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Application of electro-active biofilms

Benjamin Erable[a,b]*, Narcis M. Duţeanu[a,c], M.M. Ghangrekar[a,d], Claire Dumas[b] and Keith Scott[a]

[a]*School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK;*
[b]*Laboratoire de Génie Chimique, CNRS-Université de Toulouse, 5 rue Paulin Talabot BP1301, 31106 Toulouse, France;*
[c]*Faculty of Industrial Chemistry and Environmental Engineering, University 'Politehnica' Timisoara, 300006 Timisoara, Romania;*
[d]*Department of Civil Engineering, Indian Institute of Technology, Kharagpur 721302, India*

*Corresponding author. Email: benjamin.erable@ensiacet.fr

The concept of an electro-active biofilm (EAB) has recently emerged from a few studies that discovered that certain bacteria which form biofilms on conductive materials can achieve a direct electrochemical connection with the electrode surface using it as electron exchanger, without the aid of mediators. This electro-catalytic property of biofilms has been clearly related to the presence of some specific strains that are able to exchange electrons with solid substrata (eg *Geobacter sulfurreducens* and *Rhodoferax ferrireducens*). EABs can be obtained principally from natural sites such as soils or seawater and freshwater sediments or from samples collected from a wide range of different microbially rich environments (sewage sludge, activated sludge, or industrial and domestic effluents). The capability of some microorganisms to connect their metabolisms directly in an external electrical power supply is very exciting and extensive research is in progress on exploring the possibilities of EABs applications. Indeed, the best known application is probably the microbial fuel cell technology that is capable of turning biomass into electrical energy. Nevertheless, EABs coated onto electrodes have recently become popular in other fields like bioremediation, biosynthesis processes, biosensor design, and biohydrogen production.

Keywords: biocathodes; bioelectrochemical sensors; electro-active biofilms (EABs); electricity generation; microbial fuel cell; wastewater treatment

Introduction

Typically, biofilms have been associated with adverse effects such as biodeterioration of materials or nosocomial infections. But the discovery in the early 2000s of the ability of some biofilms, called electro-active or electrochemically active biofilms (Reimers et al. 2001), to exchange electrons directly with conductive materials, opened new perspectives. Electro-active biofilms (EABs) are the subject of a new area of expanding research involving several disciplines dominated by electrochemistry, microbiology and chemical engineering (Logan et al. 2006; Du et al. 2007). Their applications were explored in many fields, including biotechnology, sustainable energy development or bioremediation. For example, such biofilms are able to reduce heavy metals (eg chromium and uranium) playing a role in bioremediation of groundwater and contaminated soil (Li et al. 2008). EABs can be useful in electrochemical biosensors to monitor the development of biofilms in facilities, where their presence is undesirable. In addition, the EAB has a significant impact in the field of renewable energy including the development of microbial fuel cells (MFCs) and hydrogen production, through which energy production is coupled with the treatment of organic waste (Logan et al. 2008).

Biofilm coated electrodes

Discovery and historic

The conversion of chemical energy into electricity by microorganisms has attracted the attention of a large number of researchers. Due to studies on MFCs the concept of 'Bio-electricity' has really taken its meaning. EAB coated electrodes play vital role in the electron transfer and hence overall performance of the MFC. This EAB concept is being extensively studied in the MFC research in efforts to improve its performance.

Although, the concept of chemical fuel cell was discovered in 1839, it was not until 1910 that Potter discovered that microorganisms can produce electricity. In 1931, this discovery was exploited by Cohen achieving a voltage of 35 V by connecting biological fuel cells in series. This value has still not been exceeded. The development of biological fuel cells was especially enhanced in the 1960s when NASA was interested in processing organic waste into electricity in

space flights. In 1963, the first biological fuel cells were marketed as energy sources for marine equipment. However, with the cost of fossil fuels remaining low, biological fuel cells have not been more developed and were classified as commercial failure. Later, during the oil crises in the 1970–1980s, interest in biological fuel cells was rekindled. A significant improvement has been made with the addition of electrochemical mediators that increased the system efficiency and the rate of reactions (Thurston et al. 1985; Allen and Bennetto 1993). However, the power obtained was still insufficient for a real development of MFC.

Recently, the discovery of the direct electron transfer between bacteria and conductive materials has expanded the interest related to biological fuel cell. Thus, new generations of MFCs no longer require the addition of electrochemical mediators, which make the system less expensive, less polluting and more sustainable over time. In addition, this direct contact between bacteria and the electrode increases the technology performance thus achieving power of several Watts per square meter of electrode (W m^{-2}) of electrode surface.

Electron transfer

Many research articles enumerate the mechanisms of electron transfer between bacteria and conductive materials (Lovley 2006a–c; Rabaey et al. 2007; Schroder 2007). The transfer of electrons between the bacteria and the solid material can be done (i) indirectly, either by abiotic oxidation products derived from biological fermentation or through electrochemical mediators, secreted by the bacteria themselves or added to the electrolyte; or (ii) directly through the components of the cell membrane. Four mechanisms are distinguished for the transfer of electron from bacteria to the electrode as detailed below (Lovley 2006a).

Oxidation of bacterial metabolism product

The first studies on EABs/conductive material interfaces showed bio-electricity generation by oxidation of products derived from bacterial fermentation on the anode surface. These products such as H_2, alcohols, ammonia or H_2S or HS^-, are oxidized at the anode (Figure 1). Recently, Niessen et al. used strains of *Clostridium* (*Clostridium beijerinckii, Clostridium butyricum*) or other fermentative bacteria (*Escherichia coli* K12,) to produce hydrogen electrochemically on the anode (Niessen et al. 2004a, b, 2006). These studies indicated high current densities up to 30 Amps per square meter (A m^{-2}) of electrode (at + 0.20 V/ Ag-AgCl) with heat treated soil (compost-based fertilizers and manure) and tungsten carbide electrodes.

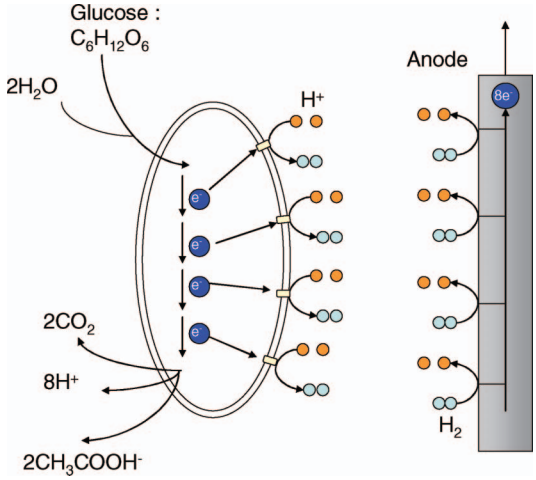

Figure 1. Indirect electron transfer involving the oxidation of product from microbial metabolism at the anode. For example, hydrogen produced by bacteria from glucose can be abiotically oxidized on the anode surface. (Adapted from Lovley 2006a.)

Compounds other than hydrogen were also exploited in biological fuel cells. For example, the sulfate-reducing bacteria present in marine sediments were able to reduce sulfate compound into HS^- or S^{2-} (depending on pH), which oxidized into S^0 directly on the anode surface (Ryckelynck et al. 2005; Lovley 2006a; Reimers et al. 2006).

Electron transfer by artificial electrochemical mediators

Some non-fermentative bacteria can use the electrode as an electron acceptor to achieve the desired conversion, but require the use of mediators for the electron transfer. Electrochemical mediators are molecules that can be oxidized and reduced and then recycled successively. In their oxidized form, they are able to cross the cell membrane, to accept electrons from at least one electron donor within the cell and then transfer throughout the cell under reduced form to finally oxidize and transfer electrons on the anode (Shukla et al. 2004) (Figure 2).

Electrochemical mediators were often used with bacterial species such as *E. coli, Pseudomonas* sp., *Proteus* and *Bacillus*, which were unable to transfer electrons from their internal metabolism outside the cell. The most used mediators were thionine, neutral red, 2-hydroxy-1,4-naphthoquinone, and different kinds of phenazin. But many disadvantages discouraged the use of these mediators. First, it has not been demonstrated that microorganisms are able to maintain their growth in the presence of electrochemical

mediators. Second, biological fuel cells have been operated continuously, requiring the permanent presence of mediators that increased the cost of their applications. Finally, electrochemical mediators were often toxic and could not be released into the environment without treatment.

Bacteria that produce their own mediators

Some microorganisms such as *Pseudomonas* spp. (Rabaey et al. 2005), *Shewanella putrefaciens* (Kim et al. 1999a, 2002) or *Geothrix fermentans* (Bond and Lovley 2005) were able to produce electrochemical mediators to increase the extracellular electron transfer (Hernandez et al. 2004). The bacterium *Pseudomonas aeruginosa* has been described as producing phenazin molecules increasing electron transfer measured on the electrode (power obtained 116 μW m^{-2}) (Rabaey et al. 2005). In contrast, mutant strains of *P. aeruginosa*, which could not synthesize electrochemical mediators have achieved only 6 μW m^{-2} representing only 5% of the power observed with the non-deficient strain (Rabaey et al. 2005).

The ability of *Shewanella oneidensis* to grow under anaerobic and aerobic conditions, to use a wide variety of electron acceptors, and to secrete mediators to aid in electron transfer (potentially enhanced power densities), made *S. oneidensis* a provocative choice for a significantly wider variety of power applications in aerobic or microaerophilic environments (Biffinger et al. 2008a). However, to date, only a limited number of carbon containing electron donors (lactate, formate, pyruvate, amino acids) have supported metal reduction by *S. oneidensis* under anaerobic conditions.

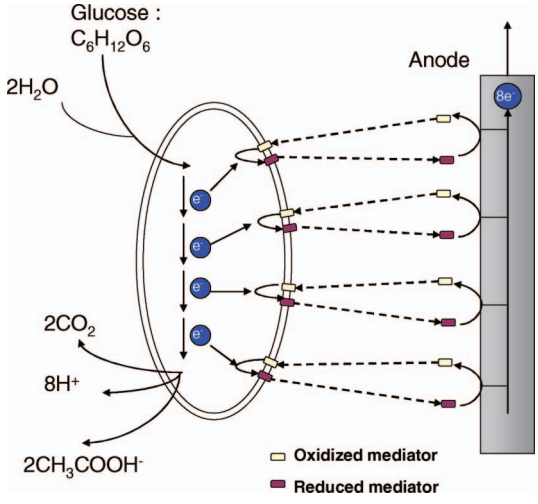

Figure 2. Indirect electron transfer *via* an electrochemical mediator that plays the role of final electron acceptor for bacteria and which transmits electrons reacting on the anode surface. (Adapted from Lovley 2006a.)

Direct electron transfer

The main element in direct electron transfer is the ability of bacterial cells to switch their metabolism from a soluble electron donor (eg hydrogen, glucose, acetate) or acceptor (eg oxygen, nitrate, fumarate) to a solid electron donor or acceptor at the surface of a conductive electrode (Figure 3). This new consideration was highlighted recently by Bond et al. (2002) and Tender et al. (2002).

In the literature, some bacteria have been described as being able to transfer electrons directly to an electrode surface, eg *Desulfuromonas acetoxidans* (Bond et al. 2002), *Geobacter sulfurreducens*, *Geobacter metallireducens, Rhodoferax ferrireducens, Desulfobulbus propionicus* (Holmes et al. 2004), *Enterococcus gallinarum* (Kim et al. 2005). However, the direct transfer of electrons from bacteria to the conductive material was controversial for *S. putrefaciens* (Kim et al. 1999b), *C. butyricum* (Park et al. 2001), *Aeromonas hydrophila* (Pham et al. 2003), and *S. oneidensis* (Ringeisen et al. 2006). The flow of electrons obtained with these strains was a combination of a direct transfer and an indirect transfer *via* mediators secreted by the bacteria.

Biocompatible materials

The material suitable for bio-electrode construction must have certain properties such as a higher biocompatibility, higher stability, a low price, and higher conductivity (Rosenbaum et al. 2007). Special attention

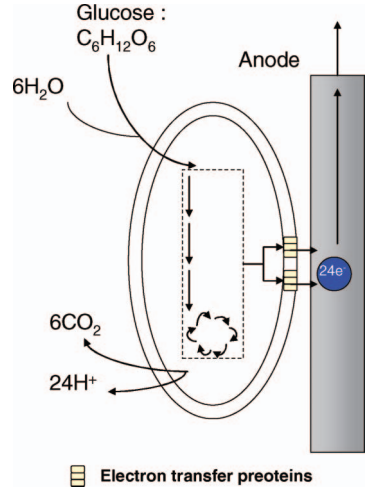

Figure 3. Direct electron transfer involving bacteria that can transfer electrons directly to a conductive material. Electrons from the oxidation of glucose, for example are transferred to the anode through the bacterial membrane by electron transport proteins such as cytochromes. (Adapted from Lovley 2006a.)

must be paid to identifying and eliminating potential toxic compounds to bacteria from the material used in bio-electrode construction.

Bio-anode materials

Tanisho et al. (1989) used stainless steel (SS) plated with platinum black anodes in MFC, but because platinum was very costly, it was necessary to find a good and cheap electrode. SS was used as an anode in MFC by other researchers and they obtained useful results (Tanisho et al. 1989; Dumas et al. 2008a; Jadhav and Ghangrekar 2009). However, the most promising material was carbon (Logan et al. 2006) because it was a cheaper and easy to use material and at the same time its specific surface could be easily increased. Also, the inert nature of this material was expected to give longer life to the electrode as compared to other metals.

The current measured on EABs coated onto electrodes is proportional to their surface area as shown in Figure 4 (Liu and Li 2007). The specific surface area of graphite materials was continuously increased using carbon paper (Liu and Logan 2004), or other forms of carbon such as, carbon fibers (Chen et al. 2001; Mano et al. 2003a, b), graphite or carbon foam, graphite or carbon cloth (Cheng et al. 2006a, b; Cheng et al. 2007; Catal et al. 2008; Sukkasem et al. 2008), graphite or carbon felt (Chang et al. 2004; Biffinger et al. 2007, 2008b; Aelterman et al. 2008; Chae et al. 2008), graphite or carbon granules (Aelterman et al. 2006), activated graphite granules (He et al. 2006), graphite wool (Aelterman et al. 2008), bamboo charcoals (ter Heijne et al. 2008; Yang et al. 2009) with or without any activation treatment.

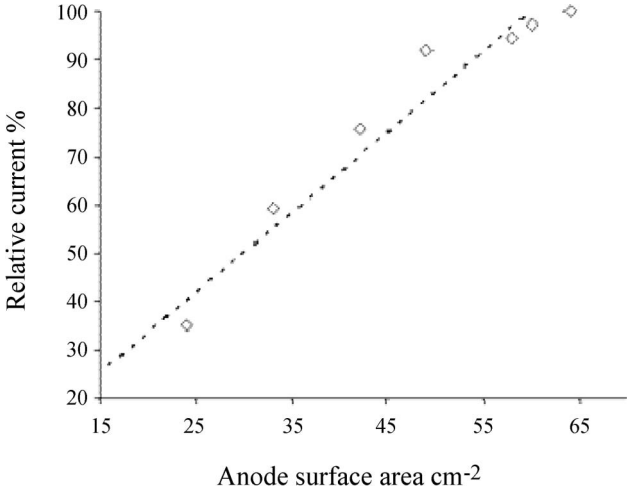

Figure 4. Relative current observed as a function of the anode surface area. (Adapted from Liu and Li 2007.)

Carbon nanotube (CN), flexible graphite (FG), and activated carbon (AC) were also used as anode material (Liang et al. 2008). The CN and FG gave higher power density, which was 31.8% and 22.6% higher than AC. The internal resistances of the cell with CN and AC were lower than FG. Recently, a unique nanostructured polyaniline (PANI)/mesoporous TiO_2 composite was synthesized and used as an anode in *E. coli* MFC and reported to give high power density (1495 mW m^{-2} of electrode), which was two fold higher than the other *E. coli* MFCs (Qiao et al. 2008).

Chemical modification of materials could be used to increase power generation by MFC. Ammonia treatment of a carbon cloth, used as bio-anode, substantially increased the surface charge of the electrode, and improved MFC performance. Increase in power to 1640 mW m^{-2} (96 W m^{-3} of reactor volume, ie W m^{-3}) using a phosphate buffer, and further to 1970 mW m^{-2} (115 W m^{-3}) using an ammonia-treated electrode was reported (Cheng and Logan 2007a). The combined effects of these two treatments boosted power production by 48% compared to air-cathode MFC without these modifications. In addition, the start up time of an MFC was reported to be reduced by 50%.

Bio-cathode materials

Relying on the extensive literature on microbial anodes, most of the work on biocathodes was conducted using 'classic' graphite as electrode materials. However, recently several new materials have been successfully used for biocathodes, such as, SS and nickel. Dumas et al. (2008b) tested plain SS following a procedure classically described in the literature, based on the catalysis of fumarate reduction by biofilms formed in pure culture of *G. sulfurreducens* (Figure 5). These authors demonstrated a 25-times increase in the current density compared to graphite under the same conditions. Maximum current densities >20 A m^{-2} were reported. Wang et al. (2008) replaced carbon-based materials by nickel foam as biocathodic matrix for electricity generation using autophytic autotrophic bacteria as cathodic catalyst (Wang et al. 2008). Based on electrical impedance spectroscopy analysis, the ohmic resistance of the nickel foam was only 2.1 Ω and they have shown a maximum power density of 4 W m^{-3}.

Application of EAB coated electrode in MFC for energy recovery

MFC working principle

The MFC is a device which converts chemical energy to electrical energy during substrate oxidation with the

help of microorganisms (Allen and Bennetto 1993; Kim et al. 1999a, b; Park and Zeikus 2000; Gil et al. 2003; Liu et al. 2004). The MFC is made up of two chambers, anode and cathode, separated by proton/cation exchange membrane (Figure 6). Electro-active microorganisms, either grown on the anode directly or in suspension, oxidize the substrate and produce electrons and protons in the anode chamber of the MFC. Electrons collected on the anode are transported to cathode by external circuit, powering the electrical device, and protons are transferred through the membrane internally. Thus, potential difference is produced between anode and cathode chambers due to dissimilar liquid solutions. Electrons and protons are consumed in the cathode compartment by utilizing dissolved oxygen. Formation of electro-active cells on both anode and cathode has a favorable effect on the performance of the MFC, enhancing its Coulombic efficiency and power output.

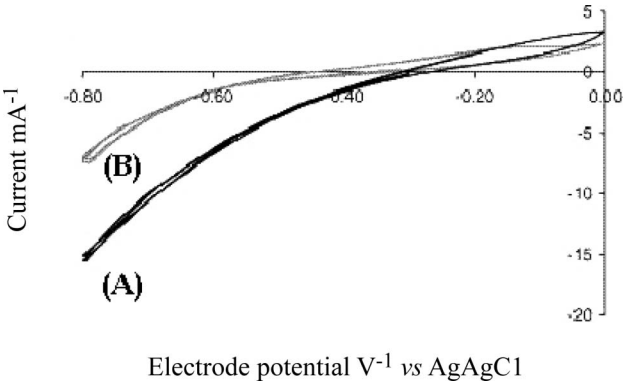

Figure 5. Cyclic voltammograms at 2 mV s^{-1} on an SS electrode with a biofilm of *G. sulfurreducens* sustaining 24.2 A m^{-2} (A) and on graphite electrode with a biofilm of *G. sulfurreducens* sustaining 0.83 A m^{-2} (B) for fumarate reduction. (Adapted from Dumas et al. 2008b.)

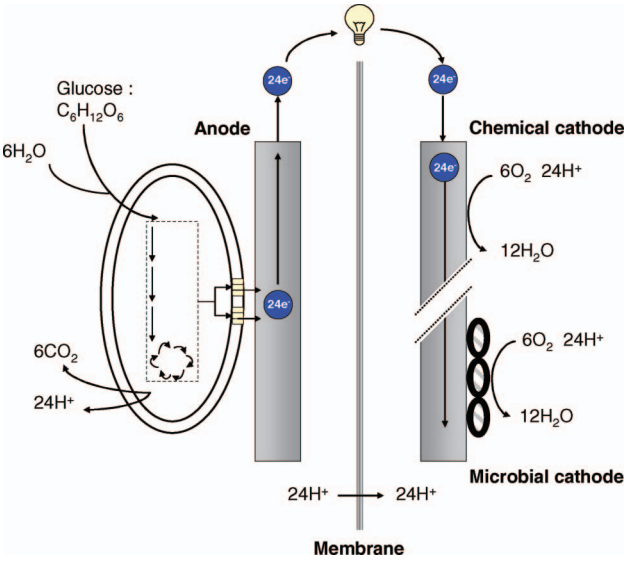

Figure 6. Schematic representation of an MFC. This type of fuel cell uses EABs as catalysts to oxidize or reduce the organic or inorganic matter and generate electric current. Most MFCs implement a microbial anode and a chemical cathode.

Marine MFCs

Marine MFCs consist typically of a graphite anode embedded in anaerobic marine sediments and connected through an electrical circuit (eg a marine scientific instrument or capacitor) to a cathode set-up in the overlying aerobic seawater as shown in Figure 7 (Reimers et al. 2001; Tender et al. 2002). A main feature of this marine MFC is sustainability, which is attributed to a constant supply of fuel and oxidant by environmental processes that are typically derived from settlement of dead phytoplankton and/or vegetative detritus, resulting in constant regeneration of its microbial electrode catalysts, and the ability of these microbial catalysts to exchange electrons with their electrodes without electron-transfer mediators.

MFCs implemented in marine sediments with plain graphite electrodes have sustained power density around 20 mW m^{-2} of anode surface area over 4 months, with maximal values up to 28 mW m^{-2} (Tender et al. 2002). A similar laboratory system has provided about 10 mW m^{-2} for 240 days with a graphite fiber anode and cathode (Reimers et al. 2001). Using carbon brush cathode, the power density reached 34 mW m^{-2} over a 125-day period (Reimers et al. 2006). The highest power densities that have been provided by field marine MFCs have been reached with graphite anodes modified with charge transfer mediators (Lowy et al. 2006). 1,6-disulfonic acid (AQDS)-modified graphite and graphite-ceramic containing Mn^{2+} and Ni^{2+} have given maximum of 98 mW m^{-2} at a cell voltage of 0.24 V, and 105 mW

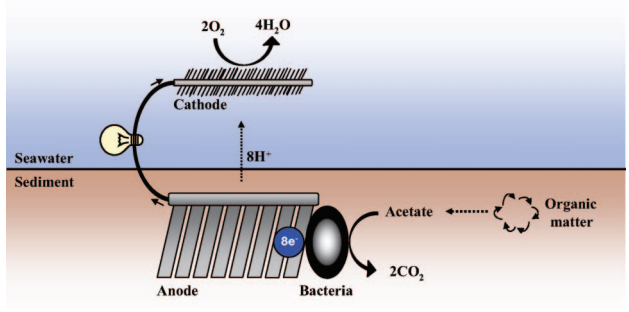

Figure 7. Schematic representation of the marine MFC principle, also called benthic MFC.

m^{-2} at 0.35 V, respectively. This performance has been matched by a cell implemented with an SS cathode supplying around 100 mW m^{-2} for 45 days (Dumas et al. 2008c).

Marine MFCs have been investigated largely with the view to operating low-power consuming marine instrumentation, such as oceanographic sensors, monitoring devices and telemetry systems as already achieved by Shantaram et al. (2005) in fresh water. Tender et al. (2008) described the first demonstration of a marine MFC as a practical alternative to batteries for a low-power consuming application. To generate enough power for the telemetry system, energy produced by the MFC was stored in a capacitor and used in short bursts when needed. The specific application reported was a meteorological buoy (*ca* 18 mW average consumption) that measured air and water temperature, pressure, relative humidity, and that was configured for real-time telemetry of data. Their prototype sustained 36 mW power equivalent of *ca* 26 alkaline D-cells per year at 25°C.

Wastewater MFCs

MFCs have been operated successfully on a variety of substrates, from pure chemicals to complex wastes (Liu et al. 2004). Using a wide variety of substrates such as glucose, acetate, butyrate (Liu et al. 2005a), cysteine (Logan et al. 2005), proteins (Heilmann and Logan 2006), and lignocellulose (Rismani-Yazdi et al. 2007), as well as complex substrates such as domestic wastewater (Cheng et al. 2006c; Ghangrekar and Shinde 2008), swine manure slurry (Min et al. 2005), landfill leachate (You et al. 2006), and meatpacking wastewater (Heilmann and Logan 2006), successful power generation from MFC was reported. The published results demonstrated the utility of MFC as a power generation device with simultaneous wastewater treatment.

The power recovery reported varied considerably due to different configurations of the reactor used, materials used for the electrodes, and the anodophilic species used, apart from operating conditions. Individual cell voltages >0.7 V have been reported by many researchers with power densities varying in the wide range between 20 mW m^{-2} and $>$ 2000 mW m^{-2} of anode surface area, depending upon the configuration of MFC, the substrate, anodophilic microorganisms, and operating conditions used. A maximum power density of 5850 mW m^{-2} was reported in a two-chamber MFC using complex electrodes system consisting of a tungsten carbide containing anode and graphite foil coated with pyrolyzed iron (III) phthalocyanine/polytetra-fluoroethylene as a cathode (Rosenbaum et al. 2006).

MFCs were reported to produce power per unit volume ranging between 2060 mW m^{-3} and 102,000 mW m^{-3} depending upon the configuration of the MFC, the type of anodophilic culture and the substrate used (Kim et al. 2007). With a miniature MFC using *S. oneidensis* and lactate as feed, power production per unit volume of 500,000 mW m^{-3} was reported by Ringeisen et al. (2006). It is evident from the literature that, smaller size MFCs are likely to generate more power by effectively harvesting the electron than larger MFCs. Although, the power density reported for an MFC using wastewater as a feed were lower compared to classical fuel cells, this drawback should be resolved soon with continuous research efforts focused on identifying the electroactive cells with multiple substrate acceptance.

Farm field MFCs

Another interesting application of MFCs was on farm fields to generate power while cultivating plants. An electrical current was generated through the *in situ* oxidation of rhizodeposits from living rice plants. The electrical power output of a sediment MFC was found to be a factor of 7 higher in the presence of actively growing plants. This process offered the potential of light-driven power generation from living plants in a nondestructive way. Sustainable power productions up to 330 W ha^{-1} could be attributed to the oxidation of the plant-derived compounds (De Schamphelaire et al. 2008). The success of such operation should be a real breakthrough by making power available to the farms located in the remote places, which are not connected with the power network as in the case of many developing countries.

MFC performance

The performance of MFCs is not only influenced by the environment used to generate the EAB but also by the nature of the substrate oxidized by the EAB. Except for experiments 'on site' where the substrate is naturally available, MFCs are fuelled by a wide variety of substrates because in theory any biodegradable organic material can be used. These substrates may be single compounds commonly used for the growth of microorganisms such as glucose, acetate, sucrose, ethanol, or butyrate. However, complex substrates such as artificial effluent containing glutamate, hospital effluent, substrates containing easily degradable compounds such as amino acids and proteins have been used increasingly. MFCs inoculated with effluent or sludge generated power about several hundred to several thousand milliwatts per square meter higher than the values obtained with the marine MFCs (a few tens of milliwatts per square meter). Generally, MFCs powered by single substrates such as glucose or acetate

give a higher performance than those fed with complex substrates (Logan and Regan 2006).

The performance of MFCs also varies with technological advances. The most significant change intended to reduce the internal resistance of the system was by removal of the proton exchange membrane (PEM). A basic MFC inoculated with domestic wastewater and fuelled with glucose furnished a power density of 262 mW m^{-2}, which got increased to 494 mW m^{-2} after removing the PEM (Liu and Logan 2004). Optimization of the cathodic reaction has also hardly been studied. Generally, the reduction of oxygen was carried out in an aqueous solution with electrodes containing platinum as a catalyst. The improvement made to this reaction was to increase oxygen concentration in the cathodic compartment (Gil et al. 2003; He et al. 2007), including the use of an air-cathode wherein the reduction occurred directly in the gas phase (Liu and Logan 2004; Liu et al. 2004; Min et al. 2005; Cheng et al. 2006a). Recently, a new cell design allowing an acidic pH in the cathodic compartment improved the performance of the reduction of the oxygen (Erable and Bergel 2009).

Better performances were also observed when oxygen reduction was replaced by the reduction of ferricyanide into ferrocyanide (Rabaey et al. 2003, 2004; Oh et al. 2004; Pham et al. 2004; ter Heijne et al. 2006). Thus, power densities of 860 mW m^{-2} (4500 mA m^{-2}) (ter Heijne et al. 2006), 3600 mW m^{-2} (2883 mA m^{-2}) (Rabaey et al. 2003), and 4310 mW m^{-2} (6491 mA m^{-2}) (Rabaey et al. 2004) were obtained. However, MFCs using ferricyanide/ferrocyanide couple at the cathode have been limited to laboratory-scale studies because this reaction was not sustainable and these products were toxic to the environment and to people. Other strategies such as temperature control were investigated to increase the performance of MFCs (Liu et al. 2005a). A power of 1030 mW m^{-2} (9030 mA m^{-2}) was observed with an MFC maintained in a water bath at 55°C (Jong et al. 2006), while these systems typically operated at room temperature. Increasing the mass transfer by mixing and/or by bubbling a gas could also increase the power supplied by MFCs (Du et al. 2007). However, these remedies were often economically discouraged because the use of such additional systems (eg pumps, potentiostats, compressors, and heaters) often consumed more energy than the MFC could produce (Du et al. 2007).

In conclusion, there is no consistency in (a) the type of reactors, (b) the material of reactors and electrodes, (c) the geometry and the size of reactors, (d) the specific surface area of the electrode required with respect to reactor volume, and for dual chamber MFCs the volumetric ratio of anode and cathode chamber, (e) the anodophilic species type, concentration and sludge age. Also, the results presented by various researchers were expressed in different units (W m^{-2} or W m^{-3}) and are difficult to compare. Harmonization of the units appears to be necessary to properly compare all the values reported in the literature.

Applications of EABs for remediation processes

Wastewater treatment

Researchers are showing increasing interest in the application of MFC as a wastewater treatment process for removal of oxidizable matter from industrial as well as domestic wastewaters. Under different operating conditions and based on the various MFC types used, chemical oxygen demand (COD) removal ranging from 60 to >90% is reported in the literature. Successful application of MFC is described in the literature for the treatment of synthetic wastewaters containing pure chemicals to complex industrial wastewaters. While treating starch, peptone, and fish extract wastewater in the single chamber air cathode MFC, COD removal efficiency of 93–95% has been reported (Shimoyama et al. 2008). But, in a single chamber MFC treating leachate, only a 53% reduction in biochemical oxygen demand (BOD) was observed at hydraulic retention time (HRT) as low as 4.7 h (Greenman et al. 2009). At an HRT of 33 h, Liu et al. (2004) reported 80% COD removal in the treatment of domestic wastewaters and at an HRT of 12 h the COD removal was in the range 50–70% (Liu et al. 2004). Min and Logan (2004) reported COD removal up to 72% in the MFC during treatment of domestic wastewater. While treating swine wastewater, COD removal up to 91% was reported in the MFC at an HRT of 72 h (Min et al. 2005).

The published results demonstrate the utility of MFC as a wastewater treatment system. With this success, after properly addressing scale-up issues, MFC is expected to replace presently used secondary wastewater treatment processes soon. Application of MFC to wastewater treatment is particularly attractive for small volume and low organic matter concentration wastewater, such as sewage. For such wastewaters, application of anaerobic processes for methane recovery is not economical because of the loss of a major fraction of the methane produced, along with the reactor effluent in soluble form, hence reducing energy recovery. For such wastewaters, MFCs have a bright future due to the ability to recover energy directly in the form of electricity.

Biocathode driven remediation

A large number of recent studies have made rapid advances concerning the bio-anodes of MFCs but only

a few papers have dealt with biocathodes. Designing new materials or developing new microbial systems for cathodes remains an open challenge in the field of fuel cells or other biofilm-driven processes. Microbial metabolism in biocathodes may be utilized to remove nitrogen during wastewater treatment by reducing nitrate compounds (ie denitrification). In addition, with the ability of accepting electrons and protons as an oxidant, the cathode chamber of MFCs could harvest considerable energy from pollutants. The potential of biocathode using a graphite electrode could be close to that observed for abiotic oxygen reduction at conventional Pt-coated electrodes in the cathode chamber. A four-fold increase in the current output was achieved using a biocatalyzed cathode, compared with the non-catalyzed graphite cathode (Freguia et al. 2008). Therefore, biocathodes could prove to be an efficient catalyst for oxygen reduction, and a feasible alternative to abiotic systems in wastewater-fed MFCs.

Although, graphite can be used for the cathode, the high current density obtained by using SS proved that it was a remarkable material to support biocathodes (Dumas et al. 2008b). Previous studies have demonstrated that the energy-efficient treatment of wastewater is one of the most promising applications of MFCs. However, biocathodes should also serve to produce useful products or remove specific pollutants.

Biocathodes for nitrogen removal

Holmes et al. (2004) found that microbes on the cathode of a benthic MFC participated in biological reactions, such as ammonia oxidation and denitrification. Gregory et al. (2004) proved that the cathode served as the sole electron donor for nitrate reduction to nitrite in a half cell reactor assisted by a potentiostat. In their study, nitrate was only reduced in the presence of *G. metallireducens* or an adapted enrichment culture, indicating the involvement of bacteria in nitrate reduction. Nitrogen is often present in problematic concentrations in wastewaters, and therefore needs to be removed along with the organic matter. This nitrogen is difficult to remove if the organic substrate concentrations are too low to sustain full denitrification. When using a cathode as electron donor, the oxidation of the organic donor is physically separated from the reduction process. Clauwaert et al. (2007) have described an MFC that linked up the oxidation of acetate with the reduction of nitrate at the cathode. The system even generated useful power up to 8 W m^{-3} of cathodic effluent and removed up to 0.146 kg equivalent nitrogen per cubic meter per day (Clauwaert et al. 2007). Selection of a proper MFC configuration, increased the removal rates up to 0.41 kg equivalent nitrogen per cubic meter of cathodic compartment per day, while producing a maximum power output of 35 W m^{-3} (Virdis et al. 2008). The three chamber configuration adopted by the authors is depicted in Figure 8. The anode effluent was aerated externally for nitrification to occur and nitrified effluents were admitted to the cathode for denitrification to occur.

Cathodes for metal oxidation

Metals can be extracted from minerals when they are used as terminal electron acceptors in the cathode. The microbial reduction of Fe(III), Mn(IV), or Cr(VI) was investigated in MFC applications. The feasibility of Fe(II) compounds as cathodic reactants was studied. *Thiobacillus ferrooxidans* was added to the cathodic compartment in order to biologically convert Fe(II) into Fe(III) releasing electrons to the oxygen. Fe(III) was spontaneously reduced accepting electrons from electrodes *via* electrochemical reactions. Rhoads et al. (2005) employed the cycle of Mn(IV) reduction in which MnO_2 was reduced abiotically on the cathode. This cycle involved manganese oxidizing bacteria (MOB), such as *Leptothrix discophora*, which oxidized in a second step Mn^{2+} to MnO_2 by releasing two electrons to oxygen. The current density delivered by using biomineralized manganese oxides as the cathodic reactant was almost two orders of magnitude higher than that delivered using oxygen. When the electrodes were connected by a 50 Ω resistor, the fuel cell delivered power density about 125 mW m^{-2}.

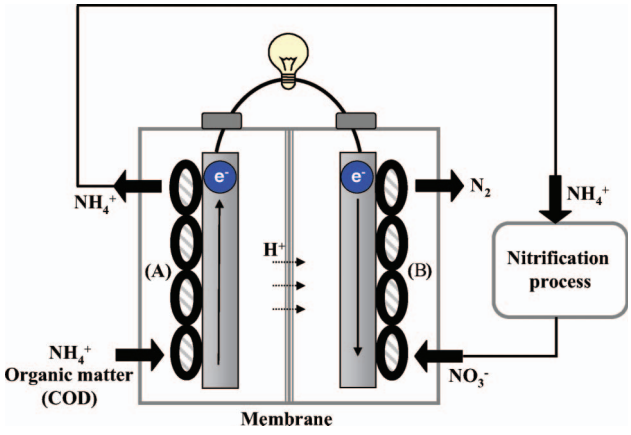

Figure 8. 'Nitrogen loop system' for the combined removal of carbon and nitrogen (Virdis et al. 2008). Organic matter containing carbon is oxidized on the bioanode (A). The resulting effluent is then treated in an external aerobic nitrification process converting ammonium into nitrate. The wastewater is finally diverted to the biocathode where nitrate reduction occurs (B).

In acidic environments Cr^{6+} has a higher oxidation potential (1.33 V *vs* SHE) than oxygen (1.23 V) and hexacynoferrate (0.36 V) (Oh et al. 2004). So it may be deduced that potassium dichromate is a more favorable electron acceptor theoretically. Li et al. (2008), using graphite paper as the cathode electrode, demonstrated successful chromium removal with a maximum power density of 1600 W m^{-2} at a Columbic efficiency of 12%. In addition, 99.5% Cr^{6+} and 66.2% total Cr were removed through reduction of $Cr_2O_7^{2-}$ to Cr_2O_3 precipitating on the surface of this graphite cathode (Li et al. 2008).

Applications of EABs as biosensors

In practice, the operation of wastewater treatment plants is a delicate task because of the lack of fast, reliable sensors for measurement of different substrates and nutrients such as ammonium, nitrate, and phosphate, and organic loading. Any improvement in process control can increase the capacity of a wastewater treatment plant and at the same time operational costs and investments can be reduced (Larsen et al. 2000). In any typical wastewater treatment plant, process control includes control of the pumps, dosage of chemicals, control of the nutrient removal process and also control of oxygen levels. Using different kinds of sensors (eg biosensors and electrochemical sensors) the performance and efficiency of wastewater treatment plants can be maximized.

In order to obtain *in situ* monitoring, useful information about microbial respiration can be obtained by using an EAB coated onto an electrode like a quantitative sensor. Information such as, the concentration of the monitored substance and the presence of some toxic species, can be made available online because the respiratory chain of microorganisms involves electron transfer to one electrode (anode), thus generating an electrical current. This can be easily measured and used to indicate a direct metabolic rate for specific respiratory processes. All respiratory processes involve stoichiometric coupling of electron donor oxidation with electrons transferred to the electron acceptor, therefore, the current generated is also a measure of the available substrate concentration (Tront et al. 2008a). Using an electrode as an electron acceptor, biosensors produce electrical current which is used like a respiration signal by microbial population (Tront et al. 2008b).

Nitrate/nitrite

Nitrite (NO_2^-) is involved in almost all biological nitrogen (N) transformations and has a central position in global nitrogen. In ecosystems, NO_2^- is of interest because of its toxicity to all organisms (microorganisms, higher organisms). For this reason, it is very important to control the NO_2^- concentration in natural bulk water and also in freshwater systems. Larsen et al. (2000) designed and used a micro-scale biosensor for *in situ* monitoring of nitrate/nitrite concentrations. This kind of biosensor was based on diffusion of nitrate/nitrite inside the concentrated mass of bacteria through a membrane. Bacteria converted these ions into nitrous oxide which could be electrochemically detected. The electrical signal provided by this type of biosensor was in very close agreement with the nitrate/nitrite concentrations. The response time of the applied biosensors was <30 s, and the detection limit was <50 μg of total nitrogen per liter ($\mu g\ N\ l^{-1}$), which were more than sufficient for its utility as a biosensor during wastewater treatment.

Nielsen et al. (2004) used the same design of nitrate/nitrite biosensor. In an attempt to choose the right EAB, they conducted experiments in order to see how the operating conditions affect sensor stability. Based on the experimental results, they concluded that in this kind of sensor two different bacteria can be used (*Stenotrophomonas nitritireducens* and *Alcaligenes faecalis*). With this sensor they obtained a linear correlation between current and concentration up to 1.5 mM (Figure 9) and they successfully used this sensor for long time online measurements in wastewater.

Glucose

Kumlanghan et al. (2007) developed a novel MFC design based on a single chamber reactor in order to use it like a BOD sensor. The EAB used in the anodic compartment was renewed for each sample by replacing the old anaerobic consortium with a new one. In

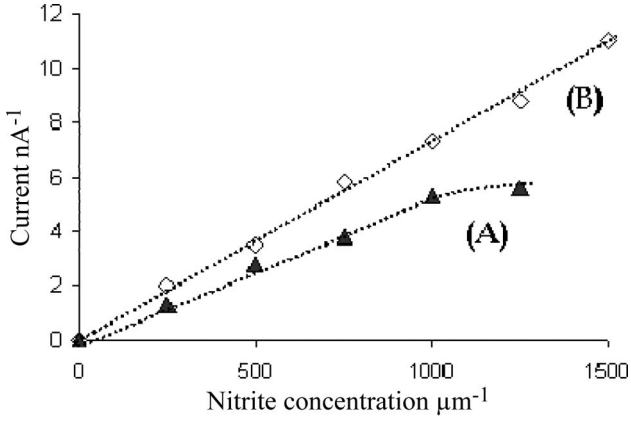

Figure 9. Nitrite biosensor. Calibration curves using *S. nitritireducens* at 20°C (A) and 30°C (B). (Adapted from Larsen et al. 2000.)

all experiments the temperature in anodic compartment was maintained at an optimum value of 37°C. Using this sensor configuration they obtained a linear response for glucose, when the concentration was increased from 0 to 25 g l^{-1}. The detection limit for this sensor was reported to be 0.025 g l^{-1}. One other advantage of this sensor was the shorter measuring time required (between 3 and 5 min). This type of sensor could also be used to estimate the quantities of biodegradable organic matter present in the wastewater.

Biological oxygen demand

Measurement of biological oxygen demand (BOD) involves an empirical test in which the relative oxygen requirements of different waters (wastewater, polluted waters, industrial effluents) are determined using standardized laboratory procedures. The result of this test is obtained after a long period (after 5 days as per the American standard and after 7 days as per the Swedish standard) (Liu and Mattiasson 2002). In an operating wastewater treatment plant it is necessary to have the result of the BOD test in a short time, so as to exercise close control over the process. Application of biosensors can offer a solution for quick measurement of BOD. The EAB-type BOD biosensor was developed as a microbial film sandwiched between a porous cellulose membrane and a gas-permeable membrane as the biological recognition element. This microbial film consisted of immobilized microbial populations that could bio-oxidize organic substrates. The response was usually represented by a change in the concentration of dissolved oxygen (DO) or by other phenomena such as light emission. Physical transducers were used to monitor this process. The result of this measurement was represented by a change in an electrical or optical signal. The signal obtained in this way was then amplified and correlated to the content of biodegradable material measured (Liu and Mattiasson 2002).

Chang et al. (2005) also performed studies on MFC to be used like a BOD sensor. They correlated the BOD concentration with the current measured on the bioanode surface as demonstrated in Figure 10. One of the major problems of this BOD sensor was related to the presence of nitrogen or oxygen in the system. In this case the signal delivered by the BOD sensor was lowered due to competition between anode respiration and some alternative electron-accepting process.

Hydrogen production

One possible solution to meet hydrogen production demands is represented by the possibility of obtaining biohydrogen using novel technology such as microbial electrolysis cells (MECs) (Logan et al. 2008) in which the organic matter is transformed in hydrogen by microbial electrolysis. In a classical MFC, during current generation, bacteria oxidize organic matter on the anode producing protons and electrons which flow to the cathode using a different pathway and are consumed in a reduction mechanism. If the presence of reducible compounds at the cathode is suppressed current production is not spontaneous. On applying a small voltage between the anode and cathode, the current generation is forced, resulting in hydrogen production at the cathodic side due to proton reduction (Figure 11).

Due to a thermodynamic barrier many organic compounds are unsuitable for use as a substrate in

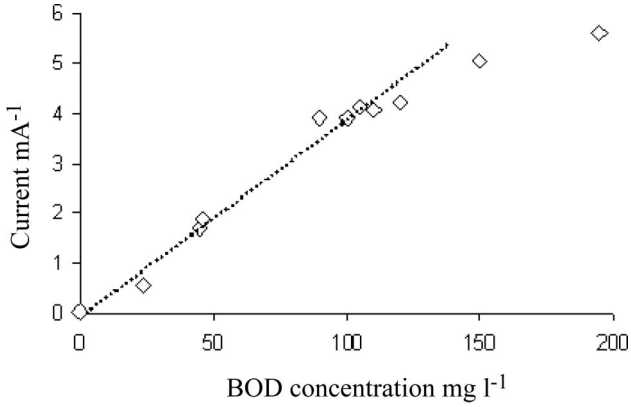

Figure 10. Relationship between BOD value and steady-state current. (Adapted from Chang et al. 2004.)

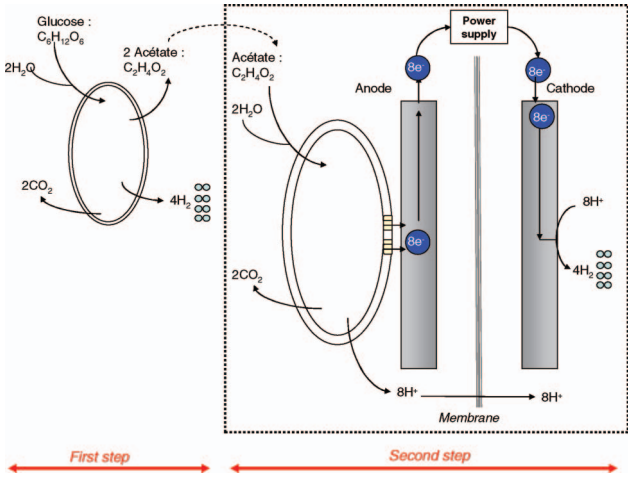

Figure 11. Schematic representation of the process of hydrogen production based on MFC technology involving a proton exchange membrane. A voltage is applied to the system by means of a power supply.

fermentative hydrogen production (Logan et al. 2008). However, they can be used in MECs, because in this case a small external voltage is applied in order to surmount the thermodynamic barrier (Sun et al. 2008; Holladay et al. 2009).

Rozendal et al. (2006) employed acetate as a model compound to produce biohydrogen using EABs. Under standard conditions biohydrogen production using acetate as a substrate requires an external energy input of 104.6 kJ mol^{-1}. Based on this, hydrogen production using EABs needs a theoretical external voltage of 0.14 V *versus* NHE. In practice, because of the internal cell losses and because EABs consume a part of the substrate for growth and maintenance, more than the theoretical value is required for this process (Rozendal et al. 2006). Data presented in literature suggest that the applied potential was typically around 0.2–0.3 V (Liu et al. 2005b; Call and Logan 2008) which was much lower in comparison with the voltage applied in classical hydrogen production through water electrolysis (Rozendal et al. 2006; Holladay et al. 2009).

Rozendal et al. (2006) obtained 0.02 m^3 hydrogen per cubic meter of reactor volume per day (m^3 m^{-3} day^{-1}) with an efficiency of 53% using a dual chamber microbial electrolysis cell at an applied voltage of 0.5 V. After optimization, they increased the hydrogen production rate about 10 m^3 m^{-3} day^{-1} with an efficiency $>90\%$ with a relatively low voltage (0.3–0.4 V). A year later, Cheng and Logan (2007b) proved that this new technology was efficient and suitable for biohydrogen production using complex organic molecules such as glucose, cellulose, and different volatile acids as the substrate, with a maximum efficiency of 99% and an external voltage of 0.8 V.

Membranes used to separate the anode and cathode chambers were associated with potential losses inside the MECs. In order to increase the cell efficiency by reducing the internal losses, Hu et al. (2008) developed and tested a single chamber membrane free MECs for biohydrogen production. Using a mixed culture and an applied voltage of 0.6 V they achieved a maximum hydrogen production rate of 0.63 m^3 m^{-3} day^{-1}. Call and Logan (2008) reported a high hydrogen recovery rate using a membrane-less single chamber MEC and showed a linear correlation between voltage and H_2 generation (Figure 12). In comparison with typical water electrolyzers they found that the MECs involved in their study had higher energy efficiencies (400%) on the same electricity energy input basis. Hydrogen production using membrane-less MECs required only 0.9 kWh m^{-3} hydrogen in comparison with classical water electrolysis which typically requires 5.6 kWh m^{-3} hydrogen (Call and Logan 2008).

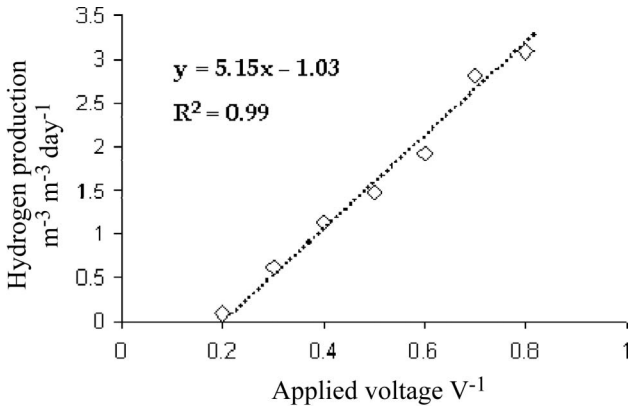

Figure 12. Hydrogen production rate as a function of applied voltage. (Adapted from Call and Logan 2008.)

Conclusions

The exploitation of microbial metabolism to catalyze or to control electrochemical reactions, which naturally occur in the environment or which are created by man, should lead to major technology breakthroughs. This will show the way for the development of new products and processes in many fields such as bio-energy, bioremediation, biofouling prevention, biosynthesis processes, bio-corrosion mitigation, and biosensor design.

A multidisciplinary approach and intensive researches are in progress exploring the possibilities of EAB applications and improvement in the process design and configuration to suit the desired applications. Implementation of such bioelectrochemical systems is not straightforward because certain microbiological, technological, and economic challenges need to be resolved. Replacement of the membrane with alternative cheaper material or proper reactor design could improve the economic feasibility of the bioelectrochemical processes. Controlling the anode potential on the first day after establishment of EABs limits the microbial competition on the anode and encourages electrogenic bacterial growth (Erable and Bergel 2009; Erable et al. 2009a, b). This helps in maximizing power harvesting from the microbial electrode without losing the substrate for competitive metabolisms (methanogenesis, for example). More studies with 'real' wastewaters and not 'synthetic' wastewaters are also required to evaluate the true potential of the technology.

Bioelectrochemical technology holds great promise toward sustainable energy through both direct generation (MFC) or hydrogen production (bio-electrolyzer). The application of this novel technology as a renewable energy source will help in minimizing the threat to the mankind and making the earth a better place in which to live. Also, development of this technology as

biosensors will facilitate faster determination of many parameters, and make real time online monitoring practically possible. Although, many possible applications of EABs are described in the literature, many more new applications may be discovered in future.

References

Aelterman P, Rabaey K, Pham HT, Boon N, Verstraete W. 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environ Sci Technol 40:3388–3394.

Aelterman P, Versichele M, Marzorati M, Boon N, Verstraete W. 2008. Loading rate and external resistance control the electricity generation of microbial fuel cells with different three-dimensional anodes. Bioresour Technol 99:8895–8902.

Allen RM, Bennetto HP. 1993. Microbial fuel cells: electricity production from carbohydrates. Appl Biochem Biotechnol 39:27–40.

Biffinger JC, Byrd JN, Dudley BL, Ringeisen BR. 2008b. Oxygen exposure promotes fuel diversity for *Shewanella oneidensis* microbial fuel cells. Biosens Bioelectron 23:820–826.

Biffinger JC, Pietron J, Ray R, Little B, Ringeisen BR. 2007. A biofilm enhanced miniature microbial fuel cell using *Shewanella oneidensis* DSP10 and oxygen reduction cathodes. Biosens Bioelectron 22:1672–1679.

Biffinger JC, Pietron J, Bretschger O, Nadeau LJ, Johnson GR, Williams CC, Nealson KH, Ringeisen BR. 2008a. The influence of acidity on microbial fuel cells containing *Shewanella oneidensis*. Biosens Bioelectron 24:906–911.

Bond DR, Lovley DR. 2005. Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. Appl Environ Microbiol 71:2186–2189.

Bond DR, Holmes DE, Tender LM, Lovley DR. 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295:483–485.

Call D, Logan BE. 2008. Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. Environ Sci Technol 42:3401–3406.

Catal T, Li K, Bermek H, Liu H. 2008. Electricity production from twelve monosaccharides using microbial fuel cells. J Power Sources 175:196–200.

Chae KJ, Choi MJ, Lee J, Ajayi FF, Kim IS. 2008. Biohydrogen production *via* biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial community analysis. Int J Hydrogen Energy 33:5184–5192.

Chang IS, Moon H, Jang JK, Kim BH. 2005. Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. Biosens Bioelectron 20:1856–1859.

Chang IS, Jang JK, Gil GC, Kim M, Kim HJ, Cho BW, Kim BH. 2004. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. Biosens Bioelectron 19:607–613.

Chen T, Calabrese Barton S, Binyamin G, Gao Z, Zhang Y, Kim HH, Heller A. 2001. A miniature biofuel cell. J Am Chem Soc 123:8630–8631.

Cheng S, Logan BE. 2007a. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochem Comm 9:492–496.

Cheng S, Logan BE. 2007b. Sustainable and efficient biohydrogen production *via* electrohydrogenesis. Proc Natl Acad Sci 104:18871–18873.

Cheng S, Liu H, Logan BE. 2006a. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. Electrochem Comm 8:489–494.

Cheng S, Liu H, Logan BE. 2006b. Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environ Sci Technol 40:364–369.

Cheng S, Liu H, Logan BE. 2006c. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. Environ Sci Technol 40:2426–2432.

Cheng S, Dempsey BA, Logan BE. 2007. Electricity generation from synthetic acid-mine drainage (AMD) water using fuel cell technologies. Environ Sci Technol. 41:8149–8153.

Clauwaert P, Rabaey K, Aelterman P, De Schamphelaire L, Pham TH, Boeckx P, Boon N, Verstraete W. 2007. Biological denitrification in microbial fuel cells. Environ Sci Technol 41:3354–3360.

Cohen B. 1931. The bacterial culture as an electrical half-cell. J Bacteriology 21:18–19.

De Schamphelaire L, Van Den Bossche L, Hai SD, Hofte M, Boon N, Rabaey K, Verstraete W. 2008. Microbial fuel cells generating electricity from rhizodeposits of rice plants. Environ Sci Technol 42:3053–3058.

Du Z, Li H, Gu T. 2007. A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25:464–482.

Dumas C, Basseguy R, Bergel A. 2008a. Electrochemical activity of *Geobacter sulfurreducens* biofilms on stainless steel anodes. Electrochim Acta 53:5235–5241.

Dumas C, Basseguy R, Bergel A. 2008b. Microbial electrocatalysis with *Geobacter sulfurreducens* biofilm on stainless steel cathodes. Electrochim Acta 53:2494–2500.

Dumas C, Mollica A, Feron D, Basseguy R, Etcheverry L, Bergel A. 2008c. Checking graphite and stainless anodes with an experimental model of marine microbial fuel cell. Bioresource Technol 99:8887–8894.

Erable B, Bergel A. 2009. First air-tolerant effective stainless steel microbial anode obtained from a natural marine biofilm. Bioresource Technol 100:3302–3307.

Erable B, Etcheverry L, Bergel A. 2009a. Increased power from a two-chamber microbial fuel cell with a low-pH air-cathode compartment. Electrochem Comm 11:619–622.

Erable B, Roncato MA, Achouak W, Bergel A. 2009b. Sampling natural biofilms: a new route to build efficient microbial anodes. Environ Sci Technol 43:3194–3199.

Freguia S, Rabaey K, Yuan Z, Keller J. 2008. Sequential anode–cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells. Water Res 42:1387–1396.

Ghangrekar MM, Shinde VB. 2008. Simultaneous sewage treatment and electricity generation in membrane-less microbial fuel cell. Water Sci Technol 58:37–43.

Gil GC, Chang IS, Kim BH, Kim M, Jang JK, Park HS, Kim HJ. 2003. Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens Bioelectron 18:327–334.

Greenman J, Galvez A, Giusti L, Ieropoulos I. 2009. Electricity from landfill leachate using microbial fuel cells: comparison with a biological aerated filter. Enzyme Microb Technol 44:112–119.

Gregory KB, Bond DR, Lovley DR. 2004. Graphite electrodes as electron donors for anaerobic respiration. Environ Microbiol 6:596–604.

He Z, Shao H, Angenent LT. 2007. Increased power production from a sediment microbial fuel cell with a rotating cathode. Biosens Bioelectron 22:3252–3255.
He Z, Wagner N, Minteer SD, Angenent LT. 2006. An upflow microbial fuel cell with an interior cathode: assessment of the internal resistance by impedance spectroscopy. Environ Sci Technol 40:5212–5217.
Heilmann J, Logan BE. 2006. Production of electricity from proteins using a microbial fuel cell. Water Environ Res 78:531–537.
Hernandez ME, Kappler A, Newman DK. 2004. Phenazines and other redox-active antibiotics promote microbial mineral reduction. Appl Environ Microbiol 70:921–928.
Holladay JD, Hu J, King DL, Wang Y. 2009. An overview of hydrogen production technologies. Catal Today 139:244–260.
Holmes DE, Bond DR, O'Neil RA, Reimers CE, Tender LR, Lovley DR. 2004. Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. Microb Ecol 48:178–190.
Hu H, Fan Y, Liu H. 2008. Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Res 42:4172–4178.
Jadhav GS, Ghangrekar MM. 2009. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresource Technol 100:717–723.
Jong BC, Kim BH, Chang IS, Liew PWY, Choo YF, Kang GS. 2006. Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. Environ Sci Technol 40:6449–6454.
Kim BH, Chang IS, Gadd GM. 2007. Challenges in microbial fuel cell development and operation. Appl Microbiol Biotechnol 76:485–494.
Kim HJ, Hyun MS, Chang IS, Kim BH. 1999b. A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*. J Microbiol Biotechnol 9:365–367.
Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH. 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb Technol 30:145–152.
Kim BH, Ikeda T, Park HS, Kim HJ, Hyun MS, Kano K, Takagi K, Tatsumi H. 1999a. Electrochemical activity of an Fe(III)-reducing bacterium, *Shewanella putrefaciens* IR-1, in the presence of alternative electron acceptors. Biotechnol Tech 13:475–478.
Kim GT, Hyun MS, Chang IS, Kim HJ, Park HS, Kim BH, Kim SD, Wimpenny JWT, Weightman AJ. 2005. Dissimilatory Fe(III) reduction by an electrochemically active lactic acid bacterium phylogenetically related to *Enterococcus gallinarum* isolated from submerged soil. J Appl Microbiol 99:978–987.
Kumlanghan A, Liu J, Thavarungkul P, Kanatharana P, Mattiasson B. 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. Biosens Bioelectron 22:2939–2944.
Larsen LH, Damgaard LR, Kjær T, Stenstrøm T, Lyngaard-Jensen A, Revsbech NP. 2000. Fast responding biosensor for on-line determination of nitrate/nitrite in activated sludge. Water Res 34:2463–2468.
Li Z, Zhang X, Lei L. 2008. Electricity production during the treatment of real electroplating wastewater containing Cr6+ using microbial fuel cell. Process Biochem 43:1352–1358.
Liang P, Fan MZ, Cao XX, Huang X, Peng YM, Wang S, Gong QM, Liang J. 2008. Electricity generation by the microbial fuel cells using carbon nanotube as the anode. Huanjing Kexue/Environ Sci 29:2356–2360.
Liu J, Mattiasson B. 2002. Microbial BOD sensors for wastewater analysis. Water Res 36:3786–3802.
Liu H, Logan BE. 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38:4040–4046.
Liu H, Ramnarayanan R, Logan BE. 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38:2281–2285.
Liu H, Cheng S, Logan BE. 2005a. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environ Sci Technol 39:5488–5493.
Liu H, Cheng S, Logan BE. 2005b. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 39:658–662.
Liu ZD, Li HR. 2007. Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell. Biochem Eng J 36:209–214.
Logan BE, Regan JM. 2006. Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 14:512–518.
Logan BE, Murano C, Scott K, Gray ND, Head IM. 2005. Electricity generation from cysteine in a microbial fuel cell. Water Res 39:942–952.
Logan BE, Call D, Cheng S, Hamelers HVM, Sleutels THJA, Jeremiasse AW, Rozendal RA. 2008. Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ Sci Technol 42:8630–8640.
Logan BE, Hamelers B, Rozendal R, Schrorder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K. 2006. Microbial fuel cells: methodology and technology. Environ Sci Technol 40:5181–5192.
Lovley DR. 2006a. Bug juice: harvesting electricity with microorganisms. Nature Rev Microbiol 4:497–508.
Lovley DR. 2006b. Microbial fuel cells: novel microbial physiologies and engineering approaches. Current Opin Biotechnol 17:327–332.
Lovley DR. 2006c. Microbial energizers: fuel cells that keep on going. Microbe 1:323–329.
Lowy DA, Tender LM, Zeikus JG, Park DH, Lovley DR. 2006. Harvesting energy from the marine sediment–water interface. II. Kinetic activity of anode materials. Biosens Bioelectron 21:2058–2063.
Mano N, Mao F, Heller A. 2003a. Characteristics of a miniature compartment-less glucose–O_2 biofuel cell and its operation in a living plant. J Am Chem Soc 125:6588–6594.
Mano N, Mao F, Shin W, Chen T, Heller A. 2003b. A miniature biofuel cell operating at 0.78 V. Chem Comm 9:518–519.
Min B, Logan BE. 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 38:5809–5814.
Min B, Kim J, Oh S, Regan JM, Logan BE. 2005. Electricity generation from swine wastewater using microbial fuel cells. Water Res 39:4961–4968.
Nielsen M, Larsen LH, Jetten MSM, Revsbech NP. 2004. Bacterium-based NO_2-biosensor for environmental applications. Appl Environ Microbiol 70:6551–6558.

Niessen J, Schroder U, Scholz F. 2004a. Exploiting complex carbohydrates for microbial electricity generation – a bacterial fuel cell operating on starch. Electrochem Comm 6:955–958.

Niessen J, Schroder U, Scholtz F. 2004b. Exploiting complex carbohydrates for microbial electricity generation – a bacterial fuel cell operating on starch. Elecrochem Comm 6:955–958.

Niessen J, Schroder U, Rosenbaum M, Scholz F. 2004c. Fluorinated polyanilines as superior materials for electrocatalytic anodes in bacterial fuel cells. Electrochem Comm 6:571–575.

Niessen J, Harnisch F, Rosenbaum M, Schroder U, Scholz F. 2006. Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation. Electrochem Comm 8:869–873.

Oh S, Min B, Logan BE. 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. Environ Sci Technol 38:4900–4904.

Park DH, Zeikus JG. 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. Appl Environ Microbiol 66:1292–1297.

Park HS, Kim BH, Kim HS, Kim HJ, Kim GT, Kim M, Chang IS, Park YK, Chang HI. 2001. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell. Anaerobe 7:297–306.

Pham CA, Jung SJ, Phung NT, Lee J, Chang IS, Kim BH, Yi H, Chun J. 2003. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. FEMS Microbiol Lett 223:129–134.

Pham TH, Jang JK, Chang IS, Kim BH. 2004. Improvement of cathode reaction of a mediatorless microbial fuel cell. J Microbiol Biotechnol 14:324–329.

Potter MC. 1910. Electrical effects accompanying the decomposition of organic compounds. Proc R Soc B84:260–276.

Qiao Y, Bao SJ, Li CM, Cui XQ, Lu ZS, Guo J. 2008. Nanostructured polyaniline/titanium dioxide composite anode for microbial fuel cells. ACS Nano 2:113–119.

Rabaey K, Lissens G, Siciliano SD, Verstraete W. 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett 25:1531–1535.

Rabaey K, Boon N, Hofte M, Verstraete W. 2005. Microbial phenazine production enhances electron transfer in biofuel cells. Environ Sci Technol 39:3401–3408.

Rabaey K, Boon N, Siciliano SD, Verhaege M, Verstraete W. 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl Environ Microbiol 70:5373–5382.

Rabaey K, Rodriguez J, Blackall LL, Keller J, Gross P, Batstone D, Verstraete W, Nealson KH. 2007. Microbial ecology meets electrochemistry: electricity-driven and driving communities. ISME J 1:9–18.

Reimers CE, Tender LM, Fertig S, Wang W. 2001. Harvesting energy from the marine sediment–water interface. Environ Sci Technol 35:192–195.

Reimers CE, Girguis P, Stecher HA, III, Tender LM, Ryckelynck N, Whaling P. 2006. Microbial fuel cell energy from an ocean cold seep. Geobiology 4:123–136.

Rhoads A, Beyenal H, Lewandowski Z. 2005. Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. Environ Sci Technol 39:4666–4671.

Ringeisen BR, Henderson E, Wu PK, Pietron J, Ray R, Little B, Biffinger JC, Jones-Meehan JM. 2006. High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. Environ Sci Technol 40:2629–2634.

Rismani-Yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH. 2007. Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. Biotechnol Bioeng 97:1398–1407.

Rosenbaum M, Zhao F, Quaas M, Wulff H, Schroder U, Scholz F. 2007. Evaluation of catalytic properties of tungsten carbide for the anode of microbial fuelmcells. Appl Catal B Environ 74:261–269.

Rosenbaum MZF, Schroder U, Scholz F. 2006. Interfacing electrocatalysis and biocatalysis with tungsten carbide: a high-performance, noble-metal-free microbial fuel cell. Angewandte Chemie Int Ed 45:6658–6661.

Rozendal RA, Hamelers HVM, Euverink GJW, Metz SJ, Buisman CJN. 2006. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int J Hydrogen Energy 31:1632–1640.

Ryckelynck N, Stecher HA, III, Reimers CE. 2005. Understanding the anodic mechanism of a seafloor fuel cell: interactions between geochemistry and microbial activity. Biogeochem 76:113–139.

Schroder U. 2007. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Phys Chem Chem Phys 9:2619–2629.

Shantaram A, Beyenal H, Veluchamy RRA, Lewandowski Z. 2005. Wireless sensors powered by microbial fuel cells. Environ Sci Technol 39:5037–5042.

Shimoyama T, Komukai S, Yamazawa A, Ueno Y, Logan BE, Watanabe K. 2008. Electricity generation from model organic wastewater in a cassette-electrode microbial fuel cell. Appl Microbiol Biotechnol 80:325–330.

Shukla AK, Suresh P, Berchmans S, Rajendran A. 2004. Biological fuel cells and their applications. Curr Sci 87:455–468.

Sukkasem C, Xu S, Park S, Boonsawang P, Liu H. 2008. Effect of nitrate on the performance of single chamber air cathode microbial fuel cells. Water Res 2008 42:4743–4750.

Sun M, Sheng GP, Zhang L, Xia CR, Mu ZX, Liu XW, Wang HL, Yu HQ, Qi R, Yu T, et al. 2008. An MEC–MFC-coupled system for biohydrogen production from acetate. Environ Sci Technol 42:8095–8100.

Tanisho S, Kamiya N, Wakao N. 1989. Microbial fuel cell using *Enterobacter aerogenes*. Bioelectrochem Bioenerget 21:25–32.

Tender LM, Reimers CE, Stecher HA, III, Holmes DE, Bond DR, Lowy DA, Pilobello K, Fertig SJ, Lovley DR. 2002. Harnessing microbially generated power on the seafloor. Nature Biotechnol 20:821–825.

Tender LM, Gray SA, Groveman E, Lowy DA, Kauffman P, Melhado J, Tyce RC, Flynn D, Petrecca R, Dobarro J. 2008. The first demonstration of a microbial fuel cell as a viable power supply: powering a meteorological buoy. J Power Sources 179:571–575.

ter Heijne A, Hamelers HVM, Saakes M, Buisman CJN. 2008. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochim Acta 53:5697–5703.

ter Heijne A, Hamelers HVM, De Wilde V, Rozendal RA, Buisman CJN. 2006. A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. Environ Sci Technol 40:5200–5205.

Thurston CF, Bennetto HP, Delaney GM. 1985. 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 131:1393–1401.

Tront JM, Fortner JD, Plotze M, Hughes JB, Puzrin AM. 2008a. Microbial fuel cell biosensor for *in situ* assessment of microbial activity. Biosens Bioelectron 24:586–590.

Tront JM, Fortner JD, Plotze M, Hughes JB, Puzrin AM. 2008b. Microbial fuel cell technology for measurement of microbial respiration of lactate as an example of bioremediation amendment. Biotechnol Lett 30:1385–1390.

Virdis B, Rabaey K, Yuan Z, Keller J. 2008. Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res 42:3013–3024.

Wang X, Feng Y, Wang E, Li C. 2008. Electricity generation using nickel foam solely as biocathodic material in a two chambered microbial fuel cell. J Biotechnol 136 (Suppl 1):S662.

Yang S, Jia B, Liu H. 2009. Effects of the Pt loading side and cathode-biofilm on the performance of a membrane-less and single-chamber microbial fuel cell. Bioresource Technol 100:1197–1202.

You SJ, Zhao QL, Jiang JQ, Zhang JN, Zhao SQ. 2006. Sustainable approach for leachate treatment: electricity generation in microbial fuel cell. J Environ Sci Health Part A 41:2721–2734.