

“Biofuelcells – Recent Advances and Applications”

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Abstract

In 2006, the journal *Biosensors and Bioelectronics* published a special issue devoted exclusively to biofuel cells, including several research papers and an extensive review of the field [Bullen *et al* 2006]. Within this review a brief description will firstly be given of the history of biofuel cells together with coverage of some of the major historical advances. The review is intended, however, to largely concentrate on and give an overview of the advances made in recent years in this area together with a discussion surrounding the practical application of biofuel cells.

There are several classes of biofuel cells: We shall firstly discuss the recent advances in biofuel cells that convert chemical fuels to produce electrical power by use of catalytic enzymes. This will be followed by a section on similar cells where microorganisms rather than enzymes are used to convert the fuel to energy. Thirdly we shall consider hybrid biofuel cells that combine the utilisation of photochemical chemistries and biological systems for the generation of electricity.

Finally we will discuss some of the proposed uses of biofuel cells together with a short consideration of future research possibilities and applications of these systems.

1. Introduction

Every year the global energy demand increases. While petroleum products currently supply much of this demand, the increasing difficulty of sustained supply and the associated problems of pollution and global warming are acting as a major impetus for research into alternative renewable energy technologies. Fuel cells offer a possible (and partial) solution to this problem, with the fuel needed for conventional cells usually being either hydrogen or methanol, although some cells have been developed which run on other fuels such as hydrocarbons [de Bruijn 2005, Bagotzky *et al* 2003]. Hydrogen is gaseous and this gives rise to storage and transport problems. Moreover many of the alternative fuels that could be used within fuel cells are still dependent on petroleum products and therefore offer few advantages. It is clear that approaches by which common waste materials and the chemical energy locked within them could be utilised would offer many benefits. For example, if a molecule of glucose is oxidised completely to CO₂, (usually with atmospheric O₂ providing the oxidant), there are 24 electrons available for current generation. Furthermore, if the glucose is produced as a by-product of photosynthesis, then the process is carbon neutral, which clearly offers environmental benefits.

Ever since Galvani first noticed the twitching of a frog's leg upon application of an electric current, it has been known that many biological pathways have a bioelectrochemical facet. Since an electrical action can induce a biological reaction, the converse in many cases is also true and in this way biological processes can be used to generate electricity. This would lead to the ability to utilise materials such as, for example, lactose (from cheese making) to power electrical equipment. One of the earliest developments in this area was described by Michael Cresse Potter in 1910, when he placed a platinum electrode into cultures of yeast or *E. Coli* and showed that a potential difference could be generated [Potter 1911]. Further work by Cohen at Cambridge led to development of batteries of microbial fuel cells capable of generating potentials in excess of 35 volts [Cohen 1931].

More recently, there has been an upsurge in research in biofuel cells. Factors driving this research include the increasing problems of supply and pollution that concern the use of fossil fuels through to the possibilities offered by the design of small devices implantable within the body - such as pacemakers. In many situations an ideal power

supply would be a fuel cell that was capable of running on the compounds such as sugars found *in vivo*. There are a number of reviews on bio-electrochemistry that include some coverage of work on biofuel cells including those by Aston and Turner [1984], Bennetto [1987], Katz *et al* [2003], Barton *et al* [2004], Shukla *et al* [2004] and Rabaey and Verstraete [2005a]. In 2006, the journal *Biosensors and Bioelectronics* published a special issue devoted exclusively to biofuel cells, including several research papers and an extensive review of the field [Bullen *et al* 2006].

There are a number of biologically based fuel cell formats that at the time of writing are the focus of active research and include:

1. Cells which use a primary fuel (usually an organic waste such as corn husks) and generate a material such as hydrogen, which is then used as a secondary fuel within a conventional hydrogen/oxygen fuel cell.
2. Cells which generate electricity directly from an organic fuel such as glucose, using either enzymes or complete micro-organisms.
3. Cells which combine the utilisation of photochemically active systems and biological moieties to harvest the energy from sunlight and convert this into electrical energy.

2. Fuel cells based on conversion of organic waste to secondary fuels.

These are not “true” biofuel cells but represent a combination of a bioreactor and a fuel cell. One of the main attractions of this type of arrangement is that it can not only generate electrical power but also consumes a wide range of organic wastes (e.g. corn husks, whey or noxious waste such as animal or human sewage). Fermentation processes can be used to produce substrates such as ethanol or hydrogen which can then be used to power a conventional H_2/O_2 or ethanol/ O_2 fuel cell. Since there is no direct generation of power by biological means, these types of cells fall outside the scope of this review.

3. Biofuel cells which directly convert fuel to electricity.

3.1. Early Work.

Cells of this type utilise biological moieties such as enzymes or living cells to directly generate power from the chemical energy contained within various organic or inorganic species. A schematic of a typical fuel cell of this format is shown in Fig. 1.

Two electrodes separated by a semi-permeable membrane are placed into solution. A biological species such as a microbial cell or enzyme can either be in solution (or as a suspension) within the anodic compartment of the cell - or alternatively be immobilised at the electrode. Once a suitable fuel is introduced, it becomes either partially or totally oxidised at the anode and the electrons released by this process are used to reduce oxygen at the cathode. The early examples by Potter and Cohen previously mentioned, utilised living cells as the active component. Later work [Davis and Yarborough 1962] involved adding either *E. Coli* or glucose oxidase to a half-cell containing glucose, which allowed small currents being generated. Much larger currents could be obtained upon addition of methylene blue to the system. This can be explained by the electron transfer from micro-organisms to the electrode being a very inefficient process - and it follows that the presence of a simple mediator compound such as methylene blue greatly increases the efficiency of the cell. Further work utilised dichloroindophenol as a mediator in a glucose oxidase based cell, with efficiencies approaching 100% [Weibel and Dodge 1975]. A schematic of a typical mediated biological reaction, in this case the ferrocene mediated oxidation of glucose, is shown in Fig. 2.

An early paper by Rao *et al* [1976] describes much of the initial work focussed towards developing glucose powered fuel cells for use within heart pacemakers. Various fuel cell constructions were surveyed within this work, although only simple inorganic electrodes were used, with the best power outputs being obtained using Pt/Ni electrodes combined with polyacrylic acid/polyvinyl alcohol copolymers. The devices were very susceptible to poisoning by amino acids, although *in vivo* animal tests displayed good performance for up to 100 days.

Once the basic principle of the biofuel cell had been established, work could then progress towards optimising the process. If a glucose molecule is completely reduced to CO₂, there are potentially 24 electrons available, however, there exists no direct simple chemical method to harvest this process. One approach is to exploit microorganisms that contain a range of enzymes to facilitate this transformation. For simpler reaction pathways it is possible to utilise enzymes. Methanol for example, can be oxidised to CO₂ using just two enzymes [Aston and Turner 1984]. In this scheme methanol can be oxidised by methanol dehydrogenase to give formaldehyde which

can then be further oxidised by formaldehyde dehydrogenase to CO₂, with the overall process generating four electrons. Much of the work on methanol cells has already been reviewed [Aston and Turner 1984]. This group developed cells based on methanol dehydrogenase along with phenazine etho-sulphate or tetramethyl *p*-phenylenediamine mediators, the best of which gave power densities per electrode area of 2 $\mu\text{W cm}^{-2}$ [Aston and Turner 1984, Turner *et al* 1982], providing the that anode compartment was kept anaerobic. Similar cells could also utilise carbon monoxide as a fuel source [Turner *et al* 1982]. Nicotinamide adenine dinucleotide (NAD) could also be successfully used as a charge carrier between glucose dehydrogenase and a Meldola Blue activated anode [Persson *et al* 1986].

Lactose waste has been utilised as fuel [Roller *et al* 1983] for a microbial fuel cell containing thionine as the mediator. Providing the lactose consumed was continuously replaced, the cell could produce a power output of 0.4 mW for over two weeks. A similar cell utilising glucose and *E. Coli* provided an electrical yield close to the theoretical maximum of 48 faradays per mole of sucrose [Bennetto *et al* 1985].

3.2 Enzyme based biofuel cells

Enzyme based fuel cells have remained a popular focus for research due to the high turnover rates associated with enzymes that lead to a high biocatalysis turnover rate. One problem associated with biofuel cells, however, is that although the biological moieties will readily produce a supply of electrons, they cannot be exploited unless they can be transferred to the electrode. Recent advances in the immobilisation of enzymes at electrode surfaces by a range of different methods [Davis and Higson 2005, Scouten *et al* 1995] have greatly facilitated the transfer rate that can be achieved, making these molecules a more attractive prospect.

Although much of the early attention was devoted to the study of reactions occurring at anodes, biological molecules can also be utilised to catalyse the reduction of oxygen at the cathode as an alternative to the classical use of platinum. Microperoxidase [Willner *et al* 1998a] can be immobilised onto a gold cathode and along with a quinone modified cathode be utilised in a biofuel cell fuelled by NADH and H₂O₂. Similar cathodes were used along with apoglucose oxidase/quinone/flavin

adenine dinucleotide phosphate modified anode [Willner *et al* 1998b] to construct a glucose/H₂O₂ fuel cell capable of generating 310 mV with a power output of 32 μ W. The same anodes and cathodes could also be utilised in a biofuel cell containing two immiscible solvents with the anode being immersed in aqueous glucose and the cathode being immersed in cumene peroxide/dichloromethane [Katz *et al* 1999a]. The resultant cells were reported to be capable of generating 1 V with a power output of 520 μ W.

Many of the biofuel cells mentioned so far require separation of the anode and cathode into separate compartments, usually by means of a semipermeable membrane, since quite often the fuels required for the separate reactions at the anode and cathode will interfere with the reaction at the opposing electrode. However it has been shown that suitably immobilised glucose oxidase molecules [Katz *et al* 1999b] give a rate of reaction (as measured by the anodic current), that is very close to the theoretical maximum. This indicates very efficient electron transfer between the enzyme and the electrode, thereby indicating that the presence of oxygen in the anodic environment would not affect the bioelectrocatalytic activity of the enzyme. Following this reasoning, a fuel cell was constructed containing a glucose oxidase immobilised anode along with a cathode with cytochrome c and cytochrome oxidase covalently immobilised at the surface [Katz *et al* 1999b]. This arrangement is known to electrocatalyse the reduction of oxygen allowing rapid electron transfer to the cathode. The resultant cell which contained no separation of anode and cathode compartments gave a power output of up to 4 μ W, (when the cell solution was saturated with oxygen and supplied with 1 mM glucose), with the power output being stable over a 48 hour period.

Further studies [Palmore and Kim 1999] also demonstrated the potential of using the fungal enzyme laccase along with a mediator to catalyse oxygen reduction within a H₂/O₂ fuel cell. When fungal laccase was added to the cathode compartment of the fuel cell, it catalysed the reduction of oxygen at a glassy carbon cathode. The resultant cell gave a maximum power density of 42 μ W cm⁻² with a cell potential of 0.61 V, whereas uncatalysed cells gave densities of 15 μ W cm⁻² - with a cell potential 0.26 V (Pt cathode) or 2.9 μ W cm⁻² at 0.28 V (using a glassy carbon cathode), thereby

demonstrating the enhanced reaction kinetics afforded by use of the enzyme. The reduction of hydrogen at graphite electrodes modified by a hydrogenase enzyme (from *Allochromatium Vinosum*) was also compared to a classical platinum electrode [Jones *et al* 2002]. Results showed the reduction at the enzyme electrode to be diffusion controlled and match those obtained at the platinum electrode. These findings were confirmed by other workers who also found that the enzyme based electrodes could match the performance attainable at platinum electrodes [Karyakin *et al* 2002]. A hydrogenase enzyme immobilised onto a carbon filament electrode gave very similar results when included in a H₂/O₂ fuel cell and was found not to be affected by carbon monoxide, which can be a serious poison for conventional platinum catalysts. A bilirubin oxidase “wired” carbon electrode [Mano *et al* 2003a] has also been used to reduce oxygen to water at a much lower overpotential than that required at a platinum electrodes (at -0.31 V the current density for the enzyme electrode was reported to be 9.5 mA cm⁻²; for the corresponding platinum electrode, a current density 0.6 mA cm⁻² is possible). The electrode could also be operated at physiological pH, at which platinum tends to corrode and was found moreover to be much less susceptible to inhibition from chloride.

Other work concerning methanol fuel cells has been focussed towards the problem of regeneration of the NAD cofactor often used with the dehydrogenase [Palmore *et al* 1998]. In the majority of cases the oxidation of NADH requires potentials which lower the operating voltage of the cell. This reaction could be catalysed by the use of diaphorase along with benzyl viologen. These materials along with dehydrogenase enzymes in solution were incorporated within methanol/O₂ fuel cells which were found to be capable of giving a maximum power output of 670 μW cm⁻² at 0.49 V vs a standard calomel electrode (SCE).

For any type of implantable biofuel cell, it is important that it must operate under physiological conditions (pH 7.4, 0.15 M NaCl, 37°C). “Wiring” redox centres from bilirubin oxidase to a carbon electrode via a redox polymer [Mano *et al* 2002] gave rise to a cathode capable of reducing oxygen to water under physiological conditions, albeit with a 5% loss in activity per day of operation. Other workers also immobilised bilirubin oxidase (cathode), and similarly glucose hydrogenase (anode), onto glassy

carbon modified with an Os-based redox polymer [Tsujimura *et al* 2002]. These electrodes could be used in the construction of a one compartment glucose/O₂ biofuel cell, capable of operating under physiological conditions, with a maximum power density of 58 $\mu\text{W cm}^{-2}$.

Fuel cells have the potential for high levels of miniaturisation due to their simplicity of construction without any moving parts. For example miniaturised methanol-air (non-biological) fuel cells with an active area of 0.25 cm² were constructed using a polymer electrolyte rather than a solution and gave power densities similar to large cells [Kelley *et al* 2000]. However the active area was reduced by a factor of 60 [Chen *et al* 2001] by these workers who used 2 cm x 7 μm carbon fibre electrodes with immobilised glucose oxidase (anode) and laccase (cathode), both with immobilised osmium-containing polymers as electron transfer agents, held 400 μm apart on a polycarbonate support. This arrangement was immersed in an aerated 15 mM glucose solution and was found to generate 64 $\mu\text{W cm}^{-2}$ at 23°C and 137 $\mu\text{W cm}^{-2}$ at 37°C, power densities which they claim were 5 and 10 times greater than any previously reported for glucose/O₂ fuel cells. This type of cell was improved by modification of the redox polymers and utilising bilirubin oxidase as the cathodic enzyme to give 50 $\mu\text{W cm}^{-2}$ at an operating potential of 0.5 V under physiological conditions [Kim *et al* 2003], although after two days continuous operation the current fell to 30 $\mu\text{W cm}^{-2}$. A fuel cell of this type was found to produce a power output of up to 430 $\mu\text{W cm}^{-2}$ at an operating potential of 0.52 V when implanted inside a living plant, namely a grape (Fig 3) [Mano *et al* 2003b]. Similar performance was also demonstrated in a physiological solution [Heller 2004]. The operating voltage could be further increased to 0.78 V by using the enzyme laccase and modifying the redox polymer [Mano *et al* 2003c]. Under physiological conditions a similar cell proved capable of generating 48 $\mu\text{W cm}^{-2}$ at an operating potential of 0.60 V [Mano *et al* 2004]. Inclusion of an eight carbon atom chain between the redox functionality and the backbone of the polymer increased the electron diffusion coefficient of the polymer 100-fold, leading to a cathode with superior oxygen reducing capabilities to platinum [Soukharev *et al* 2004]. When this cathode was incorporated into a miniature biofuel cell similar to those above, operating voltages as high as 0.88 V could be obtained with a power output of up to 350 $\mu\text{W cm}^{-2}$.

As an alternative to the osmium based polymers above, a polymer containing Vitamin-K3 was utilised to immobilise glucose dehydrogenase onto glassy carbon [Sato *et al* 2005]. The resultant glucose/O₂ cell was initially found to be capable of generating 14.5 $\mu\text{W cm}^{-2}$ at 0.36 V at pH 7.0/37°C, although after 4 days this fell to 4 $\mu\text{W cm}^{-2}$, but then remained stable for up to two weeks.

A solid binding matrix based on graphite particles combined with enzymes and ferrocene-based mediators which is spray-printed onto inert polymer films was utilised in the manufacture of electrodes for a glucose/hydrogen peroxide fuel cell [Pizzarello *et al* 2002]. Glucose oxidase and horseradish peroxidase were included in the anode and cathode respectively to give a cell with a power output of 0.15 $\mu\text{W cm}^{-2}$ at an operating potential of 0.021 V (1 mM H₂O₂ and 1 mM glucose). This cell could be operated continuously for up to 30 days. As an alternative to glucose, hydrolysed corn syrup was successfully used as a fuel. The structure of the carbon electrodes used within these systems has been shown to have a major effect as shown by comparison of superdispersed colloid graphite and acetylene black electrodes [Tarasevich *et al* 2003]. When laccase was immobilised on these electrodes, the rate of oxygen reduction per enzyme molecule at the graphite electrode was found to be five times higher than that at the acetylene black electrode, and this is thought to be due to better mixing of the laccase and graphite due to the fact the enzyme molecules and the graphite particles are very similar in size. Laccase was also immobilised at glassy carbon electrodes along with either osmium or ruthenium based redox polymers, with the osmium based polymer proving to be a more effective mediator of oxygen reduction in a membrane-less biofuel cell [Barriere *et al* 2004].

Laccase has been the choice of enzyme for the comparison of glassy and porous carbon to form biofuel cell cathodes [Farneth *et al* 2005], with the higher surface area of the porous carbons leading to x3-4 higher oxygen reduction currents when immersed in a solution of laccase and mediator. Further work by the same group studied encapsulation of the enzyme within silica or organic polymer binders onto porous carbon [Farneth and D'Amore 2005]. The resultant cathodes can be

incorporated into a methanol biofuel cell and display a far superior stability behaviour compared to cathodes where the laccase is simply adsorbed onto the carbon paper.

[Tsujimura *et al* 2005] has described a system consisting of multilayers of bilirubin oxidase and poly-L-lysine that can be deposited onto carbon electrodes. The resulting electrodes were effective catalysts for oxygen reduction without the presence of a mediator, and under convective conditions, current densities for oxygen reduction of about 1 mA cm^{-2} could be attained. It is thought that the bilirubin oxidase is diffusible within the multilayer. Another system capable of a mediator-less operation is one in which alcohol dehydrogenase is immobilised at the anode with glucose oxidase being co-deposited at the cathode along with microperoxidase [Ramanvicius *et al* 2005]. The reaction scheme for this mixture is for glucose to be enzymatically oxidised leading to the production of hydrogen peroxide at the anode, which is then catalytically reduced and acts as the source of electrons used to oxidise ethanol at the anode. When both glucose and ethanol are present (10 mM) in solution, current densities of $2.6 \text{ } \mu\text{A cm}^{-2}$ can be attained, although the cell has a limited half-life of approximately 2.5 days. Carbon nanotubes have also been shown to be possible constituents within anode materials for biofuel cells [Jung *et al* 2005]. Glucose oxidase could be successfully immobilised on carboxylic acid substituted carbon nanotubes, leading to a system where electrons are transferred from the enzyme to a graphite rod without the presence of a mediator. Other systems similar to this include microperoxidase immobilised onto carbon nanotubes which are in turn adsorbed onto a platinum microelectrode [Wang *et al* 2005]. Direct electron transfer between the enzyme and the microelectrode and catalytic reduction of oxygen and hydrogen peroxide have been successfully demonstrated for this system and for a similar system in which a glassy carbon electrode was modified with a hybrid carbon nanotube/gold nanoparticle mixture [Liu *et al* 2005a]. Porous carbon has also been used as a matrix to immobilise mixtures of carbon nanotubes with laccase (cathode) and glucose oxidase (anode) to construct a glucose/ O_2 biofuel cell [Liu *et al* 2005b]. Porous carbon gave enhanced power production compared to glassy carbon electrodes. The resultant biofuel cell was also capable of operating at a range of pHs, from pH 4-7, which is unusual for laccase based electrodes which often will only function at pH values of <5 . The power output was found to be dependent on pH falling from 99.8

$\mu\text{W cm}^{-2}$ at pH=4 to $2.0 \mu\text{W cm}^{-2}$ at pH=7, thereby affording the possibility of controlling power output by controlling pH.

Conducting polymers can also be utilised as a method of immobilising enzymes in biofuel cells. A variety of mutants of L-lactate dehydrogenase were successfully immobilised on polyaniline/polyvinyl sulphonate [Halliwell *et al* 2002] and were shown to display superior adsorption than a wild-type enzyme. Electrodes based on these types of films were shown to be capable of current generation when exposed to lactose [Simon *et al* 2002]. Polyaniline films containing gold nanoparticles were also investigated [Granot *et al* 2005], both in the form of thin films and as rod structures. Charge transport within polyaniline/Au nanoparticle composites were shown to be about 25 times greater than in systems without nanoparticles. The high surface area of these composites led to superior electron transfer mediator activity for the bioelectric activation of the glucose/glucose oxidase reaction.

A biofuel cell with switchable output has been recently developed [Katz and Willner 2003]. Incorporation of a Cu(II)-poly(acrylic acid) film between the electrodes and immobilised glucose oxidase (anode), cytochrome oxidase (cathode) lead to a fuel cell which could be readily switched on and off. The Cu(II)-poly(acrylic acid) film acts as an insulating layer but can be reduced by application of a potential of -0.5 V (vs SCE) potential to Cu(0)-poly(acrylic acid) which is conductive - thereby activating the fuel cell (current density $550 \mu\text{A cm}^{-2}$). Subjecting the films to a +0.5 V (vs SCE) potential regenerated the insulating film, so enabling the switching of the fuel cell between the ON and OFF states. The reduction of Cu(II) to Cu(0) is however quite slow, although this allows for fine tuning of the film structure and so control of the power output of the cell.

The use of mutant rather than wild-type enzymes has been studied [Yubashi *et al* 2005]. Glucose dehydrogenase is ostensibly an excellent enzyme for biofuel cell anodes due to high turnover of substrate, with wide substrate specificity and also insensitivity to oxygen. However the enzyme displays low stability, greatly shortening the longevity of any resultant devices. A range of mutants were synthesised with one being found to be stable up to temperatures of 70°C [Yubashi *et al* 2005]. When

immobilised onto carbon electrodes and placed in a biofuel cell along with a carbon/bilirubin oxidase cathode, power densities of $17.6 \mu\text{W cm}^{-2}$ could be obtained, which were similar those obtained from the wild type enzyme. The cell was, however, found to have an operating lifetime of up to approximately 152 hours, six times that of the cell constructed using the wild-type enzyme.

The performance of biofuel cells has been shown to be affected by magnetic fields. Anodes with either glucose oxidase or lactose dehydrogenase as the active biological component and containing cathodes with cytochrome c/cytochrome oxidase were constructed and their performance when exposed to glucose, lactose or oxygen characterised with - and without application of a magnetic field parallel to the electrode surface [Katz *et al* 2005]. A pronounced enhancement of the bioelectrocatalytic process is observed for both anodic systems although a far smaller effect was observed for the cathodic system. This enhancement was shown to be due to a magnetohydrodynamic effect which facilitates enhanced mass transport. When a fuel cell was constructed using the lactose dehydrogenase anode and the cytochrome c/cytochrome oxidase cathode, its power output could be trebled by application of a 0.92 T magnetic field [Katz *et al* 2005].

A topic that has been the subject of much research recently is the development of microchip sized devices. The lack of integrated power supplies for powering devices located on the chip has been one limitation to the development of lab-on-a-chip technologies. Recently the development of miniature ethanol/oxygen biofuel cells [Moore *et al* 2005] has been reported to help address this problem. The cell, as shown in Fig 4, contained a micro-moulded carbon ink anode with immobilised alcohol dehydrogenase and poly(methylene green) together with an external platinum cathode. It was found that current densities of $53 \mu\text{A cm}^{-2}$ could be obtained with this system. As an alternative approach, the same group have developed a different anode using the same active components immobilised in Nafion membranes [Akers *et al* 2005], as commonly used in classical H_2/O_2 fuel cells. Normally the acidic properties of Nafion are detrimental to biofuel cell performance due to enzyme inactivation, however, neutralisation of the sulphonic acids with tetrabutyl ammonium groups prevents this whilst retaining the high ionic transport characteristic of the polymer.

Single enzyme system containing alcohol hydrogenase gave power densities of 1.16 mW cm⁻² with ethanol with the addition of aldehyde hydrogenase increasing this to 2.04 mW cm⁻² with ethanol and 1.55 mW cm⁻² when methanol is used as a fuel.

3.3 Microbial based biofuel cells

Microbes offer some major advantage over enzymes in that they can catalyse a more thorough oxidation of many biofuels and can be less susceptible to poisoning and loss of activity under normal operating conditions making them a popular choice for use in biofuel cells. Several recent reviews on this subject have been published [Shukla *et al* 2004] and [Rabaey and Verstraete 2005a].

One major drawback, however, is that it can be extremely difficult to utilise the electrons generated by the reaction occurring inside the cell. One possible solution is via the use of mediators, however, the compounds chosen for this purpose must satisfy a number of criteria. Firstly they must be capable of being transported across the cell membranes of the micro-organisms and they must also be non-toxic. The potential for exploiting the full gamut of reactions that a micro-organism is capable of was realised by work which utilising glucose and *E. Coli*. and provided an electrical yield close to the theoretical maximum of 48 faradays per mole of sucrose [Bennetto *et al* 1985]. Other microbial fuel cells had volumes of up to 200 cm³ and were found to be capable of generating currents of up to 2 A [Bennetto 1987].

As an alternative to the earlier systems which had the micro-organisms freely suspended in the anodic solution, microbial cells of *Proteus Vulgaris*, have been immobilised onto graphite felt electrodes and have been used to generate currents from carbohydrates [Allen and Bennetto 1993]. This immobilisation lead to faster responses to substrate addition, whilst the use of a constant feed system gave improved efficiencies when compared to single large additions of fuel. There are also more recent reports of *Proteus Vulgaris* being used in suspension within a thionine mediator solution, to generate power from a variety of carbon sources [Kim *et al* 2000]. Galactose was found to be the optimal fuel giving a columbic efficiency of 63%. Use of a mixed bacterial culture originating from an anaerobic sludge and a hexacyanoferrate mediator, further improved this figure to 89% when using a glucose fuel and allowed power outputs of up to 360 μW cm⁻² [Rabeay *et al* 2003].

Instead of immobilising the microbe, other work studied the effect of immobilising the mediator (in this case Neutral Red) onto a graphite anode [Park *et al* 2000]. When acetate was used as the fuel together with *E. Coli* as the micro-organism, it was found that immobilising Neutral Red on the anode more than doubled the performance of the fuel cell. Immobilised Neutral Red and Mn^{4+} graphite anodes were used in conjunction with a Fe^{3+} immobilised cathode [Park and Zeikus 2003] and utilised in a microbial fuel cell. These workers found that when *E. Coli* or sewage sludge were exploited as biocatalysts, currents 1000 times greater than those using unmodified graphite electrodes could be obtained. Sewage sludge was found to be the superior catalyst, producing 14 mA at 0.45 V, corresponding to a power density of $78.8 \mu\text{W cm}^{-2}$, nearly an order of magnitude higher than for *E. Coli*.

In a further report a H_2/O_2 fuel cell was constructed using carbon felt anodes and cathodes together with a combined microbe/enzyme catalysis system. Bilirubin oxidase was utilised in the cathode compartment with *Desulfovibrio vulgaris* being introduced to the anode compartment along with suitable mediators. Under these conditions, currents of 0.9 mA at an operating potential of 1 V could be obtained [Tsuji-mura *et al* 2001a] at physiological pH.

Mediator-free microbial biofuelcells containing an Fe^{3+} reducing bacterium [Gil *et al* 2003] utilising wastewater from a starch processing plant have been constructed and run continuously for periods of up to 3 years. Effects of pH and electrolyte have been studied. Cells of this type have been shown to have potential uses as biological oxygen demand sensors as well as offering an approach for processing waste water. Other work using *R. ferrireducens* demonstrated that this micro-organism could be used to generate energy from glucose [Chauduri and Lovley 2003], and in this context it should be noted that the glucose is completely converted to carbon dioxide, a process capable of releasing 24 electrons with the efficiency of electron transfer to the anode being reported to be up to 83% - even in the absence of a mediator. Evidence is presented within the paper that it is the microbes adsorbed as a biofilm on the anode surface that produce the current rather than those in suspension in the solution. A variety of graphite electrodes were used, with graphite felt and graphite foam being

found to produce up to three times as much power than graphite rods, probably due to a more porous structure within the materials. Fructose, sucrose and xylose were also shown to be suitable fuels.

As shown earlier in this review, osmium containing polymers are especially suitable for “wiring” electrodes to electrode surfaces. An osmium containing polymer has also been utilised to “wire” *Gluconobacter Oxydans* cells directly to the anode of a biofuel cell [Vosteir *et al* 2004] by simple casting and drying of first the polymer followed by a suspension of the micro-organism onto cysteamine modified gold electrodes. The resulting electrodes were found to be capable of generating power from glucose, ethanol and glycerol. An electrodeposited conducting polymer was also utilised in this manner, for example by modifying platinum electrodes with a layer of poly(tetrafluoroaniline) and then placing these in a medium containing *Clostridium* bacteria [Niessen *et al* 2004]. It was found that current densities of $1.0\text{--}1.3\text{ mA cm}^{-2}$ could be generated from fuel cells such as these when fuels such as glucose, molasses or starch were used. These workers showed that the presence of the conductive polymer layer also helps to protect against poisoning of the platinum electrode.

Several of the papers previously mentioned utilise a mixed microbial community rather than a single micro-organism. Attempts have been made to determine whether under the conditions present in a microbial fuel cell, the community will “evolve” to make the most of the conditions and thereby increase the power output of cells. Following the reasoning, a microbial fuel cell was set up with graphite electrodes, with glucose being used as the fuel together with sludge obtained from a potato processing company as the biocatalyst [Rabaey *et al* 2004]. After the fuel cell had been operated for two weeks, harvesting the bacteria from the anode compartment of a fuel cell was performed by simply scraping the biofilm formed off the anode. This was then resuspended within fresh nutrient broth and replacing in the anode compartment. Repeating this process increased the power densities from $60\text{ }\mu\text{W cm}^{-2}$ up to $431\text{ }\mu\text{W cm}^{-2}$, corresponding to an efficiency of 81%. Analysis of the system indicated predominance of certain types of bacteria and also demonstrates that some of them were excreting redox components which acted as mediators. Further studies on bacteria that produce redox mediators [Rabaey *et al* 2005b] were performed using

a strain of *Pseudomonas aeruginosa* which produces phenazine derivatives. When the performance within glucose fuelled microbial fuel cells of mutant species that do not produce phenazines were compared with the wild type micro-organism, they were found to produce only 5% of the power output of the wild type. In mixed microbial systems the presence of the mediators produced by *Pseudomonas aeruginosa* were also found to enhance electron transfer rates from other bacterial species. Microbial fuel cells operated in continuous mode have also been investigated. Various designs of fuel cells have been studied, with the best results being obtained where the anode compartment comprised a packed bed reactor containing graphite granules [Rabaey *et al* 2005c] with the power output being found to be lower under continuous mode operation rather than under batch mode operation. Cyclic voltammetry indicated the presence of redox mediators of bacterial origin, however, addition of synthetic mediators of similar redox potential were found to only have a minimal effect on the performance of the cell.

Optimisation of microbial fuel cells is important if we are to extract the maximum performance from these systems. Work has been carried out which indicates that the power output from cells can be affected by a number of different factors [Min *et al* 2005]. One factor that lowers efficiency is diffusion of oxygen into the anode chamber and this can lower coulombic efficiency from 55% (chamber purged with nitrogen) down to 19% (no purge). Replacing the separator membrane with a salt bridge was also found to have a detrimental effect, probably due to an increase in internal resistance of the cell. However replacing the pure culture used in this cell (*Geobacter metallireducens*) with a mixed culture was found to have only a minimal effect. Other work has lead to the development of single chamber microbial fuel cells with and without a Nafion separator membrane [Liu and Logan 2004]. The power outputs of these cells when fuelled with glucose actually increased from 26.2 $\mu\text{W cm}^{-2}$ to 49.4 $\mu\text{W cm}^{-2}$ upon removal of the separator, although the coulombic efficiency dropped from 55% at best to 12%. Power densities of 14.6 $\mu\text{W cm}^{-2}$ could be obtained using a domestic wastewater as the fuel source [Liu and Logan 2004]. Attempts were made to improve the performance of microbial fuel cells produced by this group by, for example, replacing the simple carbon paper anode with an iron oxide coated anode. This approach was found to double the Coulombic efficiency

with an associated near quadrupling of the power density of the cell [Kim *et al* 2005]. The same group also used cysteine as a chemical oxygen scavenger in the anode compartment with an anaerobic marine sediment providing the source of the biocatalyst [Logan *et al* 2005a]. This work showed that cysteine itself was suitable as a fuel for the cell, with concentrations of 0.77 g/L giving power outputs of up to 3.9 $\mu\text{W cm}^{-2}$.

A novel tubular microbial fuel cell has recently been developed [Rabaey *et al* 2005d] in which a granular graphite anode is contained within a “sleeve” made from a commercial cation exchange membrane (Fig. 5). Microorganisms from an operating microbial cell were used to inoculate the cell and when combined with a sacrificial ferrocyanide cathode, the resultant cell was found to be capable of operating using fuels such as acetate with a coulombic efficiency of 75% or glucose with a coulombic efficiency of 59%. The fuel cell could also utilise either digester effluent or domestic wastewater as a fuel source. When wastewater was used, up to 22% of the organic materials were removed with a coulombic efficiency of 96%, leading to a power output of 48 W m^{-3} anodic compartment volume [Rabaey *et al* 2005d].

Most of these systems utilise a simple oxygen cathode, however, microbial fuel cells utilising micro-organisms in both the anode and cathode compartments have been formulated [Rhoads *et al* 2005]. In the anode compartment, suspended *Klebsiella pneumonia* is exploited to oxidise glucose, with a porous graphite electrode being used in conjunction with a soluble quinone mediator. The cathode used was porous graphite in a suspension of *Leptothrix discophora*. The suspension contains manganese which is oxidised to MnO_2 by the bacterium in the presence of oxygen. The resultant biomineralised MnO_2 is then reduced at the cathode surface without the necessity for a mediator. It was found in this case that power densities of 12.7 $\mu\text{W cm}^{-2}$ could be obtained from these cells.

Although carbohydrates based fuels, alcohols or hydrogen are usually the main fuels of interest, other potential systems have been considered. As previously mentioned cysteine has been utilised as a fuel [Logan *et al* 2005a]. Other fuel sources include acetate which has been utilised in a microbial fuel cell [Bond and Lovley 2003] in

which *Geobacter Sulfurreducens* was immobilised on a graphite anode and converted acetate to carbon dioxide with a 95% electron transfer efficiency being achieved without recourse to a mediator. Other work by the same group also showed that aromatic compounds such as benzoate and toluene could be utilised [Bond *et al* 2002], with a marine sediment providing the biocatalyst. In a more recent paper [Ieropoulos *et al* 2005] describes a microbial fuel cell with sewage sludge providing the biocatalyst, with a report that the power output of the cell when fuelled by glucose could be increased by a factor of five times by the simple addition of sulphate. In the paper recently published within the special edition of *Biosensor and Bioelectronics* [Lowy *et al* 2006], showed that redox potential across the interface between the organic rich reducing environment of marine sediments and relatively oxygen rich seawater can produce electrical energy. Organic compounds within the sediment are oxidised by sediment microbial life without the need for a mediator, therefore when a graphite anode is embedded in the sediment and a graphite cathode suspended in seawater, current flows in the order of 30 mW m^{-2} (90 mA m^{-2}) are observed although modification of the anode with quinones or metals could increase the current density by up to five times.

4. Photochemical biofuel cells

As an alternative to inorganic semiconductor-based or dye-based photoelectric cells, attempts have been made to use biological species to capture light and convert this into electrical energy. Some of these systems use microbes which respire thereby using a respiratory fuel and so fall within the scope of this review. For example a microbial cell has been reported utilising a soluble quinine mediator in which the anode compartment contains a carbon electrode and a suspension of *Anabaena variabilis* immobilised onto alginate beads [Yagashita *et al* 1998]. The cathode compartment contained potassium ferricyanide and a carbon electrode. When illuminated for a 10 hours light/10 hours dark cycle, an electrical current was generated (1 mA under a 500 ohms load) with low photon to electron conversion efficiency (0.2 %). A much more efficient system utilised *Synechococcus* in which a current output was obtained from photolysis of water under illumination as well as from consumption of carbohydrates in the dark [Yagishita *et al* 1997]. Up to 3.3% of the light energy could be harvested as electrical energy.

Each of the cells mentioned above required the use of a sacrificial ferricyanide cathode and therefore may be thought not to be true fuel cells. A cell containing biological moieties in both the anode (cyanobacteria) and cathode (bilirubin oxidase) compartments has also been reported [Tsujiura *et al* 2001b]. The cyanobacteria catalyse the photooxidation of water with the production of electrons which are passed to the carbon felt anode and are thereby made available for the reduction of oxygen at the cathode. Power outputs upon illumination of $30\text{--}40\text{ }\mu\text{W cm}^{-2}$ could be obtained with light energy conversion efficiencies of 2–2.5% being reported.

The combination of photochemical and biological reactions has also been of recent interest. A cell containing an indium tin oxide anode coated with a porphyrin sensitizer has been reported [de la Garza *et al* 2003]. Photooxidation of the sensitizer occurs at this electrode and causes conversion of NAD(P)H to NAD(P)⁺. Normally this reaction would cease when all the NAD(P)H has been converted, however, when glucose and glucose dehydrogenase are present within the anode compartment, consumption of glucose and regeneration of NAD(P)H occurs. When combined with a Pt/O₂ cathode, photocurrents could be obtained with a power output of $9.5\text{ }\mu\text{W cm}^{-2}$ at an operating potential of 0.45 V, with a photon to electron conversion efficiency of 12% being obtained. Other enzymes were utilised within this type of system to enable the use of other fuels such as methanol and ethanol [de la Garza *et al* 2003]. A similar system utilising nanoparticulate titanium oxide as the anode material has also been developed [Brune *et al* 2004] and showed improved performance with a power output of $37\text{ }\mu\text{W cm}^{-2}$ at an operating potential of 0.84 V and with a similar photon to electron conversion efficiency being reported.

5. Uses of biofuel cells

There are several potential uses of biofuel cells, with the ones receiving most interest being described below.

5.1 *Transport and energy generation*

At present the world's largest source of power is derived from the use of fossil fuels and especially petroleum. However the burning of hydrocarbons cannot continue indefinitely because of environmental problems and also the simple fact that we have a finite supply of these fuels. The utilisation of biofuel cells with carbohydrates as a power source would, if they could be developed, help to mitigate at least some of these problems. It has been calculated [Shukla *et al* 1999] that a litre of a concentrated carbohydrate solution could power a car for 25-30 km. It follows that if a car were to be fitted with a 50 L tank, the car could travel over 1000 km without refuelling. Not only would this offer environmentally benefits, it would also remove the risk associated with transport of large amounts of volatile, flammable fuels in addition to the risk of fire following a road traffic accident.

5.2 *Implantable power sources.*

Since biofuel cells can potentially be run in living systems, taking the oxygen and fuel required for their operation can conceivably be taken from their immediate environment, and this offers great potential as power sources within a range of possible implantable medical devices. For example, a biosensor for glucose has been developed utilising a glucose oxidase based anode and cytochrome c cathode to generate electrical current [Katz *et al* 2001]. This process can be used in a biosensor format to give a measurement of the glucose concentration in the range of 1-80 mM. A similar sensor for lactate has also been developed [Katz *et al* 2001]. Other potential uses for miniature fuel cells include power sources for drug delivery systems with biofuel cells being small enough for this purpose already having been developed [Mano *et al* 2003b, Moore *et al* 2005].

5.3 *Waste water treatment.*

Numerous fuel cells have been shown to generate power by oxidation of compounds found in waste water streams [e.g. Gil *et al* 2003, Rabaey *et al* 2004, Liu and Logan 2004]. Two useful purposes can be realised by this procedure; (a) for the removal of

the organic compounds from the waste stream and (b) for the generation of electrical power. A recent review on the subject [Logan 2005] calculates that the wastewater from a town of 150,000 people could potentially be used to generate up to 2.3 MW of power (assuming a 100% efficiency), although a power of 0.5 MW might be more realistic. It should be mentioned in this context that up to 80% of the chemical oxygen demand of wastewater can be removed by treatment in a microbial fuel cell and it is possible that the electricity generated in this manner could be used on site to power further treatment of the wastewater. An economic study within the review [Logan 2005] shows the potential for this application, although this is highly dependent on local power costs.

5.4 Robots

The concept of robots which can “live off the land” by utilising biofuel cells to generate their power and the various challenges to be overcome has been already discussed [Wilkinson 2000]. An early example is the “Slugbot” (Fig. 6), which as its name implies hunts slugs [Kelly 2003]. The “Slugbot” itself is powered by a rechargeable battery, however, once captured the slugs are held in a holding tank until the battery begins to run down. The “Slugbot” then returns to a microbial fuel cell to which it transfers the slugs and utilises the energy produced by their “digestion” to recharge its battery (www-robotics.usc.edu). Another “gastropod” named “Chew-Chew” has also been produced and is capable of “feeding” and so running via consumption of meat (www.gastrobots.com).

6. Future of biofuel cells

The development of biofuel cells for practical applications is a field which is still in its infancy, although there is unquestionably much potential for further improvement. Table 1 lists some of the great strides that have been made over the last few years in improving the current (and therefore power) densities obtainable at electrodes used within enzymatic based biofuel cells. Microbial fuel cells (Table 2) offer the promise for use as power supplies with high longevities that are capable of using complex biofuels formed from biological waste. Other possible avenues include exploiting enzymes harvested from the lysis of living cells as catalysts, thereby combining the power output of enzyme-based biofuel cells with the versatility of microbial based biofuel cells.

One of the most active areas in the field is focussed towards developing power sources for implantable devices within humans. Biofuel cells offer several possible advantages over existing technologies, such as the use of lithium-iodine batteries in implantable devices such as heart pacemakers. Ideally an implanted biofuel cell would use a biological metabolite fuel source such as glucose or lactate, both of which are readily available in physiological fluids such as blood. The high turnover of a “wired” enzyme electrode in such applications could generate power levels capable of meeting the needs of many devices without the need for a mediator. There are a number of problems which need to be addressed, however, the most important of which is that most of the enzyme modified electrodes described in the literature to date have lifetimes in the order of weeks whereas for *in vivo* implanted devices, longevities of years would be required for practical application. Unfortunately most of the biofuel cells described today would be capable of meeting demands for biomedical devices implanted for short-term application only. Although the stabilisation of enzymes has been an active area for many years, the state-of-the-art is not capable of meeting the requirements of such devices [Barton *et al* 2004]. It is probable that enzymes will have to be modified by routes such as genetic engineering if the required enzyme stabilities are to be met. A second problem that must be addressed is that of biocompatibility; the biofuel cell must be capable of existing in the physiological environment without an unacceptable degree of biofouling occurring over extended periods of time – which otherwise would lead to fouling of the device or to physiological harm to the patient.

Other possibilities for biofuel cell research include the future development of power supplies for use in remote areas. In an ideal scenario biofuel cells such as these should be capable of using readily available fuel sources. Plant saps, for example, often contain high levels of sugars which could be used as a fuel. Many conventional hydrogen or alcohol fuel cells require expensive noble metal catalysts and moreover often require extreme conditions of pH or high temperature. Biological fuel cells which usually perform optimally at near ambient temperatures and neutral pH - clearly would offer benefits in this respect. Microbial fuel cells may also in the future be used to help degrade organic waste such as sewage sludge whilst also producing electricity as a useful by-product.

Although some specialist devices for providing, for example, short lifetime implantable power devices could now be considered feasible, it is obvious that a continuing research effort needs to be made before we will see large scale use of biofuel cells. Problems of lifetime, stability and power density all need to be addressed, although the possible benefits of this technology are likely to drive continuing research. We need to improve our knowledge of biocatalysis, electron processes at surfaces, biological and other material stability to realise this vision.

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Table 1. Comparison of the construction and performance of some recent enzyme based biofuel cells.

Anode	Cathode	Separator	Fuel	Power Output	Reference
Au/glucose oxidase (GOx)	Au/micro peroxidase	Glass frit	Glucose/H ₂ O ₂	32 μ W at 0.31 V vs SCE	Willner <i>et al</i> 1998b
Au/GOx	Au/micro peroxidase	H ₂ O/CH ₂ Cl ₂ interface	Glucose/cumene peroxide	520 μ W at 1 V vs SCE	Katz <i>et al</i> 1999a
Au/GOx	Au/cytochrome c/ cytochrome oxidase	None	Glucose/O ₂	4 μ W	Katz <i>et al</i> 1999b
Pt	C or Pt with laccase in solution	Nafion	H ₂ /O ₂	42 μ W cm ⁻² at 0.61 V vs SCE	Palmore and Kim 1999
Graphite (Formate/Aldehyde/Alcohol Dehydrogenases soln.	Pt	Nafion	MeOH/O ₂	670 μ W cm ⁻² at 0.49 V vs SCE	Palmore <i>et al</i> 1998
Glassy C, Os redox polymer, GOx	Glassy C, osmium redox polymer, bilirubin oxidase	None	Glucose/O ₂	58 μ W cm ⁻²	Tsujimura <i>et al</i> 2002
7 μ M carbon fibre, Os redox polymer, GOx	7 μ M carbon fibre, Os redox polymer, bilirubin oxidase	None	Glucose/O ₂	64 μ W cm ⁻² /23°C 137 μ W cm ⁻² /37°C	Chen <i>et al</i> 2001
7 μ M carbon fibre, modified Os redox polymer, GOx	7 μ M carbon fibre, modified Os redox polymer, laccase	None	Glucose/O ₂	430 μ W cm ⁻² at 0.52 V	Mano <i>et al</i> 2003b
7 μ M carbon fibre, modified Os redox polymer, GOx	7 μ M carbon fibre, modified Os redox polymer, laccase	None	Glucose/O ₂	430 μ W cm ⁻² at 0.78 V	Mano <i>et al</i> 2003c
7 μ M carbon fibre, modified Os redox polymer, GOx	7 μ M carbon fibre, modified Os redox polymer, laccase	None	Glucose/O ₂	350 μ W cm ⁻² at 0.88 V	Soukharev <i>et al</i> 2004
Porous C/C nanotube/GOx	Porous C/C nanotube/laccase	Nafion	Glucose/O ₂	99.8 μ W cm ⁻²	Liu <i>et al</i> 2005
Carbon felt/Nafion NBu ₄ ⁺ salt alcohol+aldehyde dehydrogenase	Pt/C	Nafion	MeOH/O ₂ EtOH/O ₂	1550 μ W cm ⁻² 2040 μ W cm ⁻²	Akers <i>et al</i> 2005

Table 2. Comparison of the construction and performance of some recent microbial biofuel cells.

Anode	Cathode	Separator	Microbial source	Fuel	Power Output	Reference
Graphite	Graphite in soln $\text{K}_3\text{Fe}(\text{CN})_6^{3-}$	Ultrex membrane	Potato processing sludge	Glucose/ O_2	$360 \mu\text{W cm}^{-2}$	Rabaey <i>et al</i> 2003
Graphite	Graphite in soln $\text{K}_3\text{Fe}(\text{CN})_6^{3-}$	Ultrex membrane	Evolved potato processing sludge	Glucose/ O_2	$431 \mu\text{W cm}^{-2}$	Rabaey <i>et al</i> 2004
Graphite with Mn^{4+}	Graphite with Fe^{3+}	Ceramic	Sewage sludge	Lactate/ O_2	$78.8 \mu\text{W cm}^{-2}$	Park and Zeikus 2003
Polytetrafluoroaniline on graphite	Graphite	Nafion	Clostridium	Glucose or starch/ O_2	$1000\text{-}1300 \mu\text{W cm}^{-2}$	Niessen <i>et al</i> 2002
Carbon paper	Carbon cloth/Pt	None	Wastewater	Glucose or wastewater/ O_2	$49.4 \mu\text{W cm}^{-2}$	Liu and Logan 2004
Granular graphite	Graphite cloth soaked with $\text{K}_3\text{Fe}(\text{CN})_6^{3-}$	Ultrex membrane	Previous microbial cell	Wastewater	48 W m^{-3}	Rabaey <i>et al</i> 2005d
Graphite disk	Graphite disk	None	Marine sediment	Sediment organics / O_2 (seawater)	30 mW m^{-2}	Lowy <i>et al</i> 2006

Fig. 1. Schematic of a simple biofuel cell.

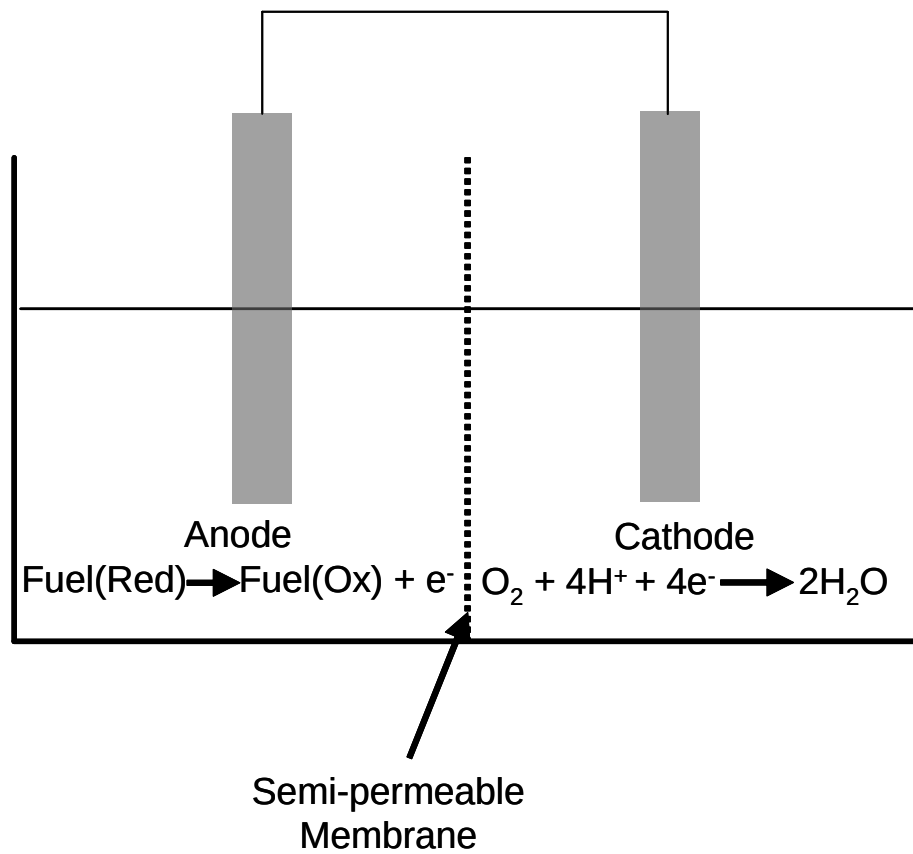


Fig. 2. The ferrocene mediated oxidation of glucose (GOD = glucose oxidase, Fc = ferrocene).

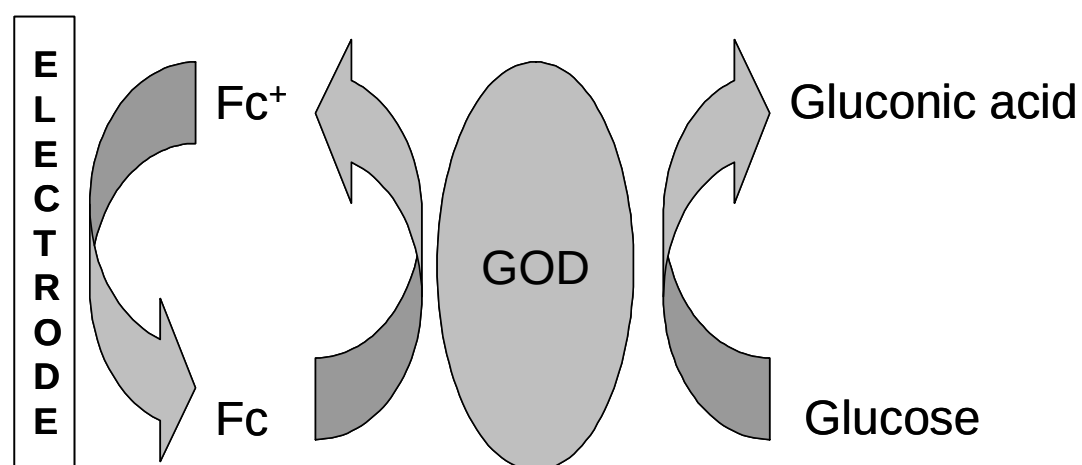


Fig.3. A miniature biofuel cell implanted within a sliced grape showing the implanted fibres and the electrical contacts. Because in the photographs the fine lines of the 7 μm fibres were barely visible, their lines are computer drawn. Reprinted with permission from [J. Am. Chem. Soc., 2003, 125, 6588]. Copyright [2003] American Chemical Society. [Mano *et al* 2003b].

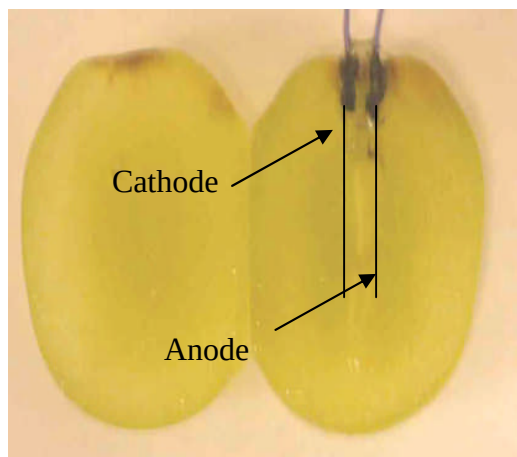


Fig. 4. A microfluidic “biofuel cell on a chip”. (A) Carbon microelectrodes printed onto glass by micromoulding technique. Dimensions: 55 μm wide, 85 μm high, and 2.5 cm long. (B) Carbon microelectrode sealed in a PDMS microchannel. Dimensions of flow channel: 200 μm wide, 100 μm in depth, and 3.0 cm long. Reproduced by permission of the Royal Society of Chemistry [Moore *et al* 2005].

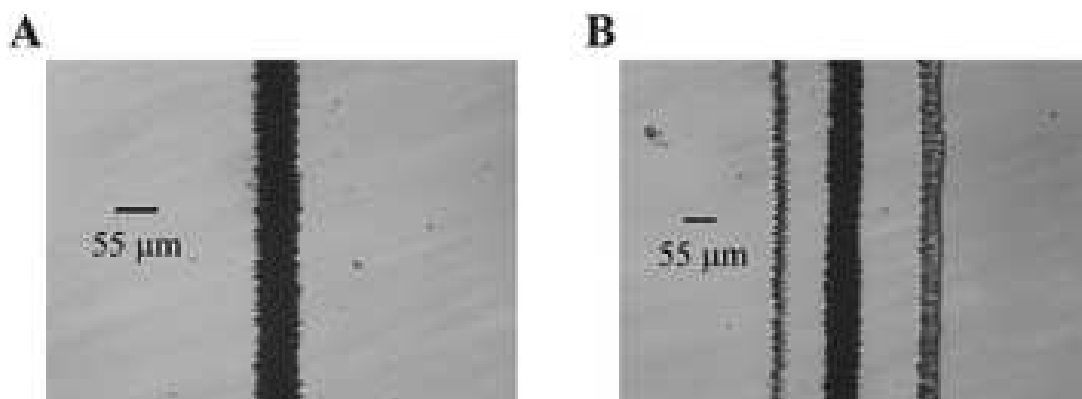


Fig. 5. A tubular microbial fuel-cell. Reprinted with permission from [Environ. Sci. Technol. 2005, 39, 8077]. Copyright [2005] American Chemical Society. {Rabaey *et al* 2005d}.

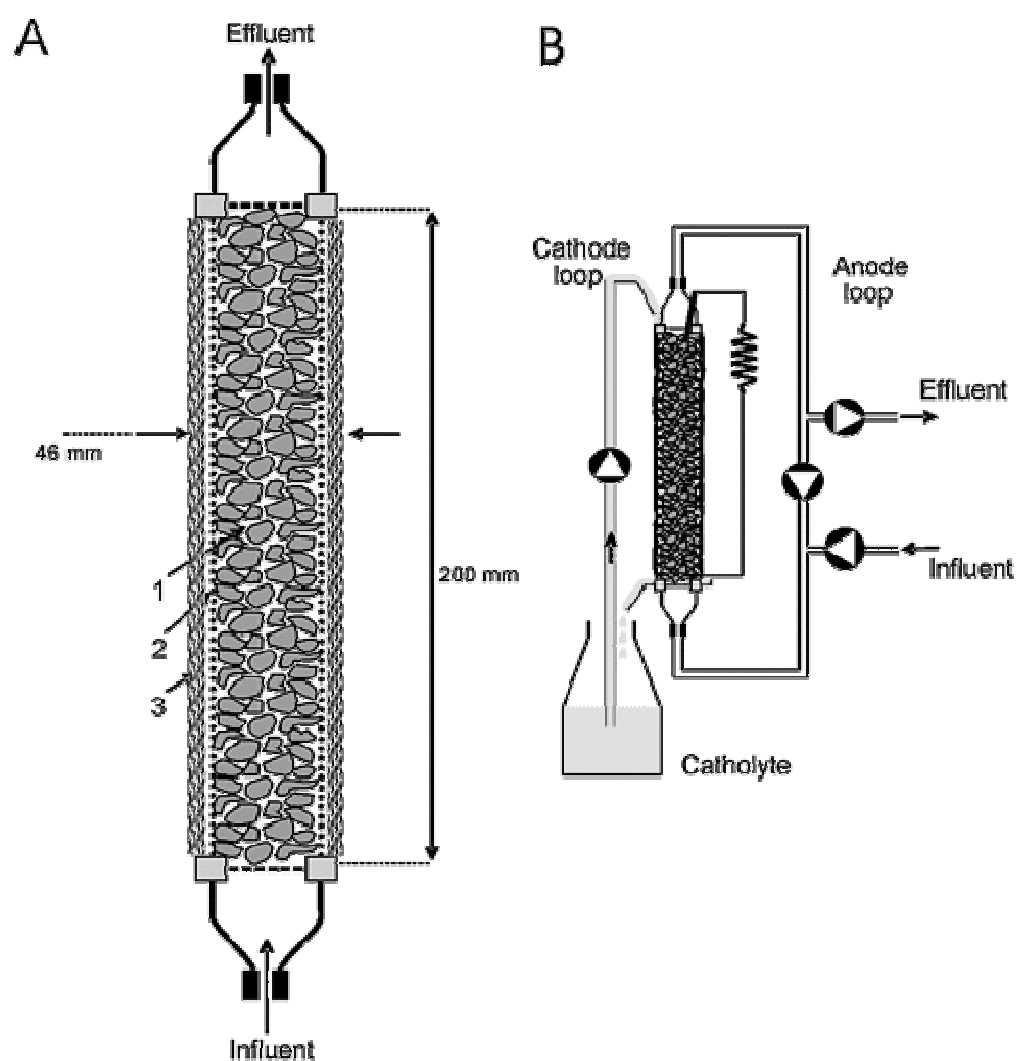
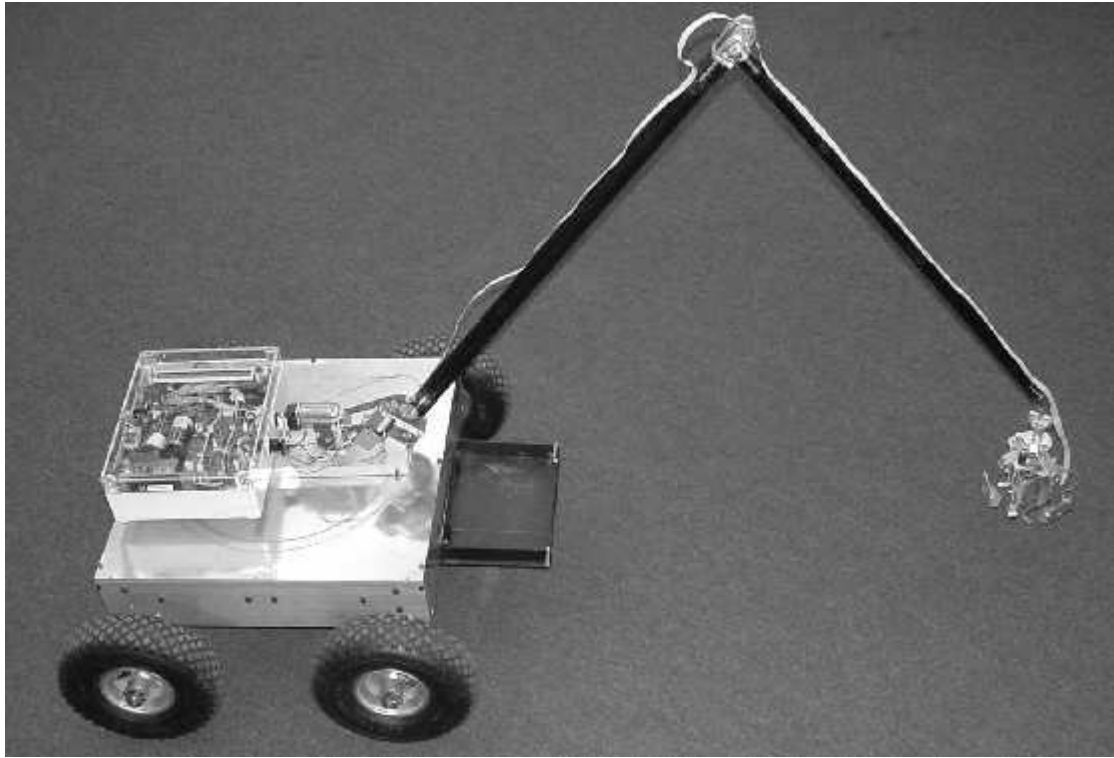


Fig. 6. “Slugbot”, a robotic slug hunter. Photograph supplied by Dr Ian Kelly.





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