltage was given.

**Glucose as anodic fuel.** A biofuel cell that could be implanted in the human body as a power source for implanted electronic devices (i.e. pacemakers and cochlear implants) would be very useful. To be practical, a fuel cell for this use should utilize endogenous substances, such as O<sub>2</sub> and glucose.

Yahiro *et al* (58) provided one of the earliest demonstrations of a glucose/O<sub>2</sub> fuel cell, using GOD as catalyst.  $V_{\text{cell}}$  for the GOD system was 300-500 mV. The introduction of elemental iron to the anode compartment increased both  $V_{\text{cell}}$  and current density to 750 mV and 17  $\mu\text{A}/\text{cm}^2$  at 300 mV, respectively. The probable mechanism by which elemental iron influenced the performance of the fuel cell is as a stoichiometric reducing agent (the authors proposed a different, less plausible, mechanism). It is not obvious from the reported information whether elemental iron functions as a catalyst or fuel or both.

Persson *et al* used N,N-dimethyl-7-amino-1,2-benzophenoxazinium ion ( $\text{MB}^+$ ) as the mediator in a GOD catalyzed glucose/O<sub>2</sub> fuel cell (59).  $\text{MB}^+$  was chosen over other mediators because of its higher stability and irreversible adsorption to graphite. The reduction potential of  $\text{MB}^+$  is -0.175 V vs. SCE; it therefore, spontaneously oxidizes NADH. An indirect fuel cell configuration was used where the immobilized enzyme was separated from the anode. During operation of the fuel cell, NADH resulting from the oxidation of glucose by the immobilized GOD was pumped to the anode compartment, where NADH reduced the adsorbed  $\text{MB}^+$  to MBH. The MBH was then electrochemically oxidized, and  $\text{NAD}^+$  was recirculated. An "O<sub>2</sub> simulated" cathode was used in this study by poisoning an electrode at 0.56 V vs. SCE with an external potentiostat. The fuel cell delivered a current density of 0.2  $\text{mA cm}^{-2}$  at ~ 0.8 V for more than 8 h using the poisoned cathode. When immobilized enzymes were used, the fuel cell was stable to storage for months. The mediator, however, needed to be replaced after several hours of use.

Laane *et al* demonstrated the applicability of a biofuel cell for the production of biochemicals (60). GOD was used as the catalyst in the anode compartment, and chloroperoxidase (CPO) was used as the catalyst in the cathode compartment. The mediator for electron transfer in the anode compartment was 2,6-dichlorophenol indophenol (DCPIP). The chemistry that occurred in the fuel cell is summarized in equations 10-13:



After operating a small fuel cell for three days, 10 mg of gluconic acid and 8 mg of 5-chlorobarbituric acid were produced. The current level was no greater than 1 mA and turnover numbers of  $1 \times 10^4$  and  $1 \times 10^7$  were obtained for glucose oxidase and chloroperoxidase, respectively.

### Assessments and New Approaches

The motivation for examining fuel cells is their potential in three areas: first, to convert the free energy of a redox reaction (typically the complete oxidation of fuel by dioxygen) to electrochemical work in a way that circumvents the thermodynamic limitations of heat engines; second, to build power-generating systems that operate without the moving parts and high internal temperatures and pressures of internal combustion and turbine engines, and that avoid the pollutants ( $\text{CO}$ ,  $\text{NO}_x$ , hydrocarbons) produced by these engines; third, to make use of fuels that cannot be used by other power sources. Underlying these general motivations is the idea that biology offers a range of catalysts (enzymes) that might be exploited for use in power generation, and that fuel cells offer one type of system in which enzymes might be used.

The research conducted so far does not establish whether biofuel cells are, in reality, contenders for practical use. Although certain enzymes do have high catalytic efficiency, most are only moderately active: the uniqueness of enzymes lies in their

selectivity and amenability to metabolic control, rather than in their ability to accelerate reactions. The competition for enzymes as catalysts in fuel cells is platinum and related metals. In terms of activity and stability as an anode operating with dihydrogen as a fuel, these metals are difficult to surpass.

There have been many small-scale demonstrations of power produced from biofuel cells (although none have demonstrated net power production: i.e., demonstrated that power produced was in excess of power consumed to produce the enzymes, fuels, reagents and cell materials, and to operate the cell). The principle question is: "Is the activity available in biological catalysts sufficient, and in the right form, to provide the basis for a practical fuel cell?" Future work should focus on this question.

In general, there seem to be two approaches to a practical biofuel cell. The first is to use microorganisms to convert fuels that cannot presently be used in conventional fuel cells into appropriate form: the conversion of glucose to dihydrogen represents an obvious example. The second is to use isolated enzymes to generate reducing equivalents that can be used in new types of fuel cells: the conversion of methanol to  $\text{CO}_2$  with production of NADH or FADH represents an example.

The fundamental difficulty with approaches to fuel cells based on microorganisms is their low volumetric catalytic activity. If containment vessels are inexpensive enough, however, the capacity for self-renewal of the catalysts by microbial growth, and their ability to accept biologically-derived substrates, might make them interesting. The report of Karube, Suzuki *et al*, in which a 200 L vessel generated sufficient dihydrogen to produce 10 W of power, but in which the power generated was only 1% of the energy budget (pumps and heaters) of the cell as it was operated, illustrates the challenge. Unfortunately, the most efficient bioconversions do not generate dihydrogen, but rather species such as acetic acid, a fuel that cannot be used by currently available fuel cells.

Approaches based on isolated enzymes have the capability, in principle, to equal transition metal-based systems in volumetric catalytic activity, but a careful study of the systems tradeoffs must be performed before the real potential of these systems can be defined. Important questions in this area are whether satisfactory redox mediators can be found or whether the fuel cell must be run in a direct configuration, the nature of membrane separators and electrodes that will resist fouling by components of biological systems, the potential of enzymes to decrease the overvoltage at the dioxygen cathode, and the potential for enzymes from thermophiles to permit operation of the fuel cell at higher temperatures.

To increase the value of research in this area, more emphasis should be placed on reporting the electrochemical details of the systems being examined. Typically, power density ( $\text{W cm}^{-2}$  of electrode) is the parameter most often used to compare the performance of different fuel cells. Unfortunately, power density is rarely reported in the biofuel cell literature (although it may not be the ideal parameter to use when one or both electrodes are inexpensive, high surface area systems such as carbon felts or packed carbon beds). If  $V_{\text{cell}}$  and current output at a given load are reported and the dimensions of both electrodes included, power density can be calculated. Examination of Tables I-III reveals that it is also rare to find all three of these parameters ( $V$ ,  $A$ ,  $\text{cm}^2$ ) reported. Future reports on biofuel cells should include  $V_{\text{cell}}$  at open-circuit, current and voltage at a given load, and the geometrical area (or other relevant descriptions) of both anode and cathode. Of the literature reviewed, the highest power density that could be calculated from the values reported for microbial biofuel cells was  $533 \mu\text{W cm}^{-2}$  (based on the calculated surface area of both sides of the anodes and cathodes) (38). The enzymatic biofuel cell with the highest power density ( $160 \mu\text{W cm}^{-2}$  based on the current density reported) was reported by Persson *et al* (59). The corresponding value for a phosphoric acid cell using dihydrogen as fuel is  $\sim 100 \text{ mW cm}^{-2}$  (2).

Table IV. Applications for Biofuel Cells and Their Desirable Features

<i>Application</i>	<i>Fuel/Type of Catalyst; Desirable Features</i>
Implantable	glucose/O <sub>2</sub> /using human derived enzymes; biocompatible.
Developing Countries	carbohydrates or agricultural and municipal wastes/using microorganisms; robust, low-cost components.
Portable	methanol or ethanol/using thermophilic enzymes; high activity (to achieve high power density).
Space	human waste/microbial; simplicity in regeneration of catalysts, dual use in environmental management and power generation.
Waste Control	waste/microbial; able to handle a large range of feeds.
Cogeneration	detoxify waste in anode and produce synthetic compound in cathode/microbial in anode, enzymatic in cathode; detoxify industrial waste while simultaneously producing a valuable chemical product in the cathode, generate power.

The open-circuit voltage indicates the loss in voltage from the theoretical maximum cell voltage. A comparison of this parameter for different fuel cells provides information on the relative rates of electron transfer of fuels or different mediators at different electrode surfaces. The highest open-circuit voltage reported for a microbial biofuel cell was 1.04 V where dihydrogen (produced by *Enterobacter aerogenes* from glucose) was oxidized at the anode and 0.01 M K<sub>3</sub>[Fe(CN)<sub>6</sub>] was reduced at the cathode (48). The highest open-circuit voltage (0.6 V) reported for an enzymatic biofuel cell used DCPIP as the mediator in the anode compartment and dioxygen in the cathode compartment (60).

What constitutes a practical biofuel cell? The answer to this question depends upon the application. For example, space applications suggest systems in which the renewability of the catalyst by fermentation is a valuable characteristic, while applications above room temperature will require thermophilic catalysts. Table IV summarizes possible applications for biofuel cells and the projected important parameters (61,62).

## Conclusions

The generation of power, albeit in small quantities, using biological catalysts in a fuel cell has been exhaustively demonstrated. The poor current and power densities reported in the literature reveal that key obstacles to developing a practical biofuel cell are increasing the catalytic rates of the system of microorganisms or enzymes used, and maximizing the rates of heterogeneous electron transfer, without losing cell voltage. In the next phase of biofuel cell research, robust and highly active enzymes appropriate for use in biofuel cells must be identified and characterized. If careful analysis indicates that these enzymes have the theoretical capacity to form the basis for practical biofuel cells, their costs must be lowered (and perhaps their properties improved) through genetic engineering. Systems that do not require NAD<sup>+</sup> should be explored as well as mediators whose E°' is equal to E°' of the fuel or the common cofactor systems (NAD<sup>+</sup>/NADH and FAD<sup>+</sup>/FADH). The engineering aspects of the



cells -- component lifetimes, membrane performance, energy budget -- require much closer attention than they have received.

The challenge to developing a practical biofuel cell is straightforward: the external circuit and the products of enzymatic catalysis must be linked in a manner that maximizes cell voltage, charge flow and systems life, and minimizes costs.

### Legend of Symbols and Selected Chemical Structures

ADH = alcohol dehydrogenase

AldDH = aldehyde dehydrogenase

FAD<sup>+</sup>/FADH = oxidized/reduced form of flavin adenine dinucleotide

FDH = formate dehydrogenase

FeCyDTA = Fe(III)-cyclohexane-1,2-diamine-N,N,N',N'-tetraacetic acid

FeDTPA = Fe(III)-diethylenetriamine pentaacetic acid

FeEDADPA = Fe(III)-ethylenediamine diacetic acid dipropionic acid

FeEDTA = Fe(III)-ethylenediamine tetraacetic acid

FeTTHA = Fe(III)-triethylenetetramine hexaacetic acid

GC = glassy carbon

HNQ = 2-hydroxy-1,4-naphthoquinone

NAD<sup>+</sup>/NADH = oxidized/reduced form of nicotinamide adenine dinucleotide

NSIDCP = 1-naphthol-2-sulfonate indo-2,6-dichlorophenol

RVC = reticulated vitreous carbon

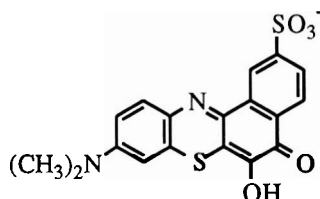
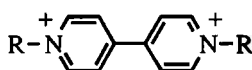
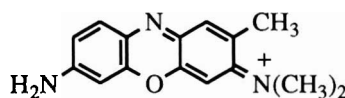


ABB = Alizarin Brilliant Blue

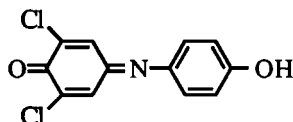


R = benzyl: BV<sup>2+</sup> = Benzyl Viologen

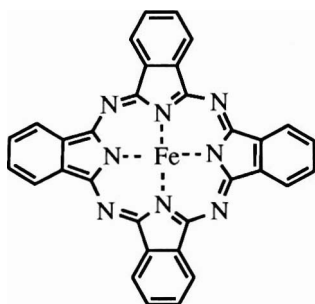
R = Me: MV<sup>2+</sup> = Methyl Viologen



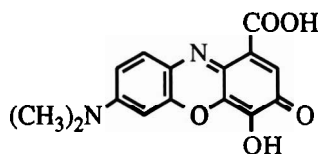
BCB = Brilliant Cresyl Blue



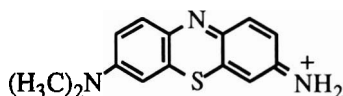
DCPIP = 2,6-dichlorophenolindophenol



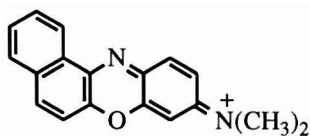
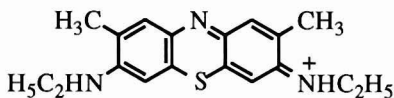
Fe(phthal) = Iron phthalocyanine



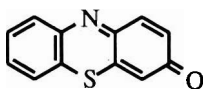
GCN = Gallocyanine



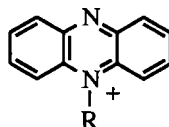
MB = Methylene Blue

MB<sup>+</sup> = Meldola's Blue

NMB = New Methylene Blue

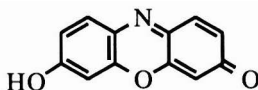


PTZ = Phenothiazinone

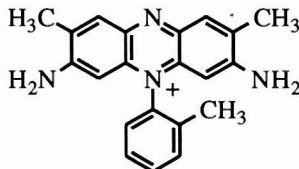


R = Me: PMS = Phenazine methosulphate

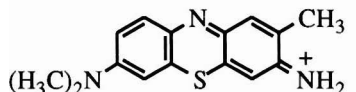
R = Et: PES = Phenazine ethosulphate



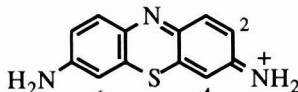
RS = Resorufin



SF = Safranin-O



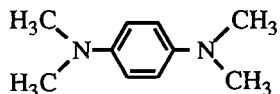
TB = Toluidine Blue-O

TH<sup>+</sup> = Thionine

DMST-2 = N,N-dimethyl-disulfonated thionine

DST-2 = 2,6-disulfonated thionine

TST-1 = 2,4,6-trisulfonated thionine



TMPD = N,N,N',N'-tetramethyl-4-phenylene diamine

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## Literature Cited

1. Vijh, S. K. *J. Chem. Ed.* **1970**, *47*, 680.
2. Kleywegt, G. J.; Driessen, W. L. *Chem. Br.*, **1988**, *24*, 447.
3. Prentice, G. *Chem. Tech.* **1984**, *14*, 684.
4. Oniciu, L. In *Fuel Cells*; Hammel, J., Ed.; Abacus Press; Tunbridge Wells, UK, 1976.

5. de Bethune, A. J. *J. Electrochem. Soc.* **1960**, *107*, 937.
6. Liebhafsky, H. A. *J. Electrochem. Soc.* **1959**, *106*, 1068.
7. Liebhafsky, H. A. *J. Electrochem. Soc.* **1961**, *108*, 601.
8. Kordes, K. *Ber. Bunsenges. Phys. Chem.* **1990**, *94*, 902.
9. *Assessment of Research Needs for Advanced Fuel Cells*; Penner, S. S., Ed.; Energy; Pergamon Press: NY, 1986.
10. Van Dijk, C.; Laane, C.; Veeger, C. *Recl. Trav. Chim., Pays-Bas* **1985**, *104*, 245.
11. Aston, W. J.; Turner, A. P. F. *Biotech. and Gen. Eng. Rev.* **1984**, *1*, 89.
12. Disalvo, E. A. *Bioelectrochem. Bioenerg.* **1980**, *7*, 807.
13. Wingard, Jr., L. B.; Shaw, C. H.; Castner, J. F. *Enzyme Microb. Technol.* **1982**, *4*, 137.
14. Parsons, R.; VanderNoot, T. *J. Electroanal. Chem.* **1988**, *257*, 9.
15. McNicol, B. D. *J. Electroanal. Chem.* **1981**, *118*, 71.
16. Wong, C-H.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 2816.
17. Lee, L. G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 6999.
18. Laval, J-M.; Bourdillon, C.; Moiroux, J. *J. Am. Chem. Soc.* **1984**, *106*, 4701.
19. Blankespoor, R. L.; Miller, L. L. *J. Electroanal. Chem.* **1984**, *171*, 231.
20. Gorton, L. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 1245.
21. Chenault, H. K.; Whitesides, G. M. *Appl. Biochem. Biotech.* **1987**, *14*, 147.
22. Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *Biotech. Gen. Eng. Rev.* **1988**, *6*, 221.
23. Bonnefoy, J.; Moiroux, J.; Laval, J-M.; Bourdillon, C. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 941.
24. Persson, B. *J. Electroanal. Chem.* **1990**, *287*, 61.
25. Persson, B.; Gorton, L. *J. Electroanal. Chem.* **1990**, *292*, 115.
26. Biade, A-E.; Bourdillon, C.; Laval, J-M.; Mairesse, G.; Moiroux, J. *J. Am. Chem. Soc.* **1992**, *114*, 893.
27. Miyawaki, O.; Yano, T. *Enzyme Microb. Technol.* **1992**, *14*, 474.
28. Rodkey, F. L. *J. Biol. Chem.* **1955**, *213*, 777.
29. Moiroux, J.; Elving, P. J. *Anal. Chem.* **1978**, *50*, 1056.
30. Moiroux, J.; Elving, P. J. *J. Electroanal. Chem.* **1979**, *102*, 93.
31. Alberly, W. J.; Knowles, J. R. *Biochemistry* **1976**, *15*, 5631.
32. Sisler, F. D. *Prog. Ind. Microbiol.* **1971**, *9*, 1.
33. Turner, A. P. F.; Aston, W. J.; Higgins, I. J.; Davis, G.; Hill, H. A. O. *Biotechnol. Bioeng. Symp.* **1982**, *12*, 401.
34. Suzuki, S.; Karube, I. *Appl. Biochem. Bioeng.* **1983**, *4*, 281.
35. Davis, J. B.; Yarbrough, Jr., H. F.; *Science* **1962**, *137*, 615.
36. van Hees, W. J. *Electrochem. Soc.* **1965**, *112*, 258.
37. Karube, I.; Matsunaga, T.; Tsuru, S.; Suzuki, S. *Biotechnol. Bioeng.* **1977**, *19*, 1727.
38. Suzuki, S.; Karube, I.; Matsunaga, T.; Kuriyama, S.; Suzuki, N.; Shirogami, T.; Takamura, T. *Biochimie* **1980**, *62*, 353.
39. Karube, I.; Suzuki, S.; Matsunaga, T.; Kuriyama, S. *Ann. N. Y. Acad. Sci.* **1981**, *369*, 91.
40. Suzuki, S.; Karube, I.; Matsuoka, H.; Ueyama, S.; Kawakubo, H.; Isoda, S.; Murahashi, T. *Ann. N. Y. Acad. Sci.* **1983**, *413*, 133.
41. Delaney, G. M.; Bennetto, H. P.; Mason, J. R.; Roller, S. D.; Stirling, J. L.; Thurston, C. F. *J. Chem. Technol. Biotechnol.* **1984**, *34B*, 13.
42. Roller, S. D.; Bennetto, H. P.; Delaney, G. M.; Mason, J. R.; Stirling, S. L.; Thurston, C. F. *J. Chem. Technol. Biotechnol.* **1984**, *34B*, 3.
43. Thurston, C. F.; Bennetto, H. P.; Delaney, G. M.; Mason, J. R.; Roller, S. D.; Stirling, J. L. *J. Gen. Microbiol.* **1985**, *131*, 1393.

44. Bennetto, H. P.; DeLaney, G. M.; Mason, Roller, S. D.; Stirling, J. L.; Thurston, C. F. *Biotech. Lett.* **1985**, *7*, 699.
45. Lithgow, A. M.; Romero, L.; Sanchez, I. C.; Souto, F. A.; Vega, C. A. *J. Chem. Res., Synop.* **1986**, *5*, 178.
46. Vega, C. A.; Fernandez, I. *Bioelectrochem. Bioenerg.* **1987**, *17*, 217.
47. Akiba, T.; Bennetto, H. P.; Stirling, J. L.; Tanaka, K. *Biotechnol. Lett.* **1987**, *9*, 611.
48. Tanisho, S.; Kamiya, N.; Wakao, N. *Bioelectrochem. Bioenerg.* **1989**, *21*, 25.
49. Sell, D.; Kraemer, P.; Kreysa, G. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 211.
50. Kreysa, G.; Sell, D.; Kraemer, P. *Ber. Bunsen-Ges. Phys. Chem.* **1990**, *94*, 1042.
51. Habermann, W.; Pommer, E.-H. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 128.
52. Tanaka, K.; Tamamushi, R.; Ogawa, T. *J. Chem. Technol. Biotechnol.* **1985**, *35B*, 191.
53. Tanaka, K.; Kashiwagi, N.; Ogawa, T. *J. Chem. Technol. Biotechnol.* **1988**, *42*, 235.
54. Karube, I.; Suzuki, S. *Methods Enzymol.* **1988**, *137*, 668.
55. Plotkin, E. V.; Higgins, I. J.; Hill, H. A. O. *Biotech. Lett.* **1981**, *3*, 187.
56. Davis, G.; Hill, H. A. O.; Aston, W. J.; Higgins, I. J.; Turner, A. P. F. *Enzyme Microb. Technol.* **1983**, *5*, 383.
57. Yue, P. L.; Lowther, K. *Chem. Eng. J.* **1986**, *33B*, 69.
58. Yahiro, A. T.; Lee, S. M.; Kimble, D. O. *Biochim. Biophys. Acta* **1964**, *88*, 375.
59. Persson, B.; Gorton, L.; Johansson, G.; Torstensson, A. *Enzyme Microb. Technol.* **1985**, *7*, 549.
60. Laane, C.; Pronk, W.; Granssen, M.; Veeger, C. *Enzyme Microb. Technol.* **1984**, *6*, 165.
61. Srinivasa, S. *J. Electroanal. Chem.* **1981**, *118*, 51.
62. Glazebrook, R. W. *Electric Vehicle Developments*, **1982**, *4*, 18.

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