

# Biological fuel cells and their applications

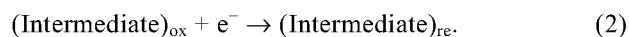
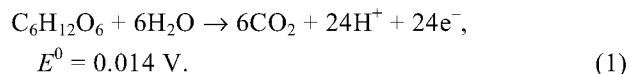
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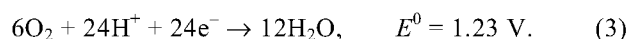
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**One type of genuine fuel cell that does hold promise in the long-term is the biological fuel cell. Unlike conventional fuel cells, which employ hydrogen, ethanol and methanol as fuel, biological fuel cells use organic products produced by metabolic processes or use organic electron donors utilized in the growth processes as fuels for current generation. A distinctive feature of biological fuel cells is that the electrode reactions are controlled by biocatalysts, i.e. the biological redox-reactions are enzymatically driven, while in chemical fuel cells catalysts such as platinum determine the electrode kinetics. This article provides a brief introduction to biological fuel cells along with their envisaged applications.**

A fuel cell is an electrochemical device that continuously converts chemical energy to electrical energy for as long as fuel and oxidant are supplied to it. Beginning with the space programme of the sixties and followed by the energy crisis of the seventies, we have seen a truly substantial improvement in generic fuel-cell technologies<sup>1-3</sup>. Albeit their high operational-efficiency, the advantages of these fuel cells are partially offset by the high cost of catalysts, high operational-temperatures, and the corrosive electrolytes employed with them. In this regard, biological fuel cells<sup>4-6</sup> are both attractive and promising. Unlike chemical fuel cells, biological fuel cells operate under mild reaction conditions, namely ambient operational temperature and pressure. They also employ neutral electrolyte and use inexpensive catalysts such as platinum. In biological fuel cells, the catalyst is either a microorganism as simple as Baker's yeast or an enzyme<sup>7-11</sup>. Biological fuel cells convert the chemical energy of carbohydrates, such as sugars and alcohols, directly into electric energy. As most organic substrates undergo combustion with the evolution of energy, bio-catalysed oxidation of organic substances by oxygen at the two electrode interfaces provides a means for the conversion of chemical energy into electrical energy. In normal microbial catabolism, a substrate such as carbohydrate is oxidized initially without participation of oxygen, while its electrons are taken up by an enzyme-active site, which acts as a reduced intermediate<sup>12</sup>, described as follows.



In the absence of oxygen, the electrons are diverted to the electrode by some means and made to pass through the outer circuit, and ultimately combine with an electron sink, namely, molecular oxygen as follows.

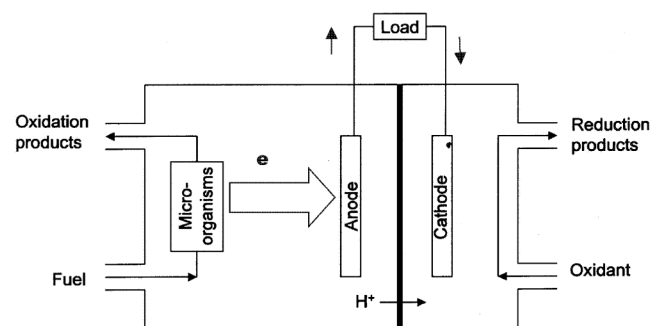


If a continuous fuel flow to the aforesaid microbial fuel cell is maintained, it acts as a biological fuel cell. A typical biological fuel cell is shown schematically in Figure 1.

After a brief outline of the long and winding history of biological fuel cells, this article deals with the operating principles of various types of biological fuel cells and their technological applications.

## History of biological fuel cells

For centuries, microorganisms, which transform food into an electron flow, were only a biological curiosity; but now scientists have made it possible to use them in watches and cameras as power sources<sup>13</sup>. Luigi Galvani, who noticed twitching of isolated frog leg when a brief electrical discharge was passed through it<sup>14</sup>, was the first to observe the bioelectric phenomenon as early as in 1790 and the term bioelectricity was coined after that observation. In 1910, Michael Cresse Potter, a professor of botany at the



**Figure 1.** A typical biological fuel cell representing current generation with the help of microorganisms. The fuel generated by microbial metabolism gets oxidized at the anode and usually oxygen is reduced at the cathode.

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University of Durham, UK demonstrated that organisms could generate a voltage and deliver current. Cohen at Cambridge revived Potter's idea in 1931. He described how a batch of biological fuel cells produced more than 35 V. But biological fuel cells became popular in the 1960s, when the National Aeronautics and Space Administration evinced interest in turning organic waste into electricity on its long-haul space flights. Algae and bacteria were among the first organisms used in biological fuel cells. During the mid 19th century, for the first time, Rohrback *et al.*<sup>15</sup> designed a biological fuel cell in which *Clostridium butyricum* was used as a biological material to generate hydrogen by glucose fermentation. In 1963, biological fuel cells were already commercially available for use as a power source in radios, signal lights and other appliances at sea. However, these fuel cells were not a commercial success and soon disappeared from the market. With the successful development of technical alternatives, e.g. solar photovoltaics for the energy supply on space flights, biological fuel cells suffered a short setback. Later, during the oil crisis of the 70s and 80s, the interest in the development of biological fuel cells was revived. In 1966, Williams<sup>16</sup> showed that biological fuel cells powered by rice husk produced 40 mA at 6 V. Rice husk is a potential source of lignocellulose, which on fermentation yields many useful enzymes and biofuels like ethanol that could be used in biological fuel cells. In 1969, Yao *et al.*<sup>17</sup> showed that glucose could be used as a fuel in the presence of platinum-black. Later, Karube *et al.*<sup>18</sup> reported the generation of about 300 mA electric current from an *Anabaena* spp.-based biological fuel cell, in which phosphoric acid was used as the electrolyte. Bennetto and co-workers<sup>19-21</sup> have made noteworthy contributions to biological fuel cells. They have developed and demonstrated improved biological fuel cells using various microorganisms and mediator systems. They showed that the mediators could enhance both the efficiency of electron-transfer and the reaction rate. Recently, Chaudhuri and Lovely<sup>22</sup>, have reported that a microorganism *R. ferrireducens* can recover an electron from glucose oxidation in the presence of Fe<sup>3+</sup> up to 83% without a mediator. Presently, efforts are being expended to improve the performance of mediator-less biological fuel cells as well as on finding an effective route to wire the microorganism to the electrode so as to promote the efficiency of electron-transfer.

### Types of biological fuel cells

There are two types of biological fuel cells, namely microbial fuel cells and enzymatic fuel cells.

#### Microbial fuel cells

The use of microorganisms in biological fuel cells eliminates the isolation of individual enzymes, thereby providing

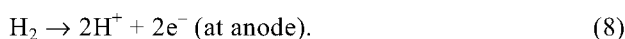
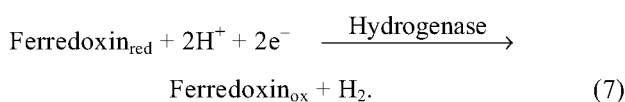
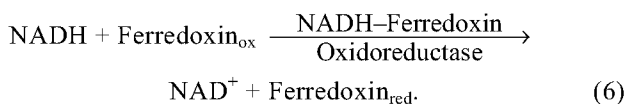
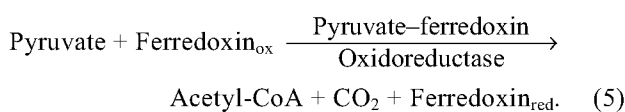
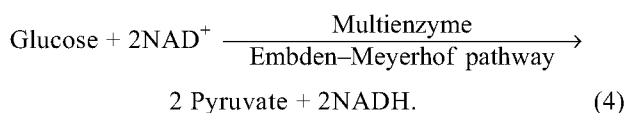
cheaper substrates for biological fuel cells. Microorganisms can be used in four ways for producing electrical energy:

- (i) Microorganisms can produce electrochemically active substances through fermentation or metabolism. For the purpose of energy generation, fuels are produced in separate reactors and transported to the anode of a conventional fuel cell. Accordingly, in this configuration, the microbial bioreactor is kept separated from the fuel cell.
- (ii) In the second configuration, the microbiological fermentation process proceeds directly in the anodic compartment of the fuel cell.
- (iii) In the third configuration, electron-transfer mediators shuttle electrons between the microbial biocatalytic system and the electrode. The mediator molecules accept electrons from the biological electron transport chain of the microorganisms and transport them to the anode of the biological fuel cell.
- (iv) In the fourth configuration, the metal-reducing bacterium having cytochromes in its outer membrane and the ability to communicate electrically with the electrode surface directly result in a mediator-less biological fuel cell.

A brief description of the aforesaid configurations is given below:

*Microbial-systems producing hydrogen as fuel for conventional fuel cells:* Various bacteria and algae, e.g. *Escherichia coli*, *Enterobacter* aero-genes, *C. butyricum*, *Clostridium acetobutylicum* and *Clostridium perfringens*, are known to be active for hydrogen production under anaerobic condition<sup>23,24</sup>. The most effective hydrogen-producing microorganism is *C. butyricum*<sup>25</sup>. *E. coli* and *Enterobacter* aero-genes are facultative anaerobes and ferment both glucose and lactose as a carbon source to produce hydrogen.

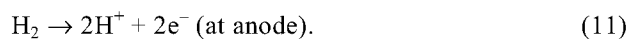
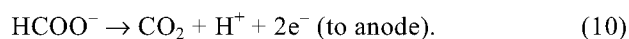
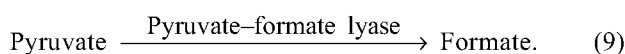
The conversion of carbohydrate to hydrogen is achieved by a multienzyme system. In bacteria, it involves the conversion of glucose to 2 mol of NADH, a reduced form of coenzyme 1, namely  $\beta$ -nicotinamide adenine dinucleotide of the vitamin niacin, and 2 mol of pyruvate formed by Embden–Meyerhof pathway. Pyruvate is then oxidized through a pyruvate–ferredoxin oxidoreductase producing acetyl-CoA, CO<sub>2</sub> and reduced ferredoxin. NADH–ferredoxin oxidoreductase oxidizes NADH and reduces ferredoxin. The reduced ferredoxin is reoxidized to form hydrogen by hydrogenase. As a result, 4 mol of H<sub>2</sub> are produced from 1 mol of glucose under ideal conditions as shown below. In practice, however, H<sub>2</sub> yield is only about 25% of the theoretical value<sup>26</sup>. Improvement in H<sub>2</sub> production is possible by genetic engineering techniques and screening of new hydrogen-producing bacteria.



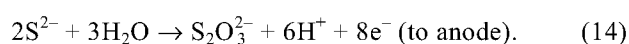
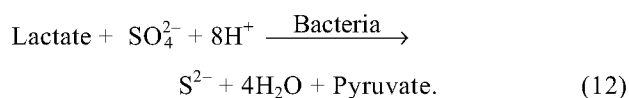
A  $\text{H}_2/\text{O}_2$  fuel cell comprising a platinum-black-nickel mesh anode and a palladium-black-nickel mesh cathode, and separated by a nylon filter operating at room temperature was connected to a bioreactor producing hydrogen<sup>27,28</sup>. The current and voltage output were dependent on the rate of hydrogen production in the fermenter. For example, an open-circuit voltage ( $V_{\text{oc}}$ ) of 0.95 V and a short-circuit current density ( $I_{\text{sc}}$ ) of 40 mA/cm<sup>2</sup> were obtained at a  $\text{H}_2$  flow of 40 ml/min. The biological fuel cell operating under steady-state conditions for a week produced a continuous current between 500 and 550 mA.

The immobilization of the biocatalyst is primarily important in a bioreactor. In the biological fuel cell configuration described above, the immobilization of hydrogen-producing bacteria, *C. butyricum* is of great value as this stabilizes the relatively unstable hydrogenase system. The immobilization steps could be the trapping of microorganisms into polymeric matrices of polyacrylamide<sup>25</sup>, agar gel<sup>27,28</sup> or filter paper<sup>26</sup>. The immobilized microbial cells continuously produced  $\text{H}_2$  under anaerobic conditions for several weeks, whereas non-immobilized bacterial cells were fully deactivated in less than two days.

*Microbial systems producing electrochemically active metabolites in the anodic compartment of biological fuel cells:* In this configuration, the fermentation process is conducted directly at the electrode surface supplying the anode with  $\text{H}_2$  fuel. Additional by-products of the fermentation process, namely formic acid, acetic acid and lactic acid are also utilized as fuels<sup>29</sup>. Besides, the base substrate glucose used for the fermentation process, by-products could as well contribute to the anodic current. Hence,  $\text{H}_2$  provided by the microorganisms can be a source of anodic current as indicated by the side reactions below.



In addition to fuels like  $\text{H}_2$ , formic acid, lactic acid and sulphur-containing electrochemically active metabolites like  $\text{S}^{2-}$  species, can be produced during the fermentation of lactate by *Desulfovibrio desulfuricans*, which are known to be sulphate-reducing bacteria, shown as follows.



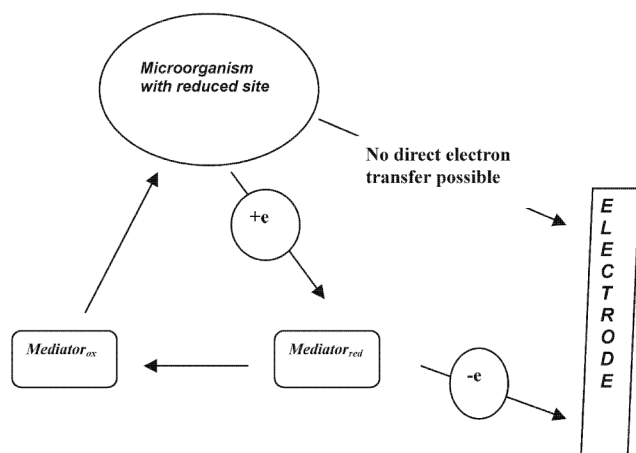
The presence of sulphides in the medium results in the inhibition of the metabolic bacterial processes because of their interaction with iron-containing proteins, e.g. cytochromes, causing the electron transport systems to be blocked.  $\text{S}^{2-}$  species poison many metallic electrodes because of their strong and irreversible adsorption. In a typical experiment, porous graphite electrode impregnated with cobalt hydroxide as catalyst was used as the anode. Cobalt hydroxide undergoes transition into a highly catalytically active cobalt oxide/cobalt sulphide mixture. A biological fuel cell has been constructed with the above biocatalytic anode along with a graphite cathode activated with iron(II) phthalocyanins and vanadium(V) compounds. The anode and cathode are separated to maintain anaerobic conditions at the anode compartment. The cell characteristics observed are  $V_{\text{oc}} = 2.8$  V and  $I_{\text{sc}} = 2.5$ –4 A. The cell was operated for a period of 18 months with about 6A being drawn from it for 40–60 min daily<sup>30,31</sup>. The poor performance of fuel cells arising due to the adsorption of by-products on the electrode surface is improved by an electrode modification process. The microorganisms processing glucose in a tank of the fermentation fluid are continuously pumped through a separate anode space. This is separated from the cathode space by a semi-permeable membrane. These bio-fuel cells have a new type of anode where a platinum electrode or a platinized graphite electrode is coated with a layer of the electrically conducting polyaniline, which is both biocompatible and electro-catalytically active. It absorbs electrons from the metabolism of the bacteria and transfers them to the anode. In this way, it plays a decisive role in current flow. During operation, the bacterial metabolic products along with the by-products of the electro-catalytic oxidation process settle on an uncoated anode and rapidly deactivate it. The polymer slows down this process considerably. Additional regular voltage pulses chemically convert the deposit and release them from the anode surface. This fuel cell continuously provides up to 1.5 mA/cm<sup>2</sup> of current<sup>32</sup>.

**Mediator-coupled microbial fuel cells:** Reductive species generated by metabolic processes inside microbial cells are isolated by a microbial membrane. Thus, the contact of the microbial cells with an electrode usually results in only a diminutive electron-transfer across the membrane of the microbes<sup>33</sup>, except in some special cases as described later. The electro-active groups responsible for the redox activity of enzymes present in the microbial cells are deeply buried inside their prosthetic groups, which leads to poor electrical communication between the cells and the electrode surface. The cells can, however, be wired to the electrode surface with the help of mediators. Low molecular weight redox species may assist the shuttling of electrons between the intracellular bacterial space and an electrode, and are referred to as mediators. The working principle of these mediators is shown schematically in Figure 2.

The mediator molecules should meet the following requirements<sup>34</sup>:

- (i) The oxidized mediator should easily penetrate through the bacterial membrane to reach the reductive species inside the bacteria.
- (ii) The redox potential of the mediator should match the potential of the reductive metabolite.
- (iii) None of the oxidation states of the mediator should interfere with other metabolic processes.
- (iv) The reduced mediator should easily escape from the cell through the bacterial membrane.
- (v) Both the oxidized and reduced states of the mediator should be chemically stable in the electrolyte solution, should be easily soluble, and should not adsorb on the bacterial cells or electrode surface.
- (vi) The electrochemical kinetics of the oxidation process for the mediator-reduced state at the electrode should be fast.

A variety of organic compounds have been studied in combination with bacteria to test the electron-transfer effi-



**Figure 2.** Working principle of redox mediators.

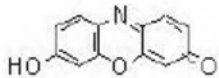
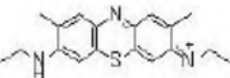
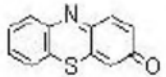
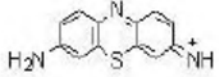
ciency of the mediator between the microorganism and anode surface. Thionine and organic dyes<sup>35-40</sup> have been frequently used as mediators in biological fuel cells. Redox potentials and structures of some of the electron relays (mediators) are summarized in Table 1. The overall efficiency of the electron-transfer mediators depends on many parameters, particularly on the electrochemical rate-constant of mediator re-oxidation, which depends on the electrode materials<sup>41,42</sup>. It is difficult to realize the perfect conditions for electron transport from a bacterial cell to an electrode. A mixture of two mediators can be useful in optimizing the efficiency. Two mediators, namely thionine and Fe(III) EDTA, are employed with the biocatalyst *E. coli* for the oxidation of glucose<sup>43</sup>. It was found that thionine is reduced over 100 times faster than Fe(III) EDTA. But the electrochemical oxidation of thionine is much slower than the oxidation of Fe(II) EDTA. Therefore, electrons obtained from the biocatalysed oxidation of glucose are transferred mainly to thionine. The reduced thionine is rapidly reoxidized by Fe(III) EDTA. The reduced Fe(II) EDTA transfers the electrons to the anode, and is kinetically fast.

Another example of a mixture of mediators promoting electron-transfer across the anode is demonstrated with the help of methyl viologen and 2-hydroxy-1,4-naphthoquinone in the case of bioelectrocatalysed oxidation of glucose in the presence of *E. coli* immobilized on graphite particles<sup>44</sup>. Table 2 summarizes the characteristics of biological fuel cells containing mediators.

The electrodes should be designed so as to facilitate electrical contact between a biocatalytic system and an anode, and to improve the cell output. The mediators can be coupled to the microorganisms in three ways<sup>19,44,45</sup> (Figure 3): (i) as diffusional mediator shuttling between the microbial suspension and the anode surface, (ii) a diffusional mediator shuttling between the anode and microbial cells covalently linked to the electrode. The microbial cells can be covalently linked to the electrode surface having  $-COOH$  groups through amino groups of the microbial membrane resulting in the formation of amide bond. Standard organic reagents like carbodiimide and acetyl chloride are used to link the microbial cells to the surface, and (iii) mediator adsorbed on the microbial cells providing electron transport from the cells to the anode.

**Mediator-less microbial fuel cells:** Fe(III)-reducing microorganisms are found to be electrochemically active as they have cytochromes in their outer membranes. It was first demonstrated with the Fe(III) reducers, *Shewanella putrefaciens*, that these can be used as a catalyst in a mediator-less microbial fuel cell<sup>46-48</sup>. Recent studies have demonstrated that Fe(III) reducing microorganisms of the family Geobacteraceae can directly transfer electrons on to electrodes. However, the range of electron donors that these organisms can use is limited to simple organic acids such as acetate<sup>49,50</sup>.

**Table 1.** Redox potential with structural formula of mediators used

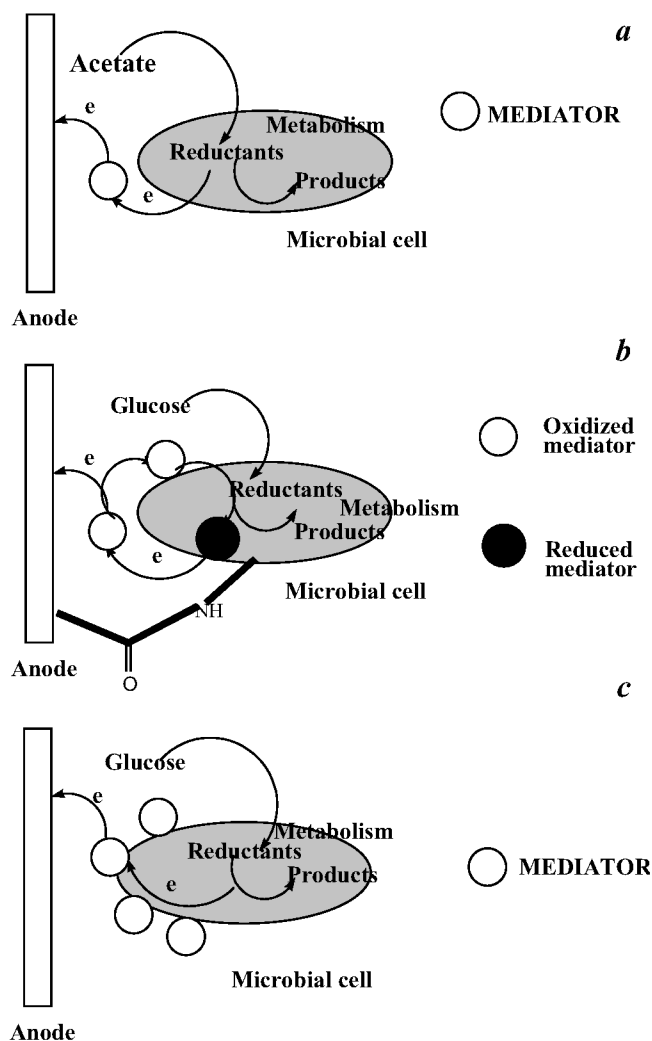
Redox relay	Structural formula	Redox potential <i>V</i> (vs NHE)	Rate of reduction $\mu\text{mol (g dry wt)}^{-1}\text{s}^{-1}$ *
Resorufin		-0.051	0.61
New methylene blue		0.021	0.20
Phenothiazinone		1.43	0.130
Thionine		0.064	7.10

\*The dye reduction by *Proteus vulgaris* at 30°C, with 50 mM dye and 0.10–0.15 mg (dry wt) ml<sup>-1</sup> microbial cells.

**Table 2.** Examples of microbial-based biological fuel cells utilizing electron relays for coupling of intracellular electron-transfer processes with electrochemical reactions at the anode<sup>76,a</sup>

Microorganism	Nutritional substrate	Mediator	Cell voltage	Current or current density	Anode <sup>c</sup>
<i>Pseudomonas methanica</i>	CH <sub>4</sub>	1-Naphthol-2-sulphonate indo-2,6-dichloro-phenol	05–06 V (oc) <sup>d</sup>	2.8 $\mu\text{A cm}^{-2}$ (at 0.35 V)	Pt-black 12.6 cm <sup>2</sup>
<i>Escherichia coli</i>	Glucose	Methylene blue	0.625 V (oc)	–	Pt, 390 cm <sup>2</sup>
<i>Proteus vulgaris</i> <i>Bacillus subtilis</i> <i>E. coli</i>	Glucose	Thionine	0.64 V (oc)	0.8 mA (at 560 $\Omega$ )	Reticulated vitreous carbon 800 cm <sup>2</sup>
<i>P. vulgaris</i>	Glucose	Thionine	350 mV (at 100 $\Omega$ ) <sup>b</sup>	3.5 mA (at 100 $\Omega$ )	Reticulated vitreous carbon 800 cm <sup>2</sup>
<i>P. vulgaris</i>	Sucrose	Thionine	350 mV (at 100 $\Omega$ ) <sup>b</sup>	350 mA (at 100 $\Omega$ )	Carbon
<i>E. coli</i>	Glucose	Thionine	390 mV (at 560 $\Omega$ ) <sup>b</sup>	0.7 mA (at 560 $\Omega$ )	–
<i>Lactobacillus plantarum</i> <i>Streptococcus lactis</i>	Glucose	Fe(III) EDTA	0.2 V (oc)	90 $\mu\text{A}$ (at 560 $\Omega$ ) <sup>b</sup>	–
<i>Erwinia dissolvens</i>	Glucose	Fe(III) EDTA	0.5 V (oc)	0.7 mA (at 560 $\Omega$ ) <sup>b</sup>	–
<i>P. vulgaris</i>	Glucose	2-Hydroxy-1,4-naphthoquinone	0.75 V (oc)	0.45 mA (at 1 k $\Omega$ )	Graphite felt 1 g (0.47 m <sup>2</sup> g <sup>-1</sup> )
<i>E. coli</i>	Acetate	Neutral red	0.25 V (oc)	1.4 $\mu\text{A cm}^{-2}$ (sc) <sup>e</sup>	Graphite 100 cm <sup>2</sup>
<i>E. coli</i>	Glucose	Neutral red	0.85 V (oc)	17.7 mA (sc)	Graphite felt 12 g (0.47 m <sup>2</sup> g <sup>-1</sup> )
<i>E. coli</i>	Glucose	2-Hydroxy-1,4-naphthoquinone	0.53 V (at 10 k $\Omega$ )	0.18 mA cm <sup>-2</sup> (sc)	Glassy carbon 12.5 cm <sup>2</sup>

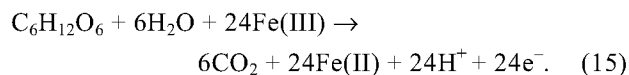
<sup>a</sup>In most of the studies, the biological anode was conjugated with an O<sub>2</sub>-cathode; <sup>b</sup>Value calculated from other data using Ohm's law; <sup>c</sup>Anode surface is given as a geometrical surface; <sup>d</sup>Open-circuit measurement; <sup>e</sup>Short-circuit measurement.



**Figure 3.** Various modes of attachment of mediators: *a*, Mediator and microorganisms are present in the solution phase; *b*, Microorganisms are covalently attached to the electrode surface; *c*, mediators are covalently linked to the outer membrane of microorganisms.

Metal-reducing bacteria are the most used species in this type of fuel cells. Studies have been conducted to understand the mechanism of Fe(III) reduction by bacteria such as *Shewanella putrefaciens* and *Geobacter metallireducens*<sup>51-53</sup>. Development of mediator-less microbial fuel cells using *Shewanella putrefaciens* IR-1 has been reported by Kim *et al.*<sup>48</sup>. In these fuel cells, the electrochemical activity of the microorganism has been confirmed by cyclic voltammetry when grown under anaerobic conditions, although no activity was found when they were grown under aerobic conditions. A gradual decrease in both coulombic yield and maximum current values was observed during the sequential batch operation of microbial fuel cells. Recently, there is a report on the bacteria, *Rhodospirillum rubrum* that can be used in microbial fuel cells effectively without a mediator<sup>22</sup>. *R. ferrireducens* was isolated from anoxic sub-surface sediments of Oyster

Bay, Virginia, USA as a dissimilatory Fe(III) reducing microorganism. *R. ferrireducens* grows on glucose in the presence of Fe(III). The stoichiometry of glucose utilization and Fe(III) reduction can be explained as below.



The performance was tested in a system that comprised a reactor containing an anaerobically growing suspension of *E. coli* K12 in a standard glucose medium (55 mM glucose) and the fuel cell consisting of an anode compartment through which the bacterial medium was pumped. The cathode was woven graphite and the catholyte was a 50 mM ferricyanide solution in a phosphate buffer, akin to the buffer in a bacterial medium.

The recovery of electrons from glucose oxidation is 83% of the theoretical value available from glucose oxidation. Microbial growth is supported by energy derived from the electron-transfer process itself and results in a stable, long-term power production. This type of microbial fuel cell exhibits many of the desirable features of secondary batteries, including the ability to be recharged to the nearly original charged state subsequent to the discharge, the ability to accept fast recharge, reasonable cycle-life and low capacity-loss under open-circuit conditions as well as on prolonged storage under idle conditions. Thus, mediator-less fuel cells have an advantage over those with mediators in terms of cost as well as non-desirability of toxic mediators. In mediator-less fuel cells, there is also ample room to increase the efficiency of electron-transfer.

### Enzymatic fuel cells

Enzymes have a complex structure comprising proteins. The electron-transferring unit of the enzyme, namely the apoenzyme and cofactor, is deeply buried inside its complex structure. Hence, efficient electrical communication between the electrode substrate and the enzyme biocatalyst is difficult. Mediated bioelectrocatalysis uses small redox molecules as electron-transfer mediators between the enzyme and the electrode. Mediated bioelectrocatalysis is useful for studying electron-transfer kinetics of the enzymes and is a key reaction in applying the enzymes to biological fuel cells<sup>54</sup>. In the case of quinoxaline, direct electron-transfer as well as mediated electron-transfer is possible between the enzyme and an electrode where the heme in the protein functions as a built-in mediator in an enzyme electrochemical reaction.

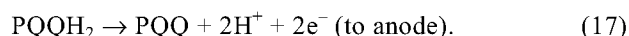
*Enzymes for the anodic reactions of biological fuel cells:* Niacin is widely distributed in plant and animal tissues. The coenzyme forms of the vitamins are the pyridine nucleotides. In accordance with the recommendations of

the Commission on Enzymes of the International Union of Biochemistry, coenzyme-I is referred to as  $\text{NAD}^+$  and coenzyme-II as  $\text{NADP}^+$  (Figure 4). The pyridine nucleotides are coenzymes for the dehydrogenase group of enzymes that are responsible for catalysis of certain biological oxidation–reduction reactions. As examples of some redox reactions in which the pyridine nucleotides participate, one might consider the action of the enzyme alcohol dehydrogenase, which oxidizes ethanol to acetaldehyde. In ethanol-based biofuel cells, the aforesaid reaction is the basis for electron generation.

Flavin is a trivial name given to the redox active prosthetic group of respiratory enzymes known as lipoproteins, which occur widely in plants and animals. Flavoproteins are enzymes that catalyse oxidation–reduction reactions in biological systems. The enzyme contains FAD (Flavin adenine dinucleotide) as the prosthetic group (Figure 4). The flavoprotein enzymes are also often found in the redox systems involving pyridine nucleotides. Figures 5 and 6 explain the participation of these coenzymes in biological oxidation–reduction processes. In order to reduce the disulphide of glutathione, glutathione reductase enzymes are employed. These enzymes contain FAD as their prosthetic group. In the presence of NADH, FAD is first reduced

to  $\text{FADH}_2$ , which then facilitates the reduction of the disulphide moiety of the glutathione. Figure 6 represents the series of oxidation–reduction reactions employed to re-oxidize the pyridine and flavin nucleotides. Molecular oxygen is the terminal electron acceptor. Such electron transport chains are associated with the respiratory processes of microorganisms. In the case of biological fuel cells, the electrode acts as the terminal electron acceptor and the electrons are channelled to the anode for current generation.

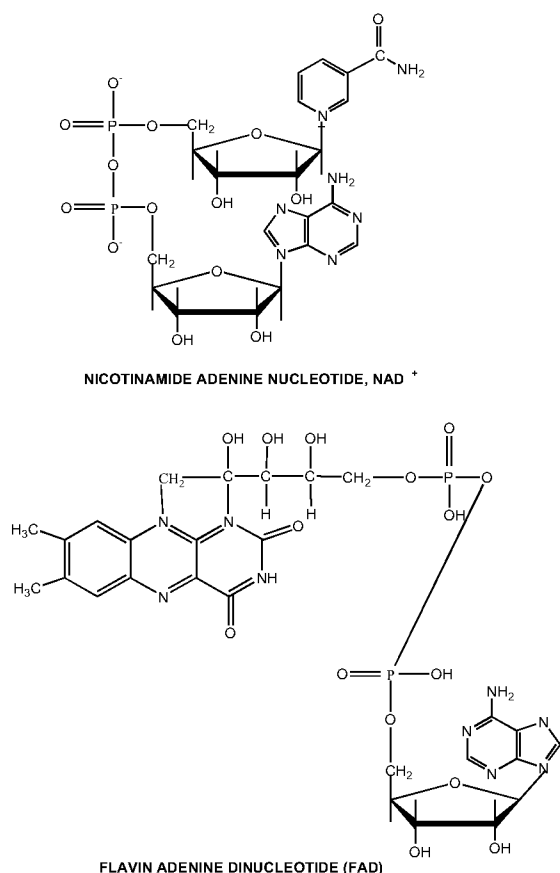
*Anodes based on the bioelectrocatalysis of NADH:* The electrochemical oxidation process  $\text{NADH}/\text{NAD}^+$  is highly irreversible and proceeds with large overpotentials. Strong adsorption of  $\text{NAD}^+$  and  $\text{NADH}$  poisons the electrode surface and inhibits the oxidation process. Hence, non-catalysed electrochemical oxidation of  $\text{NAD(P)H}$  is inappropriate for use in practical fuel cells and therefore, mediated bioelectrocatalysis is mandatory. Mediated bio-electrocatalysis proceeds through the mediator pyrroloquinoline quinone (PQQ) covalently linked to gold substrates through a self-assembled monolayer of cystamine in the presence of  $\text{Ca}^{2+}$ -ions as promoter<sup>55</sup>, as shown below.



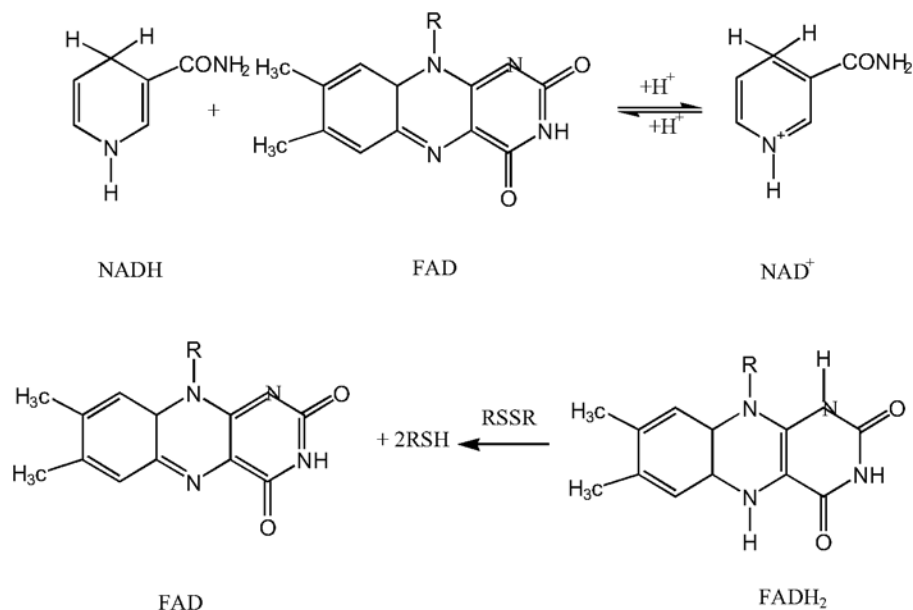
Based on the above observations, two anodic fuel-cell reactions have been standardized involving  $\text{NAD}^+$  electrocatalytic regeneration. In the first case, methanol electro-oxidation is carried out through  $\text{NAD}^+$ -dependent dehydrogenase. Diaphorase catalyses the oxidation of  $\text{NADH}$  to  $\text{NAD}^+$  using benzyl viologen as the electron acceptor at a graphite anode (Figure 7)<sup>56</sup>. The graphite anode thus releases electrons for the reduction of dioxygen at the platinum cathode. The biocatalytic anode was conjugated with an  $\text{O}_2$  cathode to complete the biological fuel cell. The total reaction in the biological fuel cell is methanol oxidation by  $\text{O}_2$ . The biological fuel cell provided  $V_{\text{oc}} = 0.8 \text{ V}$  and a maximum power density of  $0.68 \text{ mW/cm}^2$  at  $0.49 \text{ V}$ . However, this multienzyme system was utilized in a non-organized configuration where all biocatalysts exist as diffusional components in the cell.

In the second case, lactate electrooxidation is carried out through an assembly of an integrated lactate dehydrogenase (LDH) monolayer electrode fabricated by the cross-linking affinity. The complex formed between the LDH and a  $\text{PQQ-NAD}^+$  monolayer-functionalized Au electrode<sup>57–59</sup> is shown in Figure 8.

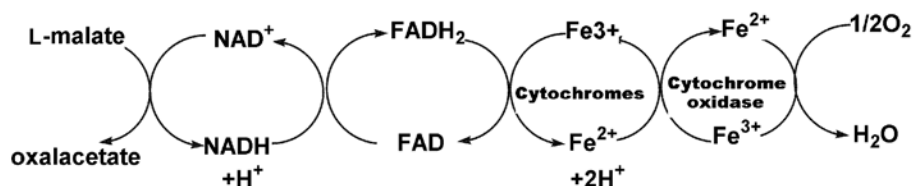
*Anodes based on bioelectrocatalysis of FAD units:* Bioelectrocatalysis of flavoenzymes can be utilized as anodic reactions in a biological fuel cell. The overall electrical efficiency of an enzyme-modified electrode depends not only on the electron transport properties of the mediator,



**Figure 4.** Chemical structure of coenzymes responsible for biological redox reactions.

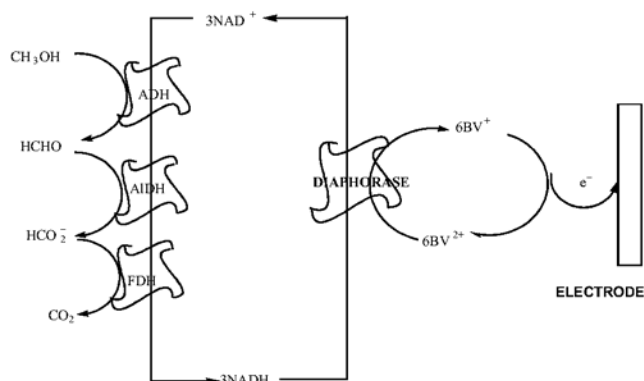
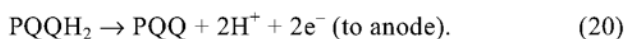
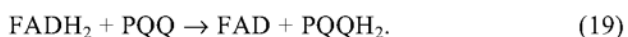
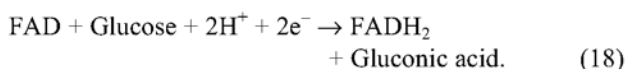


**Figure 5.** Reduction of disulphide moiety in the presence of coenzymes NADH and FAD – an example of a biological redox process.



**Figure 6.** Simplified electron transport chain for the oxidation of pyridine and flavin nucleotides by molecular oxygen.

but also on the transfer steps occurring in the assembly. In order to accomplish superior electron contact, the mediator may be selectively placed in a favourable position between the redox centre and the enzyme periphery. A novel route for establishing electrical contact between the redox centre of flavoenzymes and their environment based on a reconstitution approach has been recently demonstrated, wherein PQQ was covalently linked to a base cystamine monolayer at an Au electrode and N6-(2-aminoethyl)-FAD was then attached to the PQQ units. Subsequently, apo-glucose oxidase was reconstituted on to the FAD units of the PQQ-FAD monolayer architecture, to yield a structurally aligned, immobilized biocatalyst on the electrode surface, resulting in a high current density for glucose oxidation<sup>60-62</sup>.



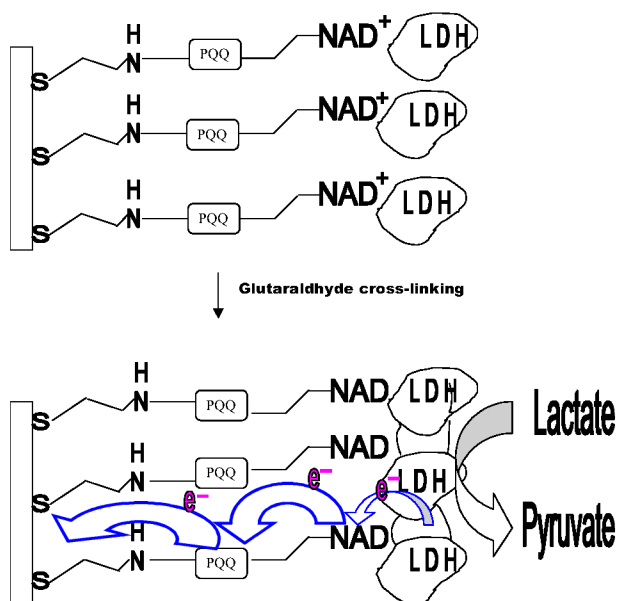
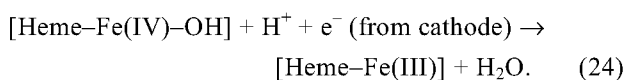
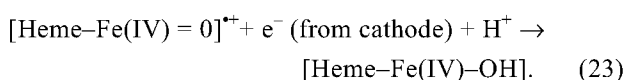
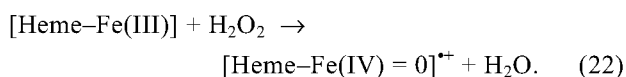
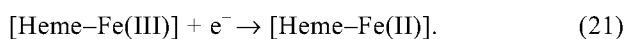
**Figure 7.** NAD<sup>+</sup>-dependent dehydrogenase oxidizes CH<sub>3</sub>OH to CO<sub>2</sub>. Diaphorase catalyses the oxidation of NADH to NAD<sup>+</sup> using benzyl viologen as electron acceptor. Benzyl viologen is oxidized back at the graphite electrode.

*Enzymes for the cathodic reactions in biological fuel cells:* Both the reduction of dioxygen and hydrogen peroxide has been studied as cathodic reactions. Various microperoxidase enzymes have been used for the reduction of H<sub>2</sub>O<sub>2</sub>. Several biological catalysts such as ascorbate oxi-



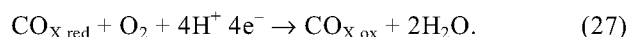
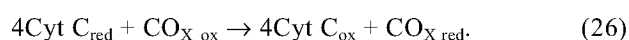
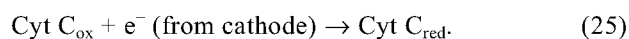
dase laccase, ceruloplasmin and cytochrome oxidase are known to catalyse the four-electron reduction of dioxygen to water. The active site of laccase contains four copper ions  $[\text{Cu}^{2+/+}]$  that couple the four-electron reduction of dioxygen to water<sup>63</sup>.

Hydrogen peroxide is a strong oxidizer; yet its electrochemical reduction proceeds with a high overpotential. The bioelectrocatalysed reduction of  $\text{H}_2\text{O}_2$  has been accomplished in the presence of various peroxidases. Microperoxidase-II (MP-II) is an oligopeptide comprising 11 amino acids and a covalently linked Fe(III)–protoporphyrin IX heme site. MP-II was covalently linked to a cystamine monolayer self-assembled on an Au electrode and the bioelectrocatalysis of  $\text{H}_2\text{O}_2$  has been characterized<sup>64</sup>. The biocatalytic reduction of organic peroxides has also been investigated in nonaqueous solvents and these studies have been useful in the development of non-compartmentalized biological fuel cells<sup>65,66</sup>.



**Figure 8.** Assembly of an integrated lactate dehydrogenase (LDH) monolayer electrode by the cross-linking affinity complex formed between the lactate dehydrogenase and a PQQ– $\text{NAD}^+$  monolayer electrode.

Direct electrochemical reduction of dioxygen proceeds with large overpotentials. Therefore, catalysts are required to utilize oxygen reduction in fuel cells. Biocatalytic systems comprising enzymes and their respective electron-transfer mediator, e.g. bilirubin oxidase or fungal laccase with 2,2'-azino-bis-(3-ethyl benzothiazoline-6 sulphonate) as mediator, are able to biocatalyse the electro-reduction of  $\text{O}_2$  to  $\text{H}_2\text{O}$  effectively<sup>63,67</sup>. These systems comprise dissolved enzymes and mediators operating through a diffusional path, which is not suitable for technological applications. A cytochrome *c*-functionalized Au electrode linked to cytochrome oxidase  $\text{CO}_x$  was found to effectively catalyse the four electron reduction of oxygen<sup>68</sup>.



*Biological fuel cells constructed with enzyme-based anodes and cathodes:* So far, we have addressed the engineering of electrode surfaces for separate biocatalytic anodes and cathodes. But, for the construction of a complete biological fuel cell, it is mandatory to couple the cathode and anode units. The integration of these units has certain limitations, for example, the oxidizer should not interact with the biocatalyst relay or cofactor units. The flow of electrons in the external circuit must be compensated by cation transport in the electrolyte solution. The catholyte and anolyte solutions are normally compartmentalized. If the bioelectrocatalytic reactions were made indifferent to the interfering components, non-compartmentalized fuel cells would become feasible. In any biological fuel cell, either the bioelectrocatalytic transformation or the transport process is a rate-determining step in controlling cell efficiency. Table 3 summarizes the characteristics of some biological fuel cells based on layered enzyme electrodes. It is worth comparing the performance of different types of biological fuel cells among themselves as well as with chemical fuel cells. Such a comparison is given in Tables 4 and 5. As can be seen from Tables 4 and 5, biological fuel cells can operate only at room temperature and only around physiological pH between 7 and 9, which may pose some limitations in their applications. However, the construction of biological fuel cells is simple, and it has been shown recently that even the construction of non-compartmentalized biological fuel cells devoid of expensive ion-exchange membranes is possible. Biological fuel cells can provide a source of low but stable power. The current upper limit is the generation of  $3.6 \text{ W/m}^2$  of power using mixed microbial culture<sup>69</sup>. From the electrochemical point of view, the choice of electrodes is seminal. It is observed that when microbial growth processes determine current generation, there is no accumulation of biomass, and electrode-

**Table 3.** Biological fuel cells based on layered enzyme electrodes and observed cell characteristics

Anode	Cathode	Separator	Cell	Reference
Au electrode functionalized with PQQ through a self-assembled monolayer of cystamin promoting NADH/NAD <sup>+</sup> in solution	Au electrode functionalized with micro peroxidase catalysing H <sub>2</sub> O <sub>2</sub> reduction	Membrane	Cell voltage = 310 mV at 50 kΩ. Short circuit current 100 μA. Short circuit current density 30 μA/cm <sup>2</sup>	77
Au electrode functionalized with PQQ and glucose oxidase catalysing glucose	Au electrode functionalized with micro peroxidase catalysing H <sub>2</sub> O <sub>2</sub> reduction	Membrane	Cell voltage 310 mV Max. power 32 μW at an external load of 3 kΩ	78
Oxidation of glucose biocatalysed by glucose oxidase in aqueous solution	Cumene peroxide reduction catalysed by micro peroxidase in dichloromethane	Liquid, liquid interface	Cell voltage 1.0 V Short circuit current density	65
Glucose oxidase reconstituted onto PQQ/FAD monolayer for biocatalysed oxidation of glucose which is indifferent to the presence of O <sub>2</sub>	Cytchrome/ <i>c</i> cytochrome oxidase couple that catalyses reduction of O <sub>2</sub> to water	No separator	Cell voltage = 155 mV	68
Carbon fibres 7 μm diameter and 2 cm long, modified with a polymer containing osmium bipyridine and glucose oxidase	Carbon fibre 7 μm diameter and 2 cm long, modified with a polymer containing Os (4,4'-dimethyl-2,2' bipyridine) 2 and lactase	No separator	Power density 64 μW/cm <sup>2</sup> at 230 operating potential 0.4 V	79
Glucose oxidase/ferrocene-modified solid binding matrix (SBM) graphite composite anode	Horse radish peroxidase/ferrocene modified SBM graphite composite cathode	Membrane	OCV 0.22 V power output 0.15 μW/cm <sup>2</sup> at 0.021 V 30 days	80

**Table 4.** Comparison between chemical and biological fuel cells

	Chemical fuel cell	Biological fuel cell
Catalyst	Noble metals	Microorganism/enzyme
pH	Acidic solution (pH < 1)	Neutral solution (pH 7.0–9.0)
Temperature (°C)	Over 80	Room temperature, 22–25
Electrolyte	Phosphoric acid, sulphuric acid, etc.	Phosphate solution
Efficiency (%)	40–60	Around 40
Voltage (V)	≈ 1	≈ 1
Fuel type	Methanol, H <sub>2</sub> , etc.	Any carbohydrate or hydrocarbon

poisoning problems are prevented. In the case of biological fuel cells, where electro-active metabolites produced by microorganisms are oxidized at the anode, the products of oxidation adsorb on the electrode and poison the surface. This can be avoided by suitable modification of the electrode surfaces. There are many modification strategies including modification by self-assembly of thiol or silane-based compounds, and polymer coatings. The modification can be achieved by simple processes such as physical adsorption, covalent linkages, electrostatic interactions, hydrogen-bonding interactions, etc. The electron-transfer kinetics between the biocatalyst (enzyme or microorganism) and the electrode can be improved by incorporating promoter molecules on the electrode surfaces.

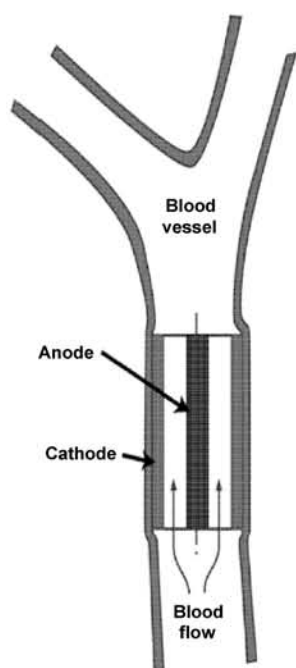
Promoters are organic compounds with terminal functional groups like –COOH, –NH<sub>2</sub>, which can anchor the protein molecules in a favourable orientation for efficient electron-transfer. By taking the above points into consideration, a proper choice of the electrode material should be made. For the mediator-less biofuel cells, activated carbon-based electrodes are ideal, while for mediator-coupled fuel cells various electrode materials like carbon, platinum, gold, ITO, etc. could be explored.

### Applications of biological fuel cells

In about 200 years from now, vehicles will not have petrol tanks because our petroleum reserves will be depleted. An alternative, which is less wasteful and cleaner, is to power vehicles directly with carbohydrates using biological fuel cells<sup>70</sup>. The energy liberated during the complete oxidation of a monosaccharide like glucose or a disaccharide such as sucrose to carbon dioxide and water is about  $16 \times 10^6$  J/kg, which is about 5 kWh of electrical energy and is just less than half the energy that can be obtained from equivalent amounts of fuels such as octane. But the efficiency of burning carbohydrate in biological fuel cells is potentially greater than burning gasoline. For example, a medium-sized car that needs about 200 Wh/km, could travel 25–30 km on one kilogram of a concentrated solution of a carbohydrate<sup>71</sup>. Accordingly, 50 l of a strong sugar solution would give the car a range of more than 1000 km.

**Table 5.** Comparison of direct methanol fuel cell, microbial fuel cell and enzymatic fuel cell

	DMFC	Microbial fuel cell	Enzymatic fuel cell
Fuel	3% MetOH-H <sub>2</sub> O	Glucose, fish meat	10% MetOH-H <sub>2</sub> O
Temperature (°C)	90	22–25	22–25
Output	0.5 V @ 300 mA/cm <sup>2</sup> (50 A/electrode)	0.5 V @ 0.21 mA/cm <sup>2</sup> (26 mA/electrode)	0.5 V @ 3.56 mA/cm <sup>2</sup> (161 mA/electrode)
Size (electrode)	4" × 6" 154.8 cm <sup>2</sup>	3.5" × 5.5" 117.4 cm <sup>2</sup>	2" × 3.5" 45.2 cm <sup>2</sup>
Time (operation; h)	1500	≈ 700	≈ 700
Catalyst	Pt and Pt-Rh	Bacteria	Methanol dehydrogenase

**Figure 9.** A biological fuel cell directly attached to the blood vessel (from Haselkom<sup>72</sup>).

A biological fuel cell that is just 0.07 cm<sup>2</sup> in area, has been designed to generate as much as 300 μV for 2 h, an amount sufficient to operate tiny devices, including microscopic drug-delivery systems<sup>72</sup>. Such a microbial fuel cell could power implantable medical devices as shown in Figure 9 and could help individuals who require regular doses of drugs, for example, AIDS patients. Apart from its small size, the system is unique because it utilizes glucose, a sugar present in the blood stream, as fuel<sup>73</sup>. Such miniaturization has become possible due to the advent of BIOMEMS (Bio-micro electromechanical systems). Since biological fuel cells are known to supply low but stable power density, they are suitable for running devices constructed using MEMS technology. It has been shown by researchers at the University of California that a miniaturized microbial fuel cell could be integrated with MEMS-

**Figure 10.** A Slugbot (from www.robotics.usc.edu).

based implantable medical devices. In comparison to lithium batteries as power sources for implantable devices, microbial fuel cells are smaller, less expensive and have a longer shelf-life.

A mobile robot platform has been built that derives its propulsive power from the digestion of real food in a bio-reactor<sup>34</sup>. This robot called 'Gastrobot' used harmless yeast and adopted a gas propulsion system without an electrical generation. When a carbohydrate-rich aqueous solution is mixed with yeast in a reaction chamber, fermentation takes place with liberation of carbon dioxide and it leads to substantial pressure build-up when constrained. Energy available in this pressurized CO<sub>2</sub> gas is used to propel the Gastrobot, which is achieved purely by mechanical means.

'Slugbot' is a robot that utilizes the electrical power produced from biomass (Figure 10). Slugs are common pests found on agricultural land in the UK, where farmers spend about £20 million per annum on buying and spreading molluscicides. Slugbot ferments slug mass and converts it into electrical energy, which it uses to catch the slugs in the field. Stuart Wilkinson at the University of South Florida, who is currently interested in the development of hybrid biological fuel cells<sup>74</sup>, has developed a meat-eating robot 'Chew Chew', which is powered by a microbial fuel cell and uses meat as a fuel. This has three wagons, each about a metre long. These robots could even kill human beings accidentally. The future applications of biological fuel cells will be more attractive. One could construct molecular biological cells, which could deliver electrical energy to remove tumours and cancerous cells, and could act as drug-delivery systems.

Another important application of microbial fuel cells is in the field of waste water engineering. Microorganisms can discharge the dual duty of degrading effluent and generating power. When microorganisms oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current. If power generation in these systems can be increased, microbial fuel cells may provide a new method to offset operating costs of waste water treatment plant, making advanced waste water treatment more affordable in both developing and industrialized nations. Different designs of fuel cell reactors based on chemical engineering principles like fluidized bed reactors, packed bed reactors, etc. are under trial. Tests have been conducted using single chamber microbial fuel cell (SCMFC) containing eight graphite electrodes as anodes and a single air cathode. The system was operated under continuous-flow conditions with waste water. The prototype SCMFC reactor generated an electric power of 26 mW/m<sup>2</sup>, while removing up to 80% of chemical oxygen demand of the waste water<sup>75</sup>.

## Conclusions

Biological fuel cells fall into two categories, namely microbial fuel cells and enzymatic fuel cells. The main challenge is in the electrical coupling of the biological component of the system with the electrodes of the fuel cells. By tapping the complete multi-enzyme metabolic pathways inside living cells, microbial fuel cells could last long and could utilize complex biofuels. Enzymatic fuel cells are compatible with immobilization and wiring, and, consequently, can offer high current densities, especially when used in concentrated form. A detailed characterization of the interfacial electron-transfer rates, biocatalytic rate constants and cell resistance is essential for the construction of biological fuel cells. Identification of rate-limiting steps would facilitate the development of strategies to improve and enhance cell output. Considering the pros and cons of microbial and enzymatic fuel cells, researchers are working towards hybrid biological fuel cells, where the cells after lysis, i.e. breaking and exposing the enzymes for better electrical communication, will be used along with the mediator to produce power. Although biological fuel cells are in their infancy, the prospects of their applications look attractive, particularly in robotics.

1. Larminie, J. and Dicks, A., *Fuel Cell Systems Explained*, Wiley, 2003.
2. Shukla, A. K., Electrochemical power sources. *Resonance*, 2001, **6**, 72–81.
3. Kordesch, K. and Simander, G., in *Fuel Cells and their Applications*, VCH Weinheim, 1996.
4. Akiba, T., Bennetto, H. P., Stirling, J. L. and Tanaka, K., Electricity generation from alkalophilic organisms. *Biotechnol. Lett.*, 1985, **9**, 611–616.
5. Mano, N., Mao, F. and Heller, A., A miniature bio fuel cell operating in a physiological buffer. *J. Am. Chem. Soc.*, 2002, **124**, 12962–12963.
6. Tanaka, K., Kashiwagi, N. and Ogawa, T., Effect of light on the electrical output of bioelectrochemical fuel cells containing *Anabaena variabilis* M-2: mechanism of the post-illumination burst. *J. Chem. Technol. Bio-Technol.*, 1988, **42**, 235–240.
7. Bennetto, H. P., *Microbial Fuel Cells, Life Chemistry Reports* (eds Michaelson, A. M. and Bannister, J. V.), Harwood Academic, London, 1984, vol. 2, pp. 363–453.
8. Aston, W. J. and Turner, A. P. F., Biosensors and biofuel cells. *Biotechnol. Gen. Eng. Rev.*, 1984, **1**, 89–120.
9. Wingard, Jr. L. B., Shaw, C. H. and Castner, J. F., Bioelectrochemical fuel cells. *Enzyme Microb. Technol.*, 1982, **4**, 137–142.
10. Yahiro, A. T., Lee, S. M. and Kimble, D. O., Bioelectrochemistry. I. Enzyme utilizing biofuel cell studies. *Biochim. Biophys. Acta*, 1964, **88**, 375–383.
11. Sisler, F. D., Biochemical fuel cells. In *Progress in Industrial Microbiology* (ed. Hockenhull, D. J. D.), Churchill, London, 1971, vol. 9, p. 1.
12. Bennetto, H. P., Electricity generation by microorganisms. *Biotechnol. Educ.*, 1990, **1**, 163–168.
13. Bennetto, H. P., Mason, J. R., Stirling, J. L. and Thurston, C. F., Research and development in non-mechanical electrical power sources. In *Power Sources 11* (ed. Pearce, L. J.), 1987, p. 373.
14. Cole, K. S., In *Membranes, Ions and Impulses*, University of California Press, 1972.
15. Rohrback, G. H., Scott, W. R. and Canfield, J. H., Biochemical fuel cells. In Proceedings of the 16th Annual Power Sources Conference, 1962, p. 18.
16. Williams K. R., In *An Introduction to Fuel Cells*, Elsevier, Amsterdam, 1966, p. 248.
17. Yao, S. J., Appleby, A. J., Geise, A., Cash, H. R. and Wolfson, S. K., Anodic oxidation of carbohydrates and their derivatives in neutral saline solution. *Nature*, 1969, **224**, 921–922.
18. Karube, I., Ikemoto, H., Kajiwara, K., Tamiya, E. and Matsuok, H., Photochemical energy conversion using immobilized blue-green algae. *J. Biotechnol.*, 1986, **4**, 73–80.
19. Allen, R. M. and Bennetto, H. P., Microbial fuel cells – electricity production from carbohydrates. *Appl. Biochem. Biotechnol.*, 1993, **39/40**, 27–40.
20. Bennetto, H. P., Dew, M. E., Stirling, J. L. and Tanaka, K., Rates of reduction of phenothiazine redox dyes by *E. coli*. *Chem. Ind.*, 1981, **7**, 776–778.
21. Thurston, C. F., Bennetto, H. P., Delaney, G. M., Mason, J. R., Roller, S. D. and Stirling, J. L., Glucose metabolism in a microbial fuel cell. Stoichiometry of product formation in a thionine mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. *J. Gen. Microbiol.*, 1985, **131**, 1393–1401.
22. Chaudhuri, S. K. and Lovely, D. R., Electricity generation by direct oxidation of glucose in mediator-less microbial fuel cells. *Nature Biotechnol.*, 2003, **21**, 1229–1232.
23. Gottschalk, G., In *Bacterial Metabolism*, Springer Verlag, New York, 1979, 2nd edn.
24. Heyndrickx, M., Devos, P. and De Ley, J. U., Hydrogen production from chemostat fermentation of glucose by *Clostridium butyricum* and *Clostridium pasteurianum* in ammonium and phosphate limitation. *Biotechnol. Lett.*, 1990, **12**, 731–736.
25. Suzuki, S. and Karube, I., Energy production with immobilized cells. *Appl. Biochem. Bioeng.*, 1983, **4**, 281–310.
26. Suzuki, S., Karube, I., Matsuoka, H., Ueyama, S., Kawakubo, H., Isoda, S. and Murahashi, T., Biochemical energy conversion by immobilized whole cells. *Ann. N.Y. Acad. Sci.*, 1983, **413**, 133–143.
27. Karube, I., Suzuki, S., Matsunaga, T. and Kuriyama, S., Biochemical energy conversion by immobilized whole cells. *Ann. N.Y. Acad. Sci.*, 1981, **369**, 91–98.

28. Suzuki, S., Karube, I., Matsunaga, T., Kuriyama, S., Suzuki, N., Shirogomi, T. and Takamura, T., Biochemical energy conversion using immobilized whole cells of *Clostridium butyricum*. *Biochimie*, 1980, **62**, 353–358.
29. Karube, I., Matsunaga, T., Tsuru, T. and Suzuki, S., Biochemical fuel cell utilizing immobilized cells of *Clostridium butyricum*. *Biotechnol. Bioeng.*, 1977, **19**, 1727–1733.
30. Cooney, M. J., Roschi, E., Marison, I. W., Comniellis, C. and von Stockar, U., Physiologic studies with the sulfate-reducing bacterium *Desulfovibrio desulfuricans*: evaluation for use in a biofuel cell. *Enzyme Microbiol. Technol.*, 1996, **18**, 358–365.
31. Habermann, W. and Pommer, E. H., Biological fuel cells with sulphide storage capacity. *Appl. Microbiol. Biotechnol.*, 1991, **35**, 128–133.
32. Schroder, U., Nießer, J. and Scholz, F., A generation of microbial fuel cells with current output boosted by more than one order of magnitude. *Angew. Chem.*, 2003, **115**, 2986–2989.
33. Allen, M. J., Biofuel cells. In *Methods in Microbiology* (eds Norris, J. R. and Ribbon, D. W.), Academic Press, New York, 1972, pp. 247–283.
34. Wilkinson, S., Gastrobots – benefits and challenges of microbial fuel cells in food powered robot applications. *Autonomous Robots*, 2000, **9**, 99–111.
35. Delaney, G. M., Bennetto, H. P., Mason, J. R., Roller, S. D., Stirling, J. L. and Thurston, C. F., Electron transfer coupling in microbial fuel cells: 2. Performance of fuel cells containing selected microorganism–mediator–substrate combination. *J. Chem. Technol. Biotechnol. B*, 1984, **34**, 13–27.
36. Kim, N., Choi, Y., Jung, S. and Kim, S., Effect of initial carbon sources on the performance of microbial fuel cells containing *Proteus vulgaris*. *Biotechnol. Bioeng.*, 2000, **70**, 109–114.
37. Roller, S. D., Bennetto, H. P., Delaney, G. M., Mason, J. R., Stirling, S. L. and Thurston, C. F., Electron-transfer coupling in microbial fuel cells: 1. Comparison of redox mediator reduction rates and respiratory rates of bacteria. *J. Chem. Technol. Biotechnol. B*, 1984, **34**, 3–12.
38. Ikeda, T., Kato, K., Maeda, M., Tatsumi, H., Kano, K. and Matsushita, Electro catalytic properties of *Acetobacter aceti* cells immobilized on electrodes for the quinone-mediated oxidation of ethanol. *J. Electroanal. Chem.*, 1997, **430**, 197–204.
39. Park, D. H. and Zeikus, J. G., Electricity generation in microbial fuel cells using neutral red as an electroionophore. *Appl. Environ. Microbiol.*, 2000, **66**, 1292–1297.
40. Ikeda, T., Kato, K., Tatsumi, H. and Kano, K., Mediated catalytic current for the oxidation of ethanol produced by *Acetobacter aceti* cells suspended in solution. *J. Electroanal. Chem.*, 1997, **440**, 265–269.
41. Kano, K. and Ikeda, T., Fundamentals and practices of mediated bioelectrocatalysis. *Anal. Sci.*, 2000, **16**, 1013–1021.
42. Davis, J. B. and Yarbrough, Jr. H. F., Preliminary experiments on a microbial fuel cell. *Science*, 1962, **137**, 615–616.
43. Tanaka, K., Vega, C. A. and Tamamushi, R., Thionine and ferric chelate compounds as coupled mediators in microbial fuel cells. *Bioelectrochem. Bioeng.* 1983, **11**, 289–297.
44. Sell, D., Kramer, P. and Kreysa, G., Use of an oxygen gas diffusion cathode and a three-dimensional packed bed anode in a bioelectrochemical fuel cell. *Appl. Microbiol. Biotechnol.*, 1989, **31**, 211–213.
45. Park, D. H., Kim, S. K., Shin, I. H. and Jeong, Y., Electricity production in biofuel cells using modified graphite electrode with neutral red. *J. Biotechnol. Lett.*, 2000, **22**, 1301–1304.
46. Kim, B. H., Kim, H. J., Hyun, M. S. and Park, D. H., Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.*, 1999, **9**, 127–131.
47. Kim, J. H., Hyun, M. S., Chang, I. S. and Kim, B. H., A microbial fuel cell type lactate biosensor using a metal reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.*, 1999, **9**, 365–367.
48. Kim, H. J., Park, H. S., Hyun, M. S., Chang, I. S., Kim, M. A. and Kim, B. H., A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enzyme Microbiol. Technol.*, 2002, **30**, 145–152.
49. Bond, D. R., Holmes, D. E., Tender, L. M. and Lovely, D. R., Electrode-reducing microorganisms that harvest energy from marine sediments. *Science*, 2002, **295**, 483–485.
50. Bond, D. R. and Lovely, D. R., Electricity production by *Geobacter sulfurireducens* attached to electrodes. *Appl. Environ. Microbiol.*, 2003, **69**, 1548–1555.
51. Dichristina, T. J. and DeLong, E. F., Isolation of anaerobic respiratory mutants of *Shewanella putrefaciens* and genetic analysis of mutants deficient in anaerobic growth on Fe<sup>3+</sup>. *J. Bacteriol.*, 1994, **176**, 1468–1474.
52. Lovley, D. R., Giovannoni, S. J., White, D. C., Champine, J. E., Phillips, E. J. P., Gorby, Y. A. and Goodwin, S., *Geobacter metallireducens* gen nov sp. nov, a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Arch. Microbiol.*, 1993, **159**, 336–344.
53. Myers, C. R. and Myers, J. M., Localization cytochromes to the outer membrane of anaerobic ally grown *Shewanella putrefaciens* MR-1192. *J. Bacteriol.*, 1992, **174**, 3429–3438.
54. Turner, A. P. F., Karube, I. and Wilson, G. S., *Biosensors Fundamentals and Applications*, Oxford University Press, New York, 1987.
55. Katz, E., Lotzbeyer, T., Schlereth, D. D., Schuhmann, W. and Schmidt, H. L., Electro catalytic oxidation of reduced nicotinamide coenzyme at gold and platinum electrode surface modified with a monolayer of pyrroloquinoline quinone. Effect of Ca<sup>2+</sup> ions. *J. Electroanal. Chem.*, 1994, **373**, 189–200.
56. Palmore, G. T. R., Bertschy, H., Bergens, S. H. and Whitesides, G. M., A methanol/dioxygen biofuel cell that uses NAD<sup>+</sup> dependent dehydrogenases as catalysts: application of an electro enzymatic method to regenerate nicotinamide adenine dinucleotide at low overpotentials. *J. Electroanal. Chem.*, 1998, **443**, 155–161.
57. Kharitonov, A. B., Alfonta, L., Katz, E. and Willner, I., Probing bio affinity interactions at interfaces using impedance spectroscopy and chronopotentiometry. *J. Electroanal. Chem.*, 2000, **487**, 133–141.
58. Katz, E., Heleg-Shabtai, V., Bardea, A., Willner, I., Rau, H. K. and Haehnel, W., Fully integrated biocatalytic electrodes based on bio affinity interactions. *Biosens. Bioelectron.*, 1998, **13**, 741–756.
59. Bardea, A., Katz, E., Buckmann, A. F. and Willner, I., NAD<sup>+</sup>-dependent enzyme electrodes: electrical contact of cofactor-dependent enzymes and electrodes. *J. Am. Chem. Soc.*, 1997, **119**, 9114–9119.
60. Riklin, A., Katz, E., Willner, I., Stocker, A. and Buckmann, A. F., Improving enzyme–electrode contact redox modifications cofactors. *Nature*, 1995, **376**, 672–675.
61. Willner, I., Heleg-Shabtai, V., Blonder, R., Katz, E., Tao, G., Buckmann, A. F. and Heller, A., Electrical wiring of glucose oxidase by reconstitution of FAD modified monolayers assembled onto Au electrodes. *J. Am. Chem. Soc.*, 1996, **118**, 10321–10322.
62. Katz, E., Riklin, A., Heleg-Shabtai, V., Willner, I. and Buckmann, A. F., Glucose oxidase electrodes via reconstitution of the apoenzyme: tailoring of novel glucose biosensors. *Anal. Chim. Acta*, 1999, **385**, 45–48.
63. Tayhas, G., Palmore, R. and Kim, H. H., Electro enzymatic reduction of dioxygen to water in the cathode compartment of a biofuel cell. *J. Electroanal. Chem.*, 1999, **464**, 110–117.
64. Lotzbeyer, T., Schuhmann, W., Katz, E., Falter, J. and Schmidt, H. L., Direct electron-transfer between the covalently immobilised enzyme microperoxidase MP-11 and a cystamine modified gold electrode. *J. Electroanal. Chem.*, 1994, **377**, 291–294.

65. Li, J., Tan, S. N. and Oh, J. J., Silica gel immobilised amperometric enzyme electrode for peroxide determination in the organic phase. *J. Electroanal. Chem.*, 1998, **448**, 69–77.
66. Katz, E., Filanovsky, B. and Willner, I., A biofuel cell based on two immiscible solvents and glucose oxidase and microperoxidase-11 monolayer functionalized electrodes. *New J. Chem.*, 1999, **23**, 481–488.
67. Tsujimura, S., Tatsumi, H., Ogawa, J., Shimizu, S., Kanok, S. and Keda, T., Bioelectrocatalytic reduction of dioxygen to water at neutral pH using bilirubin oxidase as an enzyme and 2,2'-azino-bis-(3-ethyl benzothiazolin-6 sulphonate) as an electron-transfer mediator. *J. Electroanal. Chem.*, 2001, **496**, 69–75.
68. Katz, E., Willner, I. and Kotlyar, A. B., A non-compartmentalized glucose/O<sub>2</sub> biofuel cell by bioengineered electrode surfaces. *J. Electroanal. Chem.*, 1999, **479**, 64–68.
69. Rabaey, K., Lissenes, G., Siciliano, S. D. and Verstraete, W., A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.*, 2003, **25**, 1531–1535.
70. Bennetto, H. P., Microbes come to power. *New Sci.*, 1987, **114**, 36–39.
71. Shukla, A. K., Avery, N. R. and Muddle, B. C., Future cars: the electric option. *Curr. Sci.*, 1999, **77**, 1141–1146.
72. Haselkorn, A., Microbial fuel cells to power future. *Daily Californian*, Berkeley, California, 28 August 2002.
73. Chia, M., A miniaturized microbial fuel cell. Technical Digest of Solid State Sensors and Actuators Workshop, Hilton Head Island, June 2002, pp. 59–60.
74. Wilkinson, S. and Applegarth, S., A hybrid biofuel cell approach using cellular disruption. 204 Meeting of the Electrochemical Society Inc., Abstr. 1243, 2003.
75. Liu, H., Ramnarayanan, R. and Logan, B. E., Production of electricity during waste water treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.*, 2004, **38**, 2281–2285.
76. Katz, E., Shipway, A. N. and Willner, I., In *Handbook of Fuel Cells – Fundamental, Technology, Applications* (eds Vielstich, W., Gastieger, H. and Lamin, A.), Wiley, Chichester, 2003.
77. Willner, I., Arad, G. and Katz, E., A biofuel cell based on pyroloquinoline quinone and microperoxidase-11 monolayer functionalized electrodes. *Bioelectrochem. Bioeng.* 1998, **44**, 209–214.
78. Willner, I., Katz, E., Patolsky, F. and Buckmann, A. F., Biofuel cell based on glucose oxidase and microperoxidase-11 monolayer functionalized electrodes. *J. Chem. Soc., Perkin Trans.*, 1998, **2**, 1817–1822.
79. Chen, T., Barton, S. C., Binyamin, G., Gao, Z., Zhang, Y., Kim, H. H. and Heller, A., A miniature biofuel cell. *J. Am. Chem. Soc.*, 2001, **123**, 8630–8631.
80. Pizzariello, A., Stred'ansky, M. and Miertus, S., A glucose/hydrogen peroxide biofuel cell that uses oxidase and peroxidase as catalyst by composite bulk-modified bioelectrode based on a solid binding matrix. *Bioelectrochemistry*, 2002, **56**, 99–105.

Received 5 March 2004; revised accepted 24 May 2004