

Electrochemically Active Bacteria (EAB) and Mediator-Less Microbial Fuel Cells

CHANG, IN SEOP^{1,2}, HYUNSOO MOON^{1,3}, ORIANNA BRETSCHGER⁴, JAE KYUNG JANG¹, HO IL PARK¹, KENNETH H. NEALSON⁴, AND BYUNG HONG KIM^{1*}

¹Bioelectrochemistry Laboratory, Water Environment & Remediation Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea

²Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

³Department of Biochemical Engineering, Yanbian University of Science and Technology, Beishan St. Yanji City, Jilin Province 133000, China

⁴Department of Earth Science, University of Southern California, Los Angeles, CA 90089, U.S.A.

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Microbial fuel cells (MFCs) are devices that convert chemical energy into the form of electricity through the catalytic activity of microorganisms [1, 2, 5, 6, 22, 37, 42, 86, 90, 91, 95, 97]. Many different bacteria can produce a modicum of electricity in an MFC if a mediator (electron shuttle) is used to facilitate the transfer of electrons between the bacterial cell and the working electrode (anode) in the system [22, 86, 90]. However, such mediated fuel cells (Fig. 1A) tend to be inefficient, expensive, and produce low levels of power.

Recently, a number of bacteria (dubbed “electrochemically active bacteria, or EAB) have been found to possess the ability to transfer electrons from oxidized fuel (substrate) to a working electrode without a mediator [37, 42, 43], making it possible to establish mediator-less MFCs (Fig. 1B). Dissimilatory metal reducing bacteria, which are capable of the reduction of solid metal oxides have been particularly noteworthy with regard to this ability, and to date, many species of EAB are known, including *Aeromonas hydrophilia* [21], *Clostridium Butyricum* [77], *Desulfohalobium propionicum* [30], *Enterococcus gallinarum* [41], *Geobacter sulfurreducens* [8], *Rhodospirillum rubrum* [13] and *Shewanella putrefaciens* [37, 42]. Perhaps not surprisingly, the EAB, which are defined as those bacteria capable of current production in the absence of a mediator, are also capable of electron exchange activity with a working electrode in

cyclic voltammetry tests [37]. To this end, it was shown that anaerobically grown intact cells of *S. putrefaciens* were electrochemically active both in cyclic voltammetry experiments [37, 42] and in a mediator-less MFC [42]. Furthermore, in the MFC system, growth and metabolism of *S. putrefaciens* was dependent on the presence of the active anode - in essence, it served as the electron acceptor for growth and metabolism.

Given that the MFC anode can act as the sole electron acceptor for *Shewanella* and other EAB, it was reasoned, and subsequently demonstrated, that the anode could be used to isolate other EAB from nature, simply by enrichment experiments in anoxic environments. Using this approach, single strains, as well as bacterial consortia have been enriched on anodes of MFCs [38, 47, 80], some of which have remained active in our laboratory for many years with minimal maintenance. Thus, it is clear that EAB and mediator-less MFC systems are closely linked, and both will be discussed throughout this review, with the goal of assessing the state of the art in MFC systems, assessing the challenges that must be overcome, and looking to the possible applications of this exciting technology.

Life and Electron Flow

From one point of view, life can be viewed as a series of electron exchanges and electron flow: life extracts energy in the form of electrons from any of a variety of substrates, and uses the energy of these electrons to create biologically useful energy. Almost all life works in a similar way, using the electron flow to “charge” a biological membrane, and then using this charged membrane either directly (i.e., to power transport, or motility), or indirectly (i.e., to use the membrane potential to drive the synthesis of biologically

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*Corresponding author
Phone: 82-2-958-5831; Fax: 82-2-958-5839;
E-mail: bhkim@kist.re.kr

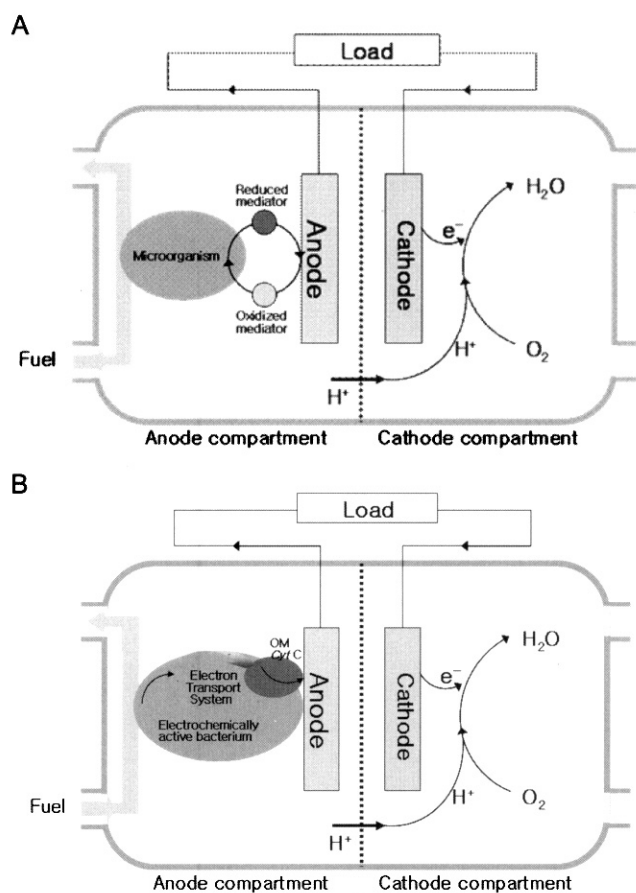


Fig. 1. Two microbial fuel cell systems.
A. Mediated-MFC, B. Mediator-less MFC.

useful energy in the form of ATP). Inherent in this mechanism is the notion that electrons must flow in order to drive the mechanism that charges the membrane. This is usually, but not always, the pumping of protons across the membrane that establishes a pH/electrical gradient called the proton motive force. If electrons flow, protons are pumped, and the “biological capacitor” is charged.

Thus, for an aerobic organism, if oxygen is taken away, electron flow stops, and the organism suffocates. For many bacteria, other electron acceptors (nitrate, sulfate, thiosulfate, fumarate, metal oxides, and CO_2 , etc.) can be used instead of oxygen (Table 1), although, as shown, considerably less energy is obtained when other electron acceptors are used. In the discussion that follows, it will become obvious to the reader that it should be possible to make totally anaerobic MFCs that utilize other electron acceptors than oxygen. However, the current yields of these MFCs will be predictably lower, and in proportion to the expected electron flow.

It is this fundamental property of living systems that allow us to capture some of the respiratory energy in the form of electrons, and use this to directly create electricity.

Table 1. Mediator-less microbial fuel cell operation using metal reducing bacteria.

Metal reducing bacteria	Reference
<i>Aeromonas hydrophila</i>	[21]
<i>Clostridium butyricum</i>	[68]
<i>Desulfobulbus propionicus</i>	[30]
<i>Enterococcus gallinarum</i>	[41]
<i>Geobacter sulfurreducens</i>	[8]
<i>Rhodospirillum rubrum</i>	[13]
<i>Shewanella putrefaciens</i> (<i>Shewanella oneidensis</i>)	[37, 42]

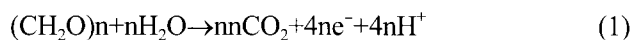
Using a fuel cell (as described below), it is possible to interact with the microbe in a way that, in the absence of an electron acceptor, it views the anode of the MFC as an acceptable alternative. Thus the flow of electrons can proceed, and the organism can continue to metabolize. The anode is effectively a substitute for respiration. The issue of importance for the microbe, and for the MFC designer and user, however, is that electrons must flow, and when they do the microbes and the MFC function properly.

Fuel Cells

Chemical fuel cells are devices that convert chemical energy directly into electricity. Chemical conversion occurs at an anaerobic electrode, where a catalyst is used to speed up the oxidation of a specific fuel (H_2 , CH_4 , CH_3OH). Electrons from this oxidation are passed through an exterior load to the cathode, while protons diffuse across a proton-permeable membrane to the cathode. The protons and electrons are then used to reduce oxygen to water, via a catalyzed reaction on the cathode electrode.

Microbial Fuel Cells

MFCs share many similarities with chemical fuel cells. The fuel is oxidized in an anaerobic anode chamber, but rather than a elemental catalyst, living bacteria are used to catalyze fuel oxidation. As the microorganisms oxidize electron donors (fuels), the resulting electrons are transferred to the anode, which is separated from the cathode by a cation exchange membrane (anode oxidation reaction, equation 1);



The electrons from the anode are then transferred across an external circuit to an aerated cathode. This transfer is induced by the potential difference between the anode and cathode electrodes. An oxygen reduction reaction takes place at the cathode utilizing the transferred electrons, protons and molecular oxygen. (Cathode reduction reaction, equation 2);



Under the closed circuit condition, the available power of MFC (P_{cell}) depends on cell voltage (V_{cell}) and cell

current (I_{cell}) (equation 3). The latter factors are linked by the applied resistance using Ohm's law in which R represents resistance (equation 4). The ideal cell voltage is the difference in the potentials of the oxidant and fuel compounds ($E_{\text{ox}} - E_{\text{fuel}}$), however irreversible losses (overpotential, η) are incurred as a result of kinetic limitations (equation 5) [36].

The kinetics of an electrochemical system can be diffusion controlled (mass transport control) and/or activation controlled (charge transfer control). Diffusion control arises when the concentration and flow of fuel or oxidant becomes limited. Charge transfer control dominates when there are high resistance values of electrode materials, electrolyte and/or cation exchange membrane. Charge transfer and mass transport are also affected by the dimensions and geometry of the membrane electrode assembly (MEA) in the MFC [36]. Ultimately, both types of kinetic limitations can decrease power generation. The specific factors affecting MFC performance have been well documented [24, 40].

$$P_{\text{cell}} = V_{\text{cell}} \times I_{\text{cell}} \quad (3)$$

$$V_{\text{cell}} = I_{\text{cell}} \times R \quad (4)$$

$$V_{\text{cell}} = (E_{\text{ox}} - E_{\text{fuel}}) - \eta \quad (5)$$

The MFCs have some notable differences from chemical fuel cells, all of which relate to the fact that they employ living, rather than chemical, catalysts. The bacteria, as a rule are not limited to one substrate, but exhibit a range of different compounds they can utilize for fuel. Furthermore, they are inexpensive, and have the ability to reproduce and to self-repair. Third, they can be custom-made and optimized to a wide variety of different physical and chemical conditions, using the robustness of the microbial cells and communities.

Mediated- MFCs

Most microbes are electrochemically inactive because the proteins associated with electron transport are contained within the cell membrane. Mediators can be used to facilitate the transfer of electrons from the microbial membrane to the MFC electrode for these microbes [1, 17–22, 45, 86, 95, 96]. Mediators are preferentially reduced during the metabolic oxidation of organic materials, and the reduced form of the mediator is then re-oxidized at the working electrode (anode), which is maintained at a sufficiently high electric potential (Fig. 1a).

Nearly any bacterium can be used to generate current in a mediated MFC, and a wide range of mediators are known, including thionine [5, 45], viologens [1, 86], methylene blue [86], 2-hydroxy-1,4-napthoquinone [1, 96] and other hydrophobic compounds [1, 2, 5, 6, 22, 86, 90, 91, 95, 97]. In general, these mediators are toxic to microorganisms in the high concentrations required for

good current generation. This, coupled with the facts that the mediators can be expensive, and that high concentrations are required (i.e., scaling up to large volumes is prohibitively expensive), make the commercialization of mediated MFCs unlikely.

Mediator-less MFCs

A mediator-less MFC was first demonstrated by Kim *et al.* [37], in which anaerobically grown cell suspensions of the metal reducing bacterium, *S. putrefaciens* produced a quasi-reversible cyclic voltammogram (CV) with a reductive peak at -0.32 V and an oxidation peak at 0.03 V against a saturated calomel electrode (SCE). The apparent redox potential was -0.15 V against a SCE, which is about 0.05 V against a normal hydrogen electrode (NHE) [37]. No redox peaks were observed in the CV test of the aerobically grown *S. putrefaciens* cells. These results suggested that, as long as anaerobic conditions were maintained, direct electron exchange should be possible using *S. putrefaciens*.

Direct electron transfer from cells of *S. putrefaciens* to an electrode was also tested using an MFC-type electrochemical device and lactate as the fuel [37, 42]. When the circuit was not connected between anode and cathode electrodes (i.e., open circuit), the cells did not consume lactate [43], while under the closed circuit condition, *S. putrefaciens* consumed lactate and generated electricity [37, 42]. To our knowledge, this was the first experimental verification of a mediator-less MFC operation. Based on both CV and MFC results, Kim *et al.* [37] also proposed that the electrochemical activity of bacterial cell suspensions was due to the presence of electrochemically active

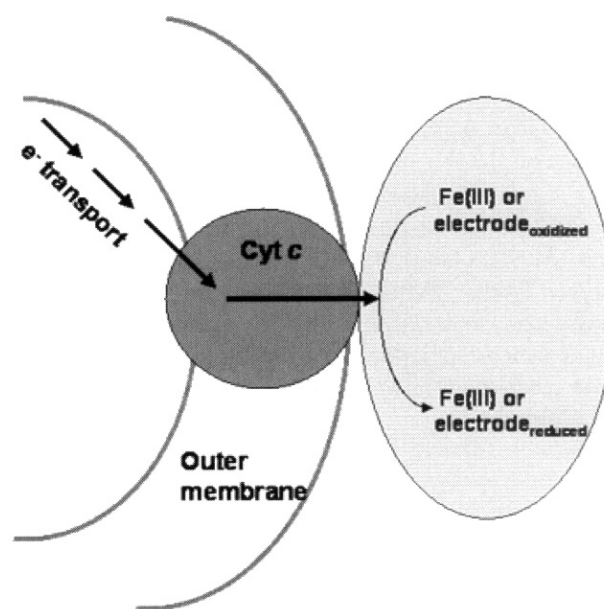


Fig. 2. Proposed electron transport by outer membrane cytochrome to oxidized metal or electrode.

compounds on the cell surface such as cytochromes, as depicted in (Fig. 2). With regard to this, it is notable that, based on genome analyses, *S. oneidensis* strain MR-1 has 43 possible cytochrome *c* genes on the whole genome sequence [28, 60], while another electrochemically active DMRB (dissimilatory metal reducing bacteria), *Geobacter sulfurreducens* has more than 100 [66, 59]. It is also notable for both organisms, that a number of these *c*-type cytochromes are located on the outer membrane of the organism, well suited for their role in extracellular electron exchange.

Electrochemically Active Bacteria and Mediator-less MFCs

Recently, a number of microorganisms have been isolated based on their ability to use oxidized metal ions including Fe(III) and Mn(IV) as their electron acceptors [62, 84]. In anoxic environments, most microbial electron acceptors such as nitrate, sulfate and carbon dioxide are essentially water soluble both before and after reduction. However, environmental Fe(III) and Mn(IV) minerals, which are used as electron acceptors by the (DMRB), usually exist as insoluble (solid) oxyhydroxide minerals at neutral pH levels. Thus the DMRB face the problem of communicating electrochemically with solid substrates that are by definition unavailable to the membrane-bound enzymes usually involved in respiration. To overcome this obstacle, the DMRB employ several strategies including: 1) the utilization of naturally existing electron shuttles (such as humic substances) as mediators [9, 56, 57]; 2) the production of their own mediators [71]; and, 3) the use of external (outer membrane) components to effect directly electron transfer to the metals.

Multiheme *c*-type cytochromes are thought to play a major role in this unique electron transport system (ETS) and outer membrane cytochromes are believed to be the contact point to externally located Fe(III) and Mn(IV)-bearing minerals. Among the DMRB, *Shewanella oneidensis* (formerly *S. putrefaciens*) [68, 78, 70] and *Geobacter sulfurreducens* [10, 46, 66, 59] are found to localize some of the *c*-type cytochromes on the outer membrane, rendering the cells electrochemically active in mediator-less MFC systems [7, 8, 37, 42]. Electrochemical activities have been observed in other DMRB such as *Aeromonas hydrophila* [21], *Rhodospirillum rubrum* [13], *Desulfobulbus propionicus* [30] as well as some fermentative microorganisms such as *Clostridium butyricum* [77] and *Enterococcus gallinarum* [41] (Table 1).

ENRICHMENT OF ELECTROCHEMICALLY ACTIVE BACTERIA

Enrichment

As noted above, early experiments with *Shewanella putrefaciens* showed that under conditions where electron

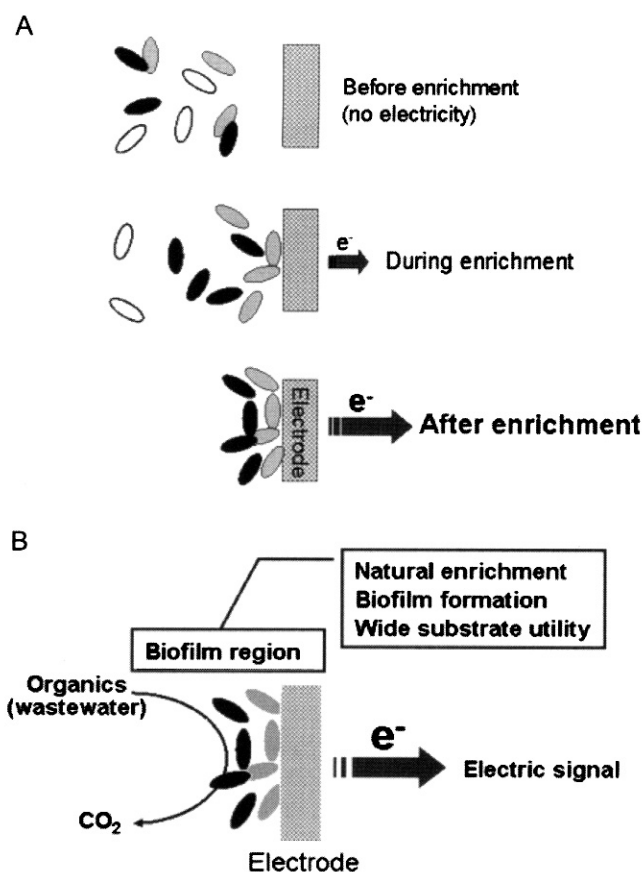


Fig. 3. Enrichment steps (A) and enriched electrode (B) in microbial fuel cell.

acceptors were not present, its metabolism was stimulated by the presence of the MFC anode [37]. Based on these simple observations, it was posited that the anode itself should offer a pathway for the isolation of EAB via anaerobic enrichment. Thus, “Enrichment” using an MFC system was initially proposed [31] as a tool for selecting electrochemically active consortia (Fig. 3). This technique was explored using sludge collected from a corn-processing wastewater treatment plant as the inoculum. The anode compartment of the MFC was inoculated with the sludge and fed with wastewater from the same source. The cathode compartment contained a buffer under continuous aeration and the two compartments were separated by a cation exchange membrane (i.e., it was an MFC!) [38]. An open circuit potential (OCP) of around 0.6 V was observed immediately after the inoculation. When the MFC was connected through a 10 Ω resistance the potential dropped to 20 mV, which corresponds to a current of 20 μ A. When an aliquot of anode solution was replaced with new wastewater (fuel), the current increased. This current increase was concomitant with COD reduction. Repeated wastewater replacements were coupled with current increase up to 1.2 mA (Fig. 4). Similar patterns were also observed

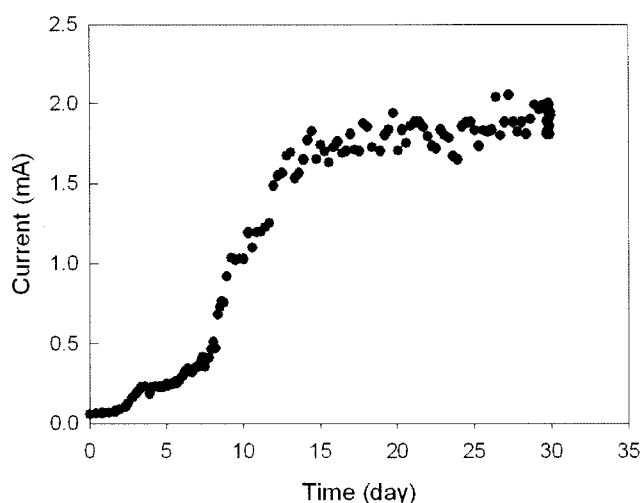


Fig. 4. Current changes during the enrichment process.

in all MFCs which had been inoculated with activated sludge or anaerobic digester sludge [38].

These results suggest that EAB propagated in the MFC and that wastewater and/or sludge contained EAB at low concentrations at the initial stage of enrichment (Fig. 3A top). The electricity production might be the result of electrons transferred to the electrode by EAB after they metabolized electron donor(s) in the wastewater in the absence of any other electron acceptors (Fig. 3A middle). Because it is thought that the electrode reducing step is an energy conserving microbial respiration process [7, 38], EAB could be enriched during MFC operation. If the enrichment step is stabilized, the electron donor(s) consumption in the wastewater could be metabolized faster than that of previous step (Fig. 3A bottom).

Furthermore, enrichment cultures were made with various nutritional characteristics, including copiotrophic cultures enriched with artificial wastewater containing acetate [47], propionate or artificial wastewater containing glucose and glutamate [20], and oligotrophic cultures with artificial wastewater [34] or river water [34, 80].

Fermentable substrates showed more diverse bacterial populations in the MFC than that of non-fermentable substrates (such as acetate) based on 16S rDNA analyses. This could be due to nutritional characteristics of electron donors. Non-EAB may also be present in the electrochemically active MFC consortia. These microbes may play a critical role in generating electron donor(s) for the EAB as a result of their metabolism.

Microscopy

An enriched MFC was operated for about a year using wastewater from a corn-processing factory and the electrodes from this system were used for microscopic observation [38]. Microscopic observations showed clear evidence of biofilm formation on the anode electrode. A thick biofilm

was observed on the surface of the electrode, indicating that the EAB colonized on the electrode surface to use the electrode as an electron acceptor. It is well documented that the microbial community in a biofilm is more stable than a free-living community [89], which would explain why the biofilm that developed on the electrode was better than artificially immobilized microbial cells for the performance of the MFC. In addition to the electrode biofilm, surface electron microscopy revealed microbial clumps loosely attached to the electrode [38]. These clumps were not observed in MFCs enriched with a non-fermentable fuel, such as acetate [47]. Confocal laser scanning microscopic observations showed that the biofilm and bacterial clumps consisted of micro-colonies of Gram positive and negative bacteria [38]. Thus, it is hypothesized that the microbial clumps are inhabited by fermentative bacteria that ferment the complex sugar substrates (complex electron donors) into products that the microbes in the biofilm oxidize and then transfer the resulting electrons to the electrode (Fig. 3B).

Culture-Independent Molecular Biological Analyses

DNA was extracted from the MFC anode communities, enriched, and evaluated using various nutritional characteristics for molecular ecological analyses. The enrichments utilized different substrates including artificial wastewater (AWW) containing glucose and glutamate and AWW containing acetate. Denaturing gradient gel electrophoresis (DGGE) revealed that the bacterial population in the MFC was different from that in the original inoculum [38, 47, 80], and the dominant microbial populations in the MFCs were dependent on the substrate used.

Analyses of the 16S rDNA sequences also showed that the dominant bacterial classes differed based on the fuel used. In most cases, Gram negative bacteria were dominant. The MFC enriched with AWW containing glucose and glutamate featured *Gammaproteobacteria* (36.5%) and *Firmicutes* (27.0%) [20]. The MFC enriched with AWW containing acetate had less diversity with approximately 70% of the bacterial population as *Deltaproteobacteria* and 17.3% *Gammaproteobacteria* [47]. In contrast, when MFCs were enriched with corn-processing wastewater [38], the majority of the bacterial clones amplified from the latter were *Betaproteobacteria* (40.9%) and *Alphaproteobacteria* (27.2%), and devoid of *Deltaproteobacteria* and *Gammaproteobacteria*.

Interestingly, high percentage of *Firmicutes* (>20%) was found in an acetate-enriched MFC in a marine environment [8] but a low population found in a fresh water environment [47].

Different microbial populations have been reported between oligotrophic MFCs enriched and run with AWW containing fuel at 10 mg/l as the BOD, and MFCs enriched and run with river water with a BOD of around 5 mg/l

[80]. *Alphaproteobacteria* were identified as the majority population in the MFCs enriched with a low concentration of AWW, followed by *Betaproteobacteria*. The high population of *Alphaproteobacteria* was apparently due to the enrichment of nitrilotriacetate (NTA) oxidizing *Aminobacter aminovorans* [80], where NTA was used as the chelating agent to dissolve metal ions. Meanwhile, *Betaproteobacteria* were the major population in the river water-enriched MFCs [80]. Thus, it would appear that *Betaproteobacteria* are the major electrochemically active bacteria under oligotrophic conditions.

It has been shown that *Deltaproteobacteria* are the major bacterial population in acetate-enriched MFCs [8, 47]. However, *Deltaproteobacteria* were not found in the MFCs enriched with corn-processing wastewater [38] and AWW with a low fuel concentration [80]. Instead, these MFCs were found to contain a high population of *Betaproteobacteria*, which was also the dominant bacterial population in the MFCs enriched with river water. *Rhodospirillum rubrum*, a member of *Betaproteobacteria*, can reduce an electrode using glucose as fuel [13].

Large populations of *Gammaproteobacteria* were also found in most of the MFCs analyzed. Members of *Gammaproteobacteria*, known to be electrochemically active, include *Shewanella putrefaciens* [42] and *Aeromonas hydrophila* [21]. Thus, electrochemical activity may be a widely distributed characteristic among bacteria, including *Firmicutes* [77]. Consequently, novel methods should be developed to characterize the role of *Gammaproteobacteria* in the natural ecosystem and confirm the hypothesis that electrode reduction is a form of anaerobic respiration.

ELECTRON TRANSPORT SYSTEM

One of the big challenges facing the MFC field will be the delineation of the mechanisms involved with electron transfer, and the optimization of this process. To this point in time, there is little information involving the components of any of the EAB in the process of current generation. This being said, both biochemical and genomic work are proceeding, and it is expected to have major revelations with regard to electron transport components and processes in the near future.

Several metabolic inhibitors have been used to establish the electron transport chain of the electrochemically active bacterial population in an MFC [38, 47]. The current increased slightly with the addition of terminal oxidase inhibitors such as cyanide and azide, but antimycin A (inhibitor of cytochrome *b*) had no effect on the current generation. Meanwhile, the current generation was inhibited in the presence of rotenone (inhibitor of NADH:CoQ oxidoreductase), 2-heptyl-4-hydroquinolone-N-oxide (HQNO, inhibitor of Quinones), dicyclohexylcarbodiimide (DCCD, ATPase inhibitor), and 2, 4-dinitrophenol (DNP, uncoupler).

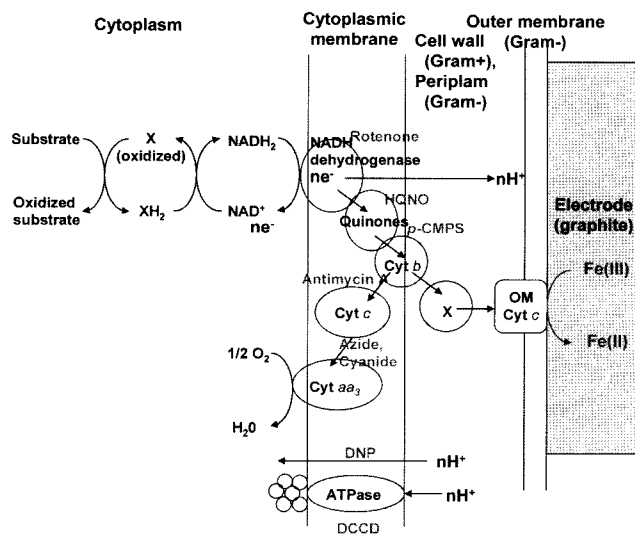


Fig. 5. Proposed electron transport system in an MFC enriched with acetate.

These results indicate that the microbial electron transport for MFC electrode reduction shares an early part of the electron transport chain (NADH dehydrogenase and coenzyme Q) with aerobic bacteria, although cytochrome *b* and terminal oxidases do not appear to be involved [38, 40, 47]. These results were used to propose electron transport chain for EAB in MFC shown in Fig. 5 [40].

DESIGN AND OPTIMIZATION OF MFCs

Electricity generation by MFC systems is determined by several physical and biochemical factors [24, 84], as shown in Fig. 6, and noted below:

- 1) Microbial oxidation of the substrate (fuel),
- 2) Electron transfer from the microbe to the anode,
- 3) Electron transfer through the circuit including external resistance,
- 4) Proton diffusion from the anode compartment to the cathode compartment
- 5) Oxygen supply and reduction at the cathode (cathode reaction),
- 6) Oxygen diffusion into the anode compartment through the membrane.

Each step is affected by MFC constituents and configuration. Among the steps, proton transfer and cathode reaction were found to be the most important steps in the electricity generation affecting the feasibility of MFC applications [24, 32, 62, 79]. Much effort has been devoted to improvement of the proton transfer and cathode reaction. However, as there are many designs and potential uses for MFCs, one can readily expect that any of the above processes could be a rate-limiting step. Surely, as one rate-limiting process is enhanced, another must take its place.

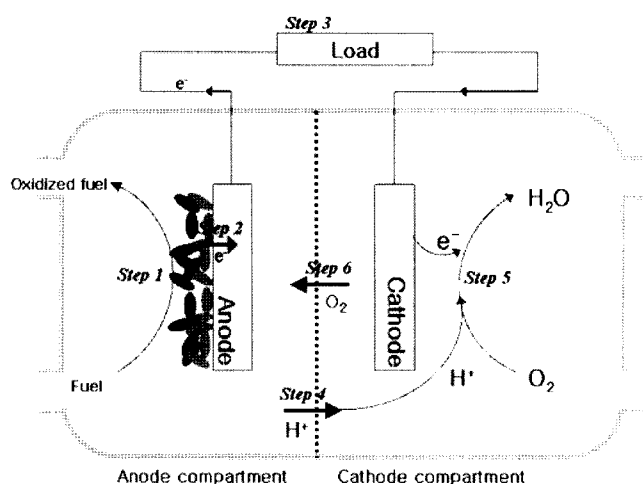


Fig. 6. Rate limiting steps in MFC enriched with electrochemically active consortia.

Proton Mass Transfer

The proton transfer can be influenced by the ionic strength of fuel and electrolyte, the resistance of proton exchange membrane, and the MFC design. A high strength buffer reduced the proton exchange limitation to some extent [24, 32], while increasing the ionic strength of the electrolyte increased the power outputs [32, 53]. Installing a proton exchange membrane between the anode and cathode compartments showed a power density up to two orders of magnitude larger than that obtained with a salt bridge MFC [71]. However, the proton exchange membrane limited power production when the surface area of the membrane was smaller than that of the electrodes due to an increase in internal resistance [72]. The commercially available cation exchange membranes can be the major cost for the construction of an MFC and attempts have been made to remove the membrane completely from the MFC system [32, 55]. Alternatively, custom-made proton exchange membranes have been utilized for some MFCs [26].

Liu and Logan [55] demonstrated that removing the cation exchange membrane from an MFC could increase power by a factor of 1.9 for glucose and 5.2 for wastewater. This increase was partly attributed to an enhancement of the proton flux from the anode to the cathode. Decreasing the distance between the anode and the cathode increased the power in an MFC system without a proton exchange membrane [32, 55].

In designing the proton exchange system, the oxygen diffusion into the anode compartment, which consumes electrons in the anode compartment, thus reducing the coulomb yield, should be considered. The use of mixed cultures may help minimize the effects of oxygen diffusion into the anode chamber because these bacteria will scavenge any dissolved oxygen, maintaining anaerobic conditions in the anode chamber [62].

Oxygen Supply and Cathode Reaction

The performance of the cathode can affect the electricity generation in MFCs. The strategies to improve the cathode reaction have involved adopting a cathode mediator such as ferricyanide or incorporating a catalyst such as platinum to enhance the kinetics of the reduction reaction. For example, addition of ferricyanide resulted in a 50–80% increase in power output [74]. Pham *et al.* [79] introduced a platinum-coated electrode as the MFC cathode to improve the oxygen reduction reaction. Platinum, although expensive, is an effective catalyst for the cathode and it has been repeatedly demonstrated in chemical fuel cells.

The effect of different Pt loading amounts on MFC performance has been examined. Cheng *et al.* [16] indicated that the performance was only slightly reduced (by a factor of 1.2) when Pt loading was decreased from 2 to 0.1 mg/cm². This difference might be acceptable given the cost of the catalyst and the large surface area needed for MFC construction. Additionally, a cobalt electrocatalyst material showed similar oxygen reduction rates to those seen with platinum catalysts under the chosen conditions of air saturated electrolyte solutions [96].

The use of peroxide for oxygenation appeared to alleviate the oxygen limitation problem and was found to improve the long-term stability of the MFC reactor [92].

Further improvements have been made through the use of air-breathing cathodes (widely used in the direct methanol fuel cells (DMFC) [14, 27]) in place of cathodes fed with air-saturated water. [6, 13, 24, 32, 37, 38, 42, 78, 79, 81, 82]. MFCs with direct air cathodes eliminated the requirement of oxygenation, which is an energy burden to MFC applications. Direct air cathodes also significantly increased MFC power generation [49–53, 78].

Some researchers have also focused on increasing the effectiveness of the cathodic reaction using a mediator such as ferricyanide [2], and to increase the oxygen reduction reaction at the cathode electrode by platinum (Pt) [32, 79] or enzyme [79] treatment or direct air-breathing assembly [78]. However, use of ferricyanide was impractical because of poor long-term operational stability. Furthermore, Pt treated electrode is not a good idea if MFC systems are to be operated in large scale applications such as wastewater treatment system. In the case of MFC's as a sensing system, Pt treated cathode electrodes may be a useful approach to increase the oxygen reduction kinetics [79].

Recently, studies have been conducted to improve the effectiveness of MFC cathode compartments by the study of membrane-less MFC's [32, 55] and biofilms on the cathode electrode [3]. Allison *et al.* [3] studied the use of manganese oxides as cathodic reactants in MFC systems and biofilms of manganese-oxidizing microorganisms (*Leptothrix discophora* SP-6) were grown on the cathode electrodes. The current density using biomineralized manganese oxides was almost 2 orders of magnitude higher than that obtained

when using oxygen. Gregory *et al.* [29] also reported improved reducing capabilities using nitrate as a cathodic reactant and using a nitrate- and iron-reducing microorganism (*Geobacter metallireducens*) with a potentiostat-poised graphite electrode as an electron donor. Park *et al.* [76] reported that nitrate reduction could occur in a biofilm at the cathode electrode. These results indicate that if oxygen is not present in MFC system, microbial nitrate reduction may be an alternative to oxygen for the cathodic reduction reaction.

Recent studies conducted by the authors show that enrichment cultures were established from an anaerobic sludge inoculum using the graphite MFC electrode as an electron donor and nitrate as the electron acceptor in cathode. Nitrate was reduced to nitrite with the production of current. The stoichiometry of electron consumption and required electron for nitrate reduction were consistent with the electrode serving as the sole electron donor for nitrate reduction.

Anode Reaction:

Limitations on power output are certainly limited by the nature of the bacterial communities on the anode, and an understanding of these communities will be needed that ranges from the mechanism(s) of electron transfer to understanding the complex interactions that occur between microbial cells (within and between species) on the membrane. To this end, some studies have explored the anodic reactions of microorganisms [3, 19, 48, 81], as well as anode modification with metals, surfactants, and organic materials [15, 23, 29]. This will be a focal point of work in the future. What we know for now is that a wide variety of different organisms exist and can be enriched on the anodes, and that eventually, as other parts of the MFCs are optimized, these will represent the rate limiting step in the production of current: rate limiting steps that can conceivably, via genetic engineering and physiological manipulation, be substantially improved upon.

With regard to materials science of the anode, Schröder *et al.* [88] obtained a power density of 6.0 W/m² using polyaniline-modified platinum as an anode. Although the power density is high, this MFC system needs improvements to overcome the inherent drawbacks such as the requirement of an additional reactor for fermentation, the use of a complex anode of polyaniline-modified platinum, and the need for a potential-pulse operation to maintain the catalytic activity of the anode [64].

DESIGN OF MEMBRANE-CONTAINING MFCs

Many MFC designs have been explored with regard to the physical and chemical nature of the cation exchange membrane (Table 2). Power density is commonly used as a representative performance index for MFCs as well as chemical fuel cells, although a value of power density

cannot clearly indicate the performance because it can be substantially varied according to the determination method of electrode area [64]. For example, in MFCs, only a fraction of the anode electrode surface may take part in the electron transfer reaction because of how the biocatalyst interacts with the electrode surface. In contrast, chemical fuel cells employ the total surface, including internal surface, for the oxidation reaction. For electrode material with internal structure, the total area of electrodes including the internal surface area [75], or apparent surface area [13, 65, 73, 92], or projected area [49, 55, 69, 81, 83] has been adopted for the computing of power density in MFCs. In this sense, volumetric power can be used as an alternative performance index to power density in large volume processes such as wastewater treatment [64].

MFCs can be designed with one or two chambers. The two-chambered MFC can be designed for fed-batch or continuous operation. A mixed reactor-type MFC and a plug flow reactor-type MFC have been used for batch operation and continuous operation, respectively. The two-chambered and mixed reactor-type MFC for the fed-batch operation generally consists of two cylinders connected by a glass tube containing a cation exchange membrane [55, 72, 75, 76]. A maximum power density of 216 W/m³ has been obtained using this type of fed-batch MFC type [81]. MFCs which are operated in continuous mode are more suitable for practical applications than fed-batch MFC's. Using this design, a continuous MFC, which was initially developed as a real-time BOD sensor [12] and then used for power generation [64], yielded power densities as high as 560 mW/m² (102 W/m³ MFC total volume).

Plug flow reactor-type MFC's have structural features that include direct contact between electrodes and membrane, and a rectangular cross section perpendicular to the direction of fuel-flow with the narrow width of 20 mm, which enabled such improvement [64]. Such a plug flow reactor type-MFC was used to continuously convert carbohydrates to electricity [83, 84], achieving a power density of 18 W/m² (37 W/m³ anode volume), using glucose as substrate.

A single-chambered MFC using a proton exchange membrane and adopting an electrode assembly technique has been developed [49, 55, 78]. Using a single-chambered MFC configuration, up to 28 mW/m² (1.6 W/m³ liquid volume) was generated using domestic wastewater [55]. A maximum of 262 mW/m² (6.6 W/m³ liquid volume) was generated using glucose with the single-chambered air-cathode MFC operated in batch mode operation [55]. It was shown using food processing wastewater as fuel that power could be increased from 81 to 371 mW/m² by using a single-chambered MFC [73].

Upflow Membrane-less MFC

Because proton exchange membranes are expensive and can be fouled during use [32], membrane-less microbial

Table 2. Comparison of MFC performances according to MFC configuration and operation method.

MFC type	Operation mode	Substrate	Cathode system	Power density ^a (mW/m ²)	Volumetric power ^b (W/m ³)	Reference
Two chambered and mixed reactor-type	Fed-batch	Acetate	Aqueous air/ferricyanide	16	0.4	[8]
		Glucose	Aqueous air/ferricyanide	8.2	0.15	[13]
		Glucose	Aqueous air/ferricyanide	3600 ^c	216 ^d	[81]
Membrane MFC	Continuous	Glucose	Direct air/platinum	212 ^e	n.a. ^e	[61]
		Glucose and glutamate	Aqueous air/platinum	560	102	[64]
Single chambered and mixed reactor-type	Continuous	Glucose	Ferricyanide	4,310 ^e	85.5	[82]
		Domestic wastewater	Direct air/platinum	26	1.6 ^f	[50]
Single chambered and plug flow reactor-type	Continuous	Glucose	Ferricyanide	n.a. ^e	90 ^d	[83]
		Food processing wastewater	Lactate	Direct air/platinum	371	21.2 ^f
Direct air/ferric graphite	788			16	[75]	
Single chambered and mixed reactor-type	Fed-batch	Domestic wastewater	Direct air/platinum	146	n.a. ^e	[49]
			Glucose and glutamate	Aqueous air/platinum	n.a. ^e	0.01
Membrane-less MFC	Continuous	Glucose and glutamate	Aqueous air/platinum	n.a. ^e	7.6	[65]
		Glucose	Peroxide	22	0.43	[92]

^aThe power densities were calculated based on the apparent surface area of electrodes.

^bThe volumetric powers were computed by using the total MFC volume.

^cThe data were reported based on the projected area of anode electrode.

^dThe data were reported based on the volume of anode compartment volume.

^en.a. means "insufficient data available", or "not applicable".

^fThe data were reported based on the volume of liquid volume.

fuel cells (ML-MFC) have been developed for wastewater treatment [32, 55, 92]. The ML-MFC is a tubular or rectangular type reactor consisting of one compartment packed with several anodes and cathodes, and is operated in an up-flow mode. (i.e., fluid elements travel upward through the ML-MFCs). An optimal configuration for the anode of an ML-MFC is thought to be an ideal plug flow reactor [65]. It is known that the organic compounds not used in the anode region are transferred to the cathode region in an ML-MFC, where they are oxidized through the aerobic bacterial respiration consuming oxygen [32]. Because the aerobic bacteria have higher affinity to oxygen than plain graphite electrodes [79], organic compounds leaving the anode region can inhibit the cathode reaction. The oxygen limitation to the cathode reaction can cause MFC instability and lead to process failure. For this reason uneven flow must be avoided. Clearly, the performance of ML-MFCs could be improved through understanding the flow characteristics at the anode and how a power density of 7.5 W/m^3 was obtained in a continuous mode [65]. By using a membrane-less air-cathode MFC, it was possible to produce as much as 12.5 W/m^3 with domestic wastewater in a fed-batch mode [55].

Sediment Batteries

In a variation on the theme of MFCs, an electrode placed in marine sediments was shown to collect electrons through microbial reactions when connected to another electrode placed at the aerobic surface [85, 93]. This system has been demonstrated without the use of a proton exchange membrane. Reimers *et al.* [85] proposed this system could be used to supply power for oceanographic instruments deployed for routine long-term monitoring operations in the coastal ocean. They also proposed that the viability of the MFC system in this environment has been established by power density.

APPLICATIONS OF MFCs

The laboratory-sized MFCs are the workhorse of the field right now, using these systems to understand how MFCs function, and how to optimize the various components. As MFCs continue to improve in performance, and they will do so, many potential applications will appear that will require moving to different size scales and different MFC designs. For example, some applications will require scaling up in size: 1) current production; and 2) waste disposal, both of which may occur from local to municipal scales, while others may require scaling down: 3) biosensors for environmental or medical applications; and 4) remote, long-lived power sources.

To date, there are few examples of MFCs that have been successfully employed in any of these applications, but

some progress has been made. For example MFCs have been used for various purposes including biosensors [11, 12, 33, 39, 66, 67] and electricity production coupled with wastewater treatment [32, 38, 44, 49–54, 62–65, 81–83]. Karube *et al.* [35] developed a BOD sensor based on an MFC using the hydrogen produced by *Clostridium butyricum* immobilized on the electrode. Particularly, mediated-MFC systems have been tested as BOD sensors [90, 94]. But these BOD sensors have a poor long-term stability as the mediators are generally toxic to microorganisms.

Mediator-less MFCs could be operated using EAB or electrochemically active consortia. The EAB at the anode of the MFC oxidizes the substrate as a fuel and resulting electrons are directly transferred to the electrode suggesting that the MFC system can monitor substrate concentration because electric signal is proportional to fuel (substrate) strength. Studies were carried out to monitor lactate concentration using *S. putrefaciens* as a biocatalyst in the MFC [37]. Furthermore, the MFC enriched with electrochemically active consortia could measure BOD values of wastewater either by reading the current or by calculating the coulombic yield [39].

Operational stability is one of critical factors to be considered in biosensors because stable sensor performance over a desired period is essential for data accuracy. MFC-type BOD sensors showed operational stability for over 5 years without any services as long as wastewater (fuel) was available. Definitely, this period is much longer than that of previously reported BOD sensors based on DO electrode systems. In addition, this MFC BOD system showed acceptable reproducibility [39] relative to conventional BOD measurement methods (BOD_5). A study was also conducted to measure the BOD of wastewater for real-time monitoring by reading current [12]). This real-time BOD monitoring system could measure BOD values up to 100 ppm based on a linear relationship while higher BOD values were measured using a lower feeding rate [12]. In addition, its repeatability was less than 10% and showed a long-term stability (over 5 years) as well [12]. However, about 30–60 min was required to reach steady-state current after the system had been changed with different BOD values of wastewater. The response time and the sensitivity of real-time BOD monitoring system were analyzed through step-change testing of fuel [66]. The results showed that newly designed smaller MFCs (5 ml) had a response time of 5 min, which was relatively shorter than that of previously used MFCs (25 ml) [66]. These MFC-type BOD sensor systems also successfully monitored low BOD values of natural river water [33] and artificial wastewater [34].

One potential problem in such systems is the presence of other electron acceptors in the effluent. For example, current generation as an indicator of BOD was reduced in the presence of nitrate and oxygen in wastewater. Nitrate and oxygen are electron acceptors with high redox potentials

suggesting that electrons could be used to reduce these alternative electron acceptors instead of an electrode. Oxygen can be removed by nitrogen purging but it is not easy to efficiently eliminate nitrate in the sample wastewater. Respiratory inhibitors such as azide and cyanide eliminated the negative effect of high redox potential electron acceptors present in the MFC [11]. As azide and cyanide did not inhibit the ETS of EAB [38, 40], the use of these respiratory inhibitors is a good recommendation for the accurate BOD measurement of sample wastewater containing nitrate and/or oxygen [11].

Another potential application is that of wastewater treatment, where MFC systems are a very attractive option due to their multi-functional characteristics. It is expected that MFC's would generate energy in the form of electricity while simultaneously remediating biodegradable contaminant in wastewater. In addition, MFC's would generate much less sludge than a conventional activated sludge process, since the major part of energy available from the oxidation of organic contaminants is converted to electricity, and the remaining energy is used for microbial growth [32]. This is another big advantage because excess sludge treatment and disposal currently represents a rising challenge for wastewater treatment plants [4].

Actually, the electrochemical activity of EAB gives an estimate of how much sludge can be reduced during MFC processing. Cyclic voltammetry of *Shewanella putrefaciens* cell suspensions showed redox activity at around 0 V against normal hydrogen electrode (NHE). This evidence suggests that electrons from substrate oxidation reduce microbial electrochemical carriers, probably *c*-type cytochrome complexes, and their redox potentials are around 0 V. If hydrogen is a representative substrate in the system, 0.42 V of potential difference may be involved in the microbial growth (sludge production), and 0.42 V is one third of the potential difference of the activated sludge process ($\Delta E'_0 = 1.24$ V).

MFC design improvements must be made if MFC systems are to be used for wastewater treatment. The model MFC structure is a two-chamber system separated by proton exchange membrane. As mentioned previously, commercial proton exchange membranes (e.g. Nafion[®]) are not suitable if MFC systems are applied for wastewater treatment processing because of cost. In addition, electrode (cathode) modification by treatment of expensive catalysts such as platinum is an expensive approach. New studies using membrane-less MFC systems offer a promising cost-reducing alternative.

Finally, a general use of the MFC might be the removal of organic matter from sediments or other polluted sites. When the rate of oxygen consumption exceeds the rate of oxygen diffusion, anaerobic conditions prevail. These conditions exist in sediment environments where organic materials are also present often as contaminants. Sulfate

and carbon dioxide can be used as alternative electron acceptors in sediment environments, but these electron acceptors are not always sufficient to facilitate the consumption of excess organic matter by microbial populations. Therefore, fermentative anaerobic bacteria are predominant in sediments and they produce fatty acids as major fermentative products. This acidic condition makes it easy to solubilize heavy metals and phosphate in the sediments and thus the water quality is deteriorated.

A two electrode MFC system was employed in a freshwater sediment environment to oxidize organic contaminants. A graphite electrode was embedded in freshwater sediment (anode), and the cathode was placed at the water surface. In this case, no proton exchange membrane was used. Power output increased with DO and proton concentrations increased at the cathode. The open circuit potential (OCP) was recorded as 0.85 V with control tests (open circuit). The ORP of anode (ORP_{anode}) was calculated as 290 mV (vs NHE) in the MFCs tested, and that of control tests was -546 mV (vs NHE). Considering ORP data of anode, the strict anaerobes such as methanogens could be predominant in the sediment of control tests, but not in the MFC tested. From experiments of this type, it is proposed that MFC type systems can be used to oxidize organic matter in place, removing pollutants and stabilizing sediments.

CHALLENGES AND PROSPECTS

The challenges facing the MFC community are many. Almost all aspect of the MFC operation can be regarded as sub-optimal at this point, including the anode, the cathode, the membrane, and MFC design! While these challenges loom as great, the opportunities are equally great.

As both bioelectrochemical activity and microbial metal reducing activity appear to share the same or similar electron transport chains, electrochemical activity of microorganisms may play an important role in the biogeochemical cycling of carbon, nitrogen, metals and other organic contaminants. Indeed, the concept of mediator-less MFC was a great finding and mediator-less MFC studies provide new insights into the function of electrochemically active bacteria directly associated with practical needs of the environmental protection.

In past years, environmental pollution control has mainly relied on how fast and feasible processes could operate to treat environmental pollutants. However, the needs of a "sustainable and renewable energy" provide pressure that will shift the focus from the treatment of pollution to resource exploitation. This concept may be a future strategy of "environmental energy technology" suggesting that pollutant treatment processing is associated with energy production.

As we know, fossil fuels as a conventional fuel source (such as petroleum and coal) have very useful properties

not shared by non-conventional energy sources (such as solar, wind and geothermal powers) that have made them popular during the last century. (i.e., they are abundant, easy to use and possess high energy). However, since the first oil crisis of 1973, we have become more aware of the need to search for alternative energy sources. As the supply of fossil fuels decreases (and the price increases) the utility of methods such as MFC power generation will become apparent. Fossil fuels are not renewable and result in the emission of CO₂ into the atmosphere. MFCs, using recently fixed biomass for energy, are renewable, and have no impact on the CO₂ budget.

Biomass can be defined as all water- and land-based organisms and vegetation. This biomass contains forestry (woody) and agricultural residues as well as industrial waste such as municipal solid waste and sewage waste. It has been thought that the only natural and renewable carbon resources that is large enough to be used as a substitute for fossil fuel is a biomass. The biomass is renewable in the sense that only a short period of time is needed to replace what is used as an energy source.

Mediator-less MFC systems can use biomass as a fuel source both directly and indirectly by the catalytic activity of microorganisms. Additionally, MFC systems directly produce electricity suggesting that they don't require advance processes to purify (separate) energy resources.

In this review, we have discussed physical and biochemical properties of electrochemically active bacteria as biocatalyst in mediator-less MFC systems and their unique electron transport chain. We also investigated several rate limiting steps. This was a very helpful approach to understand the working of MFC system, and to optimize the system for environmental process such as wastewater treatment system. Based on the discussion here, we may propose that mediator-less MFC systems can be a novel energy production system in terms of renewable, sustainable energy as well as pollutant control process. Indeed, mediator-less MFC systems have a great potential for future technology.

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REFERENCES

1. Akiba, T., H. P. Bennetto, J. L. Stirling, and K. Tanaka. 1985. Electricity generation from alkalotrophic organisms. *Biotechnol. Lett.* **9**: 11–616.
2. Allen, R. M. and H. P. Bennetto. 1993. Microbial fuel cell. *Appl. Biochem. Biotechnol.* **39/40**: 24–40.
3. Allison, R., B. Haluk, and L. Zbigniew. 2005. Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. *Environ. Sci. Technol.* **39**: 4666–4671.
4. Angenent, L. T., K. Karim, M. H. Al-Dahhan, B. A. Wrenn, and R. Domiguez-Espinosa. 2004. Production of bioenergy and biochemical from industrial and agricultural wastewater. *Trend Biotechnol.* **22**: 477–485.
5. Bennetto, H. P., G. M. Delaney, J. R. Mason, H. D. Roller, J. L. Stirling, and C. F. Thurstson. 1985. The source of fuel cell: Efficient biomass conversion using a microbial catalyst. *Biotechnol. Lett.* **7**: 699–704.
6. Bennetto, H. P., J. Box, G. M. Delaney, J. R. Mason, S. D. Roller, J. L. Stirling, and C. F. Thurstson. 1987. Redox mediated electrochemistry of whole microorganisms; from fuel cell to biosensor, pp. 291–312. In A. P. F. Turner, I. Karube, and G. S. Wilson (eds.), *Biosensors: Fundamental and Applications*. Oxford University Press, Oxford, U.K.
7. Bond, D. R., D. E. Holmes, L. M. Tender, and D. R. Lovley. 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* **295**: 483–485.
8. Bond, D. R. and D. R. Lovley. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* **69**: 1548–1555.
9. Bradley, P. M., F. H. Chapelle, and D. R. Lovley. 1998. Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene. *Appl. Environ. Microbiol.* **64**: 3102–3105.
10. Bulter, J. E., F. Kaufmann, M. V. Coppi, C. Nunez, and D. R. Lovley. 2004. MacA, a diheme *c*-type cytochrome involved in Fe(III) reduction by *Geobacter sulfurreducens*. *J. Bacteriol.* **186**: 4042–4045.
11. Chang, I. S., H. Moon, J. K. Jang, and B. H. Kim. 2005. Improvement of microbial fuel cell performance as a BOD sensor using respiratory inhibitors. *Biosens. Bioelectron.* **20**: 1856–1859.
12. Chang, I. S., J. K. Jang, G. C. Gil, M. Kim, H. J. Kim, B. W. Cho, and B. H. Kim. 2004. Continuous determination of biochemical oxygen demand sensor using a microbial fuel cell type biosensor. *Biosens. Bioelectron.* **17**: 607–613.
13. Chaudhuri, S. K. and D. R. Lovley. 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol.* **21**: 1229–1232.
14. Chen, C. Y. and P. Yang. 2003. Performance of an air-breathing direct methanol fuel cell. *J. Power Sources* **123**: 37–42.
15. Chen, P., M. A. Fryling, and R. L. McCreery. 1995. Electron transfer kinetics at modified carbon electrode surface: The role of specific surface sites. *Anal. Chem.* **67**: 3115–3122.
16. Cheng, S., H. Liu, and B. E. Logan. 2006. 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.
17. Choi, Y., E. Jung, S. Kim, and S. Jung. 2003. Membrane fluidity sensing microbial fuel cell. *Bioelectrochem.* **59**: 121–127.

18. Choi, Y., J. Song, S. Jung, and S. Kim. 2001. Optimization of the performance of microbial fuel cells containing alkalophilic *Bacillus* sp. *J. Microbiol. Biotechnol.* **11**: 863–869.
19. Choi, Y., N. Kim, S. Kim, and S. Jung. 2003. Dynamic behaviors of redox mediators within the hydrophobic layers as an important factor for effective microbial fuel cell operation. *Bull. Korean Chem. Soc.* **24**: 437–440.
20. Choo, Y. F., J. Lee, I. S. Chang, and B. H. Kim. Submitted. Bacterial community in microbial fuel cell enriched with high concentration of glucose and glutamate.
21. Cuong, P. A., S. J. Jung, N. T. Phung, J. Lee, I. S. Chang, B. H. Kim, H. Yi, and J. Chun. 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.
22. Delaney, G. M., H. P. Bennetto, J. R. Mason, H. D. Roller, J. L. Stirling, and C. F. Thurtson. 1984. Electron-transfer coupling in microbial fuel cells: 2. Performance of fuel cells containing selected microorganism-mediator-substance combinations. *J. Chem. Tech. Biotechnol.* **34B**: 13–27.
23. DuVall, S. H. and R. L. McCreey. 1999. Control of catechol and hydroquinone electron-transfer kinetics on native and modified glassy carbon electrodes. *Anal. Chem.* **71**: 4594–4602.
24. Gil, G. C., I. S. Chang, B. H. Kim, M. Kim, J. K. Jang, H. S. Park, and H. J. Kim. 2003. Operating parameters affecting the performance of a mediator-less microbial fuel cell. *Biosens. Bioelectron.* **18**: 327–334.
25. Gregory, K. B., D. R. Bond, and D. R. Lovley. 2004. Graphite electrodes as electron donors for anaerobic respiration. *Environ. Microbiol.* **6**: 596–604.
26. Grzebyk, M. and G. Pořniak. 2005. Microbial fuel cells (MFCs) with interpolymer cation exchange membranes. *Sep. Purif. Technol.* **41**: 321–328.
27. Han, J. and E. S. Park. 2002. Direct methanol fuel-cell combined with a small back-up battery. *J. Power Sources* **112**: 477–483.
28. Heidelberg, J. F., I. T. Paulsen, K. E. Nelson, E. J. Gaidos, W. C. Nelson, T. D. Read, J. A. Eisen, R. Seshadri, N. Ward, B. Methe, R. A. Clayton, T. Meyer, A. Tsapin, J. Scott, M. Beanan, L. Brinkac, S. Daugherty, R. T. DeBoy, R. J. Dodson, A. S. Durkin, D. H. Haft, J. F. Kolonay, R. Madupu, J. D. Peterson, L. A. Umayam, O. White, A. M. Wolf, J. Vamathevan, J. Weidman, M. Impraim, K. Lee, K. Berry, C. Lee, J. Mueller, H. Khouri, J. Gill, T. R. Utterback, L. A. McDonald, T. V. Feldblyum, H. O. Smith, J. C. Venter, K. H. Nealson, and C. M. Fraser. 2002. Genome sequence of the dissimilatory metal iron-reducing bacterium *Shewanella oneidensis*. *Nat. Biotechnol.* **20**: 1118–1123.
29. Hermendeaz, M. E. and D. K. Newman. 2001. Review: Extracellular electron transfer. *Cell Mol. Life Sci.* **58**: 1562–1571.
30. Holmes, D. E., D. R. Bond, and D. R. Lovley. 2004. Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* **70**: 1234–1237.
31. Hyun, M. S., H. J. Kim, and B. H. Kim. 1998. Use of a fuel cell to enrich electrochemically active Fe(III)-reducing bacteria, pp. 309–309. 98th General Meeting of American Society for Microbiology, Atlanta, U.S.A.
32. Jang, J. K., T. H., Pham, I. S. Chang, K. H. Kang, H. Moon, K. S. Cho, and B. H. Kim. 2004. Construction and operation of a novel mediator- and membrane-less microbial fuel cell. *Process Biochem.* **39**: 1007–1012.
33. Kang, K. H., J. K. Jang, J. Y. Lee, H. Moon, I. S. Chang, J. M. Kim, and B. H. Kim. 2004. A low BOD sensor using a microbial fuel cell. *J. of KSEE* **26**: 58–63.
34. Kang, K. H., J. K. Jang, T. H. Pham, H. Moon, I. S. Chang, and B. H. Kim. 2003. A microbial fuel cell with improved cathode reaction as a low biological oxygen demand sensor. *Biotechnol. Lett.* **25**: 1357–1361.
35. Karube, I., T. Matsunga, S. Mitsuda, and S. Suzuki. 1977. Microbial electrode BOD sensors. *Biotechnol. Bioeng.* **17**: 153–157.
36. Katz, E., A. N. Shipway, and I. Willner. 2003. Biochemical fuel cells, pp. 1–27. In Vielstich W., H. A. Gasteiger, and A. Lamm (eds.), *Handbook of Fuel Cells-fundamentals, Technology and Applications*, John Wiley & Sons, Ltd., Sussex, U.K.
37. Kim, B. H., H. J. Kim, M. S. Hyun, and D. H. Park. 1999. Direct electrode reaction of Fe(III) reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* **9**: 127–131.
38. Kim, B. H., H. S. Park, H. J. Kim, G. T. Kim, I. S. Chang, J. Lee, and N. T. Phung. 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Appl. Microbiol. Biotechnol.* **63**: 672–681.
39. Kim, B. H., I. S. Chang, G. C. Gil, H. S. Park, and H. J. Kim. 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnol. Lett.* **25**: 541–545.
40. Kim B. H., I. S. Chang, and H. Moon (in press). Microbial fuel cell type biochemical oxygen demand sensor In *Encyclopedia of Sensors*, Grimes, C. A., E. C. Dickey, and M. V. Pishko (eds.), American Scientific Publishers, Valencia, U.S.A.
41. Kim, G. T., M. S. Hyun, I. S. Chang, H. J. Kim, H. S. Park, B. H. Kim, S. D. Kim, J. W. T. Wimpenny, and A. J. Weightman. 2005. Dissimilatory Fe(III) reduction by electrochemically active lactic acid bacterium phylogenetically related to *Enterococcus gallinarum* isolated from submerged soil. *J. Appl. Microbiol.* **99**: 978–987.
42. Kim, H. J., H. S. Park, M. S. Hyun, I. S. Chang, M. Kim, and B. H. Kim. 2002. A mediator-less microbial fuel cell using a metal reducing bacterium *Shewanella putrefaciens*. *Enzyme Microb. Technol.* **30**: 125–152.
43. Kim, H. J., M. S. Hyun, I. S. Chang, and B. H. Kim. 1999. A fuel cell type lactate biosensor using a metal reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* **9**: 365–367.
44. Kim, J. R., B. Min, and B. E. Logan. 2005. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Appl. Microbiol. Biotechnol.* **68**: 23–30.
45. Kim, N., Y. Choi, S. Jung, and S. Kim. 2000. Effect of initial carbon sources on the performance of microbial fuel cells

- containing *Proteus vulgaris*. *Biotechnol. Bioeng.* **70**: 109–112.
46. Leang, C., M. V. Coppi, and D. R. Lovley. 2003. OmcB, a *c*-type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. *J. Bacteriol.* **185**: 2096–2103.
 47. Lee, J., N. T. Phung, I. S. Chang, B. H. Kim, and H. C. Sung. 2003. Use of acetate for enrichment of electrochemically active microbes and their 16S rDNA analyses. *FEMS Microbiol. Lett.* **223**: 185–191.
 48. Lee, S. A., Y. Choi, S. H. Jung, and S. Kim. 2005. Effect of initial carbon sources on the electrochemical detection of glucose by *Gluconobacter oxidans*. *Bioelectrochem.* **57**: 193–198.
 49. Liu, H. and B. E. Logan. 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.
 50. Liu, H., R. Ramnarayanan, and B. E. Logan. 2004. Production of electricity during wastewater using a single chamber microbial fuel cell. *Environ. Sci. Technol.* **38**: 2281–2285.
 51. Liu, H., S. Grot, and B. E. Logan. 2005. Electrochemically assisted microbial production of hydrogen from acetate. *Environ. Sci. Technol.* **39**: 4317–4320.
 52. Liu, H., S. A. Cheng, and B. E. Logan. 2005. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* **39**: 658–662.
 53. Liu, H., S. A. Cheng, and B. E. Logan. 2005. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. *Environ. Sci. Technol.* **39**: 5488–5493.
 54. Logan, B. E. 2004. Extracting hydrogen electricity from renewable resources. *Environ. Sci. Technol.* **38**: 160A–167A.
 55. Logan, B. E., C. Murano, K. Scott, N. D. Gray, and I. M. Head. 2005. Electricity generation from cysteine in a microbial fuel cell. *Water Res.* **39**: 942–952.
 56. Lovley, D. R. and E. L. Blunt-Harris. 1999. Role of humic-bound iron as an electron transfer agent in dissimilatory Fe(III) reduction. *Appl. Environ. Microbiol.* **65**: 4252–4254.
 57. Lovley, D. R., J. D. Coates, E. L. Blunt-Harris, E. J. P. Phillips, and J. C. Woodward. 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**: 445–447.
 58. Mehta, T., M. V. Coppi, S. E. Childers, and D. R. Lovley. 2005. Outer membrane *c*-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* **71**: 8634–8641.
 59. Methé, B. A., K. E. Nelson, J. A. Eisen, I. T. Paulsen, W. Nelson, J. F. Heidelberg, D. Wu, M. Wu, N. Ward, M. J. Beanan, R. J. Dodson, R. Madupu, L. M. Brinkac, S. C. Daugherty, R. T. DeBoy, A. S. Durkin, M. Gwinn, J. F. Kolonay, S. A. Sullivan, D. H. Haft, J. Selengut, T. M. Davidsen, N. Zafar, O. White, B. Tran, C. Romero, H. A. Forberger, J. Weidman, H. Khouri, T. V. Feldblyum, T. R. Utterback, S. E. Van Aken, D. R. Lovley, and C. M. Fraser. 2003. Genome of *Geobacter sulfurreducens*: Metal reduction in subsurface environments. *Science* **302**: 1967–1969.
 60. Meyer, T. E., A. I. Tsapin, I. Vandenberghe, L. de Smert, D. Fishman, K. H. Neelson, M. A. Cusanovich, and J. J. Van Beeumen. 2004. Identification of 42 possible cytochrome *C* genes in the *Shewanella oneidensis* genome and characterization of six soluble cytochromes. *OMICS* **8**: 57–557.
 61. Min, B. and B. E. Logan. 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environ. Sci. Technol.* **38**: 5809–5812.
 62. Min, B., J. Kim, S. Oh, J. M. Regan, and B. E. Logan. 2005. Electricity generation from swine wastewater using microbial fuel cells. *Water Res.* **39**: 4961–4968.
 63. Min, B., S. Cheng, and B. E. Logan. 2005. Electricity generation using membrane and salt bridge microbial fuel cell. *Water Res.* **39**: 1675–1686.
 64. Moon, H., I. S. Chang, and B. H. Kim. 2006. Continuous electricity production from wastewater using mediator-less microbial fuel cell. *Bioresource Tech.* **97**: 621–627.
 65. Moon, H., I. S. Chang, J. K. Jang, and B. H. Kim. 2005. Residence time distribution in microbial fuel cell and its influence on COD removal with electricity production. *Biochem. Eng. J.* **27**: 59–65.
 66. Moon, H., I. S. Chang, K. H. Kang, J. K. Jang, and B. H. Kim. 2004. Improving dynamic response of a mediator-less microbial fuel cell as biochemical oxygen demand (BOD) sensor. *Biotechnol. Lett.* **26**: 1917–1921.
 67. Moon, H., I. S. Chang, J. K. Jang, K. S. Kim, J. Lee, R. W. Lovitt, and B. H. Kim. 2005. On-line monitoring of low biochemical oxygen demand through continuous operation of a mediator-less microbial fuel cell. *J. Microbiol. Biotechnol.* **15**: 192–196.
 68. Myers, C. R. and J. M. Myers. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J. Bacteriol.* **194**: 3429–3438.
 69. Myers, C. R. and J. M. Myers. 1997. Outer membranes of cytochromes of *Shewanella putrefaciens* MR-1: Spectra analysis and purification of the 83-kDa *c*-type cytochrome. *Biochim. Biophys. Acta* **1326**: 307–318.
 70. Myers, J. M. and C. R. Myers. 2001. Role of outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. *Appl. Environ. Microbiol.* **67**: 260–269.
 71. Newman, D. K. and R. Kolter. 2000. A role for excreted quinines in extracellular electron transfer. *Nature* **405**: 94–97.
 72. Oh, S. E. and B. E. Logan. In press. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Appl. Microbiol. Biotechnol.*
 73. Oh, S. E. and B. E. Logan. 2005. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Res.* **39**: 4673–4682.
 74. Oh, S. E., B. Min, and B. E. Logan. 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. *Environ. Sci. Technol.* **38**: 4900–4904.

75. Park, D. H. and J. G. Zeikus. 2003. Improved fuel cell and electrode designs for producing electricity from microbial degradation. *Biotechnol. Bioeng.* **81**: 348–355.
76. Park, H. I., D. K. Kim, Y. J. Choi, and D. W. Pak. 2005. Nitrate reduction using electrode as direct electron donor in biofilm-electrode reactor. *Process Biochem.* **40**: 3383–3388.
77. Park, H. S., B. H. Kim, H. S. Kim, H. J. Kim, G. T. Kim, M. Kim, I. S. Chang, Y. K. Park, and H. I. Chang. 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.
78. Pham, T. H., J. K. Jang, H. Moon, I. S. Chang, and B. H. Kim. 2005. Improved performance of a microbial fuel cell using a membrane-electrode assembly. *J. Microbiol. Biotechnol.* **15**: 438–441.
79. Pham, T. H., J. K. Jang, I. S. Chang, and B. H. Kim. 2004. Improvement of cathode reaction of a mediatorless microbial fuel cell. *J. Microbiol. Biotechnol.* **12**: 324–329.
80. Phung, N. T., J. Lee, K. H. Kang, I. S. Chang, G. M. Gadd, and B. H. Kim. 2004. Analysis of microbial diversity in oligotrophic microbial fuel cell using 16S rDNA analyses. *FEMS Microbiol. Lett.* **233**: 77–82.
81. Rabaey, K., G. Lissens, S. D. Siciliano, and W. Verstraete. 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.* **25**: 1531–1535.
82. Rabaey, K., N. Boon, S. D. Siciliano, M. Verhaege, and W. Verstraete. 2004. Biofuel cells select for microbial consortia that self-mediated electron transfer. *Appl. Environ. Microbiol.* **70**: 1–10.
83. Rabaey, K., P. Clauwaert, P. Aelterman, and W. Verstraete. 2005. Tubular microbial fuel cells for efficient electricity generation. *Environ. Sci. Technol.* **39**: 8077–8082.
84. Rabaey, K. and W. Verstraete. 2005. Microbial fuel cells: Novel biotechnology for energy generation. *Trends Biotechnol.* **23**: 291–298.
85. Reimers, C. E., L. M. Tender, S. Fertig, and W. Wang. 2001. Harvesting energy from the marine-sediment-water interface. *Environ. Sci. Technol.* **35**: 192–195.
86. Roller, H. D., H. P. Bennetto, G. M. Delaney, J. R. Mason, J. L. Stirling, and C. F. Thurtson. 1984. Electron-transfer coupling in microbial fuel cells: 1. Comparison of redox-mediator reduction rates and respiratory rates of bacteria. *J. Chem. Tech. Biotechnol.* **34B**: 3–12.
87. Scholz, F. and U. Schröder. 2003. Bacterial batteries. *Nat. Biotechnol.* **21**: 1151–1152.
88. Schröder, U., J. Niessen, and F. Scholz, 2003. A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. *Angew. Chem. Int. Ed. Eng.* **42**: 2880–2883.
89. Stoodley, P., K. Sauer, D. G. Davies, and J. W. Costerton. 2002. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* **56**: 187–209.
90. Striling, J. L., H. P. Bennetto, G. M. Delaney, J. R. Mason, H. D. Roller, K. Tanaka, and C. F. Thurtson. 1983. Microbial fuel cells. *Biochem. Soc. Trans.* **11**: 451–453.
91. Tanisho, S., N. Kamiya, and N. Wakao. 1983. Microbial fuel cell using *Enterobacter aerogenes*. *Bioelectrochem. Bioenerg.* **21**: 25–32.
92. Tartakovsky, B. and S. R. Guiot. 2006. A comparison of air and hydrogen peroxide oxygenated microbial fuel cell reactors. *Biotechnol. Prog.* **22**: 241–246.
93. Tender, L. M., C. E. Reimers, H. A. Stecher, D. E. Holmes, D. R. Bond, D. L. Lowy, K. Pilobello, S. J. Fertig, and D. R. Lovley. 2002. Harnessing microbial power generation on the seafloor. *Nat. Biotechnol.* **20**: 821–825.
94. Thurston, C. F., H. P. Bennetto, G. M. Delaney, J. R. Mason, H. D. Roller, and J. L. Striling. 1985. Glucose metabolism in microbial fuel cell; stoichiometry of product formation a thionin-mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. *J. Gen. Microbiol.* **131**: 1393–1201.
95. Vega, C. A. and I. Fernandez. 1987. Mediating effect of ferric chelate compounds in microbial fuel cells with *Lactobacillus planetarium*, *Streptococcus lactis* and *Erwinia dissolvens*. *Bioelectrochem. Bioenerg.* **17**: 217–222.
96. Wilkinson, S. 2000. “Gastrobots:”-Benefits and challenges of microbial fuel cells in food powered robot applications. *Auton. Robot.* **9**: 99–111.
97. Zhang, X. and A. Halme. 1995. Modelling of a microbial fuel cell process. *Biotechnol. Lett.* **17**: 809–812.