Introduction

Microbial fuel cells (MFCs) are bioreactors that convert chemical energy stored in the bonds of organic matters into electricity through biocatalysis of microorganisms (Potter, 1911; Cohen, 1931; Davis and Yarbrough, 1962; Moon et al., 2006). The schematic of a typical MFC is shown in Fig. 1. In the sketch below, the anodic and cathodic chamber are separated by a proton exchange membrane (PEM) (Wilkinson, 2000; Gil et al., 2003) that allows transport protons while blocking oxygen and other compounds. Microbes in the anodic chamber degrade organic matters and produce electrons, protons and carbon dioxide. Electrons and protons produced by microbes are then transported to the cathodic chamber via external circuit and a proton exchange membrane (PEM), respectively. In the cathodic chamber, protons and electrons react with oxygen to form water. Because the terminal electron acceptor (i.e., oxygen) is kept away from the anodic chamber, electrons are allowed to pass through the external load to generate electricity (Du et al., 2007).
Typical electrode reactions are shown below using acetate as an example substrate:

Anodic reaction: \[ CH_3COOH + 2H_2O \xrightarrow{\text{microbe}} 2CO_2 + 8H^+ + 8e^- \] (1)

Cathodic reaction: \[ 8H^+ + 8e^- + 2O_2 \xrightarrow{} 4H_2O \] (2)

Overall reaction: \[ CH_3COOH + 2O_2 \xrightarrow{\text{microbe}} 2H_2O + 2CO_2 \] (3)

The overall reaction is the breaking down of acetate (fuel) into carbon dioxide and water. The anodic potential \((E_{An})\) is around -0.300 V while the cathode potential \((E_{Ca})\) is about 0.805 V. Thus, for an MFC using acetate and oxygen, the theoretical maximum cell potential is \(E_{\text{cell}} = 0.805\, \text{V} - (-0.300\, \text{V}) = 1.105\, \text{V}\) \((\text{Logan et al., 2007})\).

However, in practice, the cathode potential with oxygen as terminal electron acceptor is much less than the theoretical maximum due to the overpotential of cathodic reaction. Typically, the open circuit potential (OCP) of an air cathode is about 0.4 V, with a working potential of 0.25 V even when prohibitively expensive Pt is used as the cathodic catalyst \((\text{Liu and Logan, 2004})\). Based on the electrode reaction pair above, an MFC bioreactor can generate electricity from the electron flow from the anode to the cathode in the external circuit.

Compared with traditional technologies used for energy generation from organic matters, MFCs hold many inherent advantages and have much wider applications. Firstly, MFCs have a wide range of substrates, such as carbohydrates, proteins, lipids and even the organic matter in wastewater \((\text{Pant et al., 2010})\). Secondly, MFCs possess a high-energy transformation efficiency since it converts the chemical energy stored in substrates into electricity directly. Thirdly, single-chamber MFCs do not need energy input for aeration, which lowers operational costs \((\text{Rabaey and Verstraete, 2005})\). Finally, MFCs have great potentials for widespread applications such as wastewater treatment, biological oxygen demand (BOD) sensors, bioremediation, hydrogen production and electricity generation \((\text{Logan and Regan, 2006})\).

**MFC History**

It has been known for many years that it is possible to generate electricity directly by using bacteria to break down organic substrates. Over a century ago, Potter was the first to demonstrate that electrical current can be generated from degradation of organic compounds by bacteria or yeast \((\text{Potter, 1911})\). Two decades later, Cohen confirmed Potter’s results and produced an overall voltage of 35 V at a current of 0.2 mA using a stacked bacterial fuel cell \((\text{Cohen, 1931})\). These publications are generally considered the first reported cases of MFCs, but they didn't generate much interest since the current density and power output were very small.

It was not until the 1960s that the idea of microbial electricity generation was picked up again as a potential method to convert human wastes into electricity during long space flights \((\text{Canfield, 1963})\). It was realized that the complicated underlying bioelectrochemical processes in MFCs operations require systematic and long-term research efforts \((\text{Cohn, 1963; Lewis, 1966})\). Before MFC research could take off, the rapid advances in other
energy technologies (e.g., photovoltaics) forced MFC research to the back burner (Schroder, 2007). Another milestone of MFCs is the discovery that current density and the power output could be greatly enhanced by the addition of electron mediators in 1980s (Delaney et al., 1984; Roller et al., 1984). Many artificial dyes and metallorganics such as neutral red (NR), methylene blue (MB), thionine, meldola's blue (MelB), 2-hydroxy-1,4-naphthoquinone (HNQ), and Fe(III)EDTA were found to be exogenous mediators (Vega and Fernandez, 1987; Allen and Bennetto, 1993; Park and Zeikus, 2000; Tokuji and Kenji, 2003; Ieropoulos et al., 2005b). Unfortunately, the synthetic compounds usually tend to be cytotoxic, unstable and expensive, thus limiting their applications (Du et al., 2007) beyond laboratory tests.

The latest and most remarkable breakthrough of MFCs was made near the end of the 20th century when some microbes were found to transfer electrons directly to the anode (Kim et al., 1999). Examples of this kind of bioelectrochemical active bacteria are Shewanella putrefaciens (Kim et al., 2002), Geobacteraceae sulfurreducens (Bond and Lovley, 2003), Geobacter metallireducens (Min et al., 2005a) and Rhodoferax ferrireducens (Chaudhuri and Lovley, 2003). These microbes are able to form a biofilm on the anodic surface and transfer electrons via the cell membrane or special conductive pili (also known as nanowires). Since mediators are not needed in this kind of MFC, the operational cost can be reduced and the concern for environmental pollution caused by artificial mediators is eliminated (Ieropoulos et al., 2005). Furthermore, this form of MFC is operationally more stable and yields a high CE (Coulombic Efficiency) (Du et al., 2007). Hence, mediator-less MFCs are considered to be more suitable for wastewater treatment and power generation.

In the past decade, rapid progresses have been made in MFC research and there has been a significant increase in recent years in the number of MFC publications in the literature. Most of the studies focused on MFC design, exoelectrogenic bacteria, and cost-effective electrode materials (Logan et al., 2006; Rozendal et al., 2008; Logan and Regan, 2006). The power densities of MFCs have increased from below 0.1 to 6860 mW/m² over the past decade (Kim et al., 1999; Fan et al., 2008). The increase will eventually be limited by the sustained electron transfer rate achieved by the bacteria used in MFCs. In a practical MFC, a mixture of metabolically versatile organisms is typically present. A good understanding of the interactions of different bacteria in the biofilm community and the mechanisms of electron transfer to the anode by the dominant bacteria is essential for improving power densities (Logan and Regan, 2006).

Electricity-Producing Bacteria and Their Electron Transfer Mechanisms

Electricity-producing bacterial communities

Theoretically, most anaerobes (or facultative anaerobes) have the potential to be the biocatalyst in MFC if final electron acceptors such as oxygen, nitrate and sulfate are absent in the culture and proper electron shuttles are present. Recently, there has been an increase in the number of reports of electrochemically active microorganisms. However, the diversity of bacteria capable of electricity-producing activity is just beginning to be discovered (Logan, 2008). A variety of bacteria can produce a modicum of electricity in an MFC if a
mediator is used to facilitate the transfer of electrons between the bacterial cells and the anodic surface used in the system, while many other bacteria have been found to possess the ability to transfer electrons from fuel (substrate) oxidation to a working electrode without a mediator. There are several reviews that summarized the microbial species used in MFCs (Seop et al., 2006; Du et al., 2007; Logan, 2009). A list of microbial species used in MFCs is shown in Table 1 together with a brief comment.

Table 1. Microbes used in MFCs

<table>
<thead>
<tr>
<th>Mediator-needed electricity-producing bacteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbe</strong></td>
<td><strong>Comment</strong></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Thionin as mediator</td>
</tr>
<tr>
<td>Erwinia dissolven</td>
<td>Ferric chelate complex as mediators</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>Ferric chelate complex as mediators</td>
</tr>
<tr>
<td>Streptococcus lactis</td>
<td>Ferric chelate complex as mediators</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>Sulphate/sulphide as mediator</td>
</tr>
<tr>
<td>Actinobacillus succinogenes</td>
<td>Neutral red or thionin as electron mediator</td>
</tr>
<tr>
<td>Gluconobacter oxydans</td>
<td>Mediator (HNQ, resazurin or thionine) needed</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Mediators such as methylene blue needed.</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Thionin as mediator</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pyocyanin and phenazine-1-carboxamide as mediator</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>HNQ as mediator</td>
</tr>
<tr>
<td>Shewanella oneidensis</td>
<td>Anthraquinone-2,6-disulphonate (AQDS) as mediator</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mediator-less electricity-producing bacteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbe</strong></td>
<td><strong>Comment</strong></td>
</tr>
<tr>
<td>Shewanella putrefaciens IR-1</td>
<td>A dissimilatory metal-reducing bacterium</td>
</tr>
<tr>
<td>Desulfuromonas acetoxidans</td>
<td>Deltaproteobacteria identified from a sediment MFC</td>
</tr>
<tr>
<td>Geobacter metallireducens</td>
<td>Shown to generate electricity in a poised potential system</td>
</tr>
<tr>
<td>Geobacter sulfurreducens</td>
<td>generated current without poised electrode</td>
</tr>
<tr>
<td>Rhodoferax ferrireducens</td>
<td>Betaproteobacteria used glucose as substrate</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>Deltaproteobacteria</td>
</tr>
<tr>
<td>Desulfobulbus propionicus</td>
<td>Deltaproteobacteria</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Found to produce current after a long acclimation time</td>
</tr>
<tr>
<td>Shewanella oneidensis DSP10</td>
<td>Achieved a high power density 2 W/m²</td>
</tr>
<tr>
<td>S. oneidensis MR-1</td>
<td>Various mutants identified that increase current or lose the ability for current generation</td>
</tr>
<tr>
<td>Pichia anomala</td>
<td>Current generation by a yeast (kingdom Fungi)</td>
</tr>
<tr>
<td>Rhodopseudomonas palustris DX-1</td>
<td>Produced high power densities of 2.72 W/m²</td>
</tr>
<tr>
<td>Ochrobactrum anthropi YZ-1</td>
<td>An opportunistic pathogen, e.g., P. aeruginosa</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans</td>
<td>Reduced sulphate when growing on lactate</td>
</tr>
<tr>
<td>Acidiphilium sp. 3.2 Sup5</td>
<td>Power production at low pH</td>
</tr>
<tr>
<td>Klebsiella pneumoniae L17</td>
<td>Produced current without a mediator for the first time</td>
</tr>
<tr>
<td>Thermincola sp. strain JR</td>
<td>Phylum Firmicutes</td>
</tr>
<tr>
<td>Geopsychrobacter electrodiphilus</td>
<td>Psychrotolerant Deltaproteobacteria</td>
</tr>
</tbody>
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Mechanisms of electron transfer

Electrochemically active bacteria (EAB) are thus far known to transfer electrons from the inside of the cell to an electrode via two different mechanisms (Schroder, 2007; Rozendal et al., 2008). The first mechanism is mediated electron transfer (MET), which relies on the redox cycling of mediators between the EAB and the electrode. The mediators can either be naturally present compounds, such as humic acids and sulfur species (Stams et al. 2006), or compounds produced by the microorganisms themselves, such as quinones (Newman and Kolter, 2000) or phenazines (Rabaey et al., 2005). The second mechanism is direct electron transfer (DET), which depends on the direct physical contact between the EAB and the electrode. Some EAB take advantage of a series of membrane redox proteins (i.e., cytochromes) that transport the electrons from the inside of the cell to the electrode (Lovley, 2006), while some other EAB utilize electrically conductive pili on the surface of their cells to transfer electrons (Gorby et al., 2006 and Reguera et al., 2005).

Mediated electron transfer (MET)

Mediators are required for electron transfer by most bacteria to transport electrons from inside cells to the outside, since their cell membranes consist of non-conductive lipids, peptidoglycans and lipopolysaccharides (Du et al., 2007; Davis and Higson, 2007). Mediators in an oxidized state are reduced in the cytoplasm or periplasm by absorbing electrons released by enzyme-catalyzed organic carbon oxidation inside the bacteria. The reduced mediators migrate to an anode and release the electrons to the anode to become oxidized again (Schroder, 2007). The oxidized mediators are now ready to repeat the same process. This cyclic process is depicted in Fig. 2A. Fig. 2B shows the other scheme for mediated electron transfer in which the mediators do not cross the cell membrane. They are reduced when they encounter electron transport proteins (such as cytochrome) on the outer cell membrane instead.

![Fig. 2. Simplified schematic illustration of MET. (A) Shuttling via cytoplasmatic or periplasmatic redox couples, (B) shuttling via outer cell membrane electron transport proteins. (Figure drawn with modifications after Lovley, 2006).](image)

Judging from their sources, mediators can be divided into two groups, namely the exogenous (artificial) mediators and endogenous mediators. In nature, some microorganisms may use externally available (exogenous)
electron shuttling compounds like humic acids or metal chelators (Stams et al., 2006). In many studies, artificial redox mediators such as neutral red (Park et al., 1999), thionin (Choi et al., 2003) and methyl viologen (Park et al., 1994) were added into MFC reactors. The addition of these mediators often seemed to be essential to enhance the electric current generation. A big disadvantage of the use of exogenous redox mediators is the need for repeated addition that is costly, because they are usually unstable. Furthermore, these artificial mediators may pose environmental concerns due to their toxicity (Schroder, 2007). Thus, the application of artificial mediators is rather limited and is gradually being abandoned.

Fortunately, in some systems exogenous mediators are not needed since some bacteria possess the ability to secrete mediators by themselves. These mediators are typically produced in two ways: through the production of organic, reversibly reducible compounds (secondary metabolites) and through the generation of oxidizable metabolites (primary metabolites) (Rabaey and Verstraete, 2005). Examples for such secondary metabolites are pyocyanin, 2-amino-3-carboxy-1,4-naphthoquinone, and ACNQ (Rabaey et al., 2004; Hernandez and Newman, 2001), which are able to shuttle electrons to an electrode. The primary metabolites that are involved in the electron transfer include H₂ and H₂S, which are by Escherichia coli K12 (Niessen et al., 2007) and Sulfurospirillum deleyianum (Straub and Schink, 2004) as mediators, respectively.

**Direct electron transfer (DET)**

Certain microbes seem to also possess the ability to transport electrons from the inside of the bacterial cell to the extracellular milieu via the cell membrane or special conductive pili rather than mediators. This form of electron transfer approach is known as DET (Schroder, 2007). Since the outer layers of the majority of microbial species are non-conductive, a series of membrane bound electron transport proteins are required for the DET, such as c-type cytochromes and heme proteins (Du et al., 2007). Two proposed DET pathways are illustrated in Fig. 3. One is through the membrane bound electron transport proteins (Fig. 3A), and the other one is through the pili (nanowires) that are connected to the membrane bound electron transport proteins (Fig. 3B).

![Fig. 3. Simplified schematic illustration of DET. (A) electron transfer via membrane bound cytochromes, (B) electron transfer via pili. (Figure drawn with modifications after Schroder, 2007).](image)

In Fig. 3A, the DET via outer membrane electron transport proteins requires that the bacterial cells adhere to the surface of anode directly (Schroder, 2007). Consequently, only the bacteria in the first monolayer of the sessile cells directly on the anode surface contribute to the current generation in MFC (Lovley, 2006). The MFC current
output thus depends on the cell density in this bacterial monolayer. Thus, the power densities of these MFCs are usually very limited. For example, the maximum current densities for MFCs based on *Shewanella putrefaciens* (Kim *et al.*, 2002), *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003) and *Geobacter sulfurreducens* (Bond and Lovley, 2003) were as low as 0.6, 3 and 6.5 μA/cm², respectively.

Fig. 3B shows the simplified mechanism of DET via electronically conducting molecular pili. The pili are connected to the membrane bound electron transport proteins, via which the electron transfer to the outside of the cell is accomplished. It has been reported that some bacteria (*e.g.*, Geobacter and Shewanella) strains can utilize their electronically conducting molecular pili as nanowires to transfer electrons (Gorby *et al.*, 2006; Reguera *et al.*, 2005). The nanowires may allow the microorganisms to utilize more space around the anode and develop thicker electrochemically active biofilms beyond the first monolayer of sessile cells on the anode, whereby increasing the functional bacteria density and boosting the current generation (Schroder, 2007). For instance, Reguera and co-workers reported that the nanowires of *G. sulfurreducens* may represent an electronic network permeating the biofilm that can promote long-range, multi-layer electrical transfer in an energy-efficient manner, increasing electricity production by more than 10-fold (Reguera *et al.*, 2005).

**Enrichment of electricity-producing bacteria**

Currently, electricity-producing bacteria are generally isolated from anaerobic sludge using an MFC reactor as a selection tool. This method was initially proposed based on the observation that the metabolism of *S. putrefaciens* was stimulated by the presence of an MFC anode when electron acceptors were not present (Hyun *et al.*, 1998). Kim *et al.* (2004) described a process to enrich microbes for MFCs. They used sludge collected from a corn-processing wastewater treatment plant as inoculum for the anodic chamber of an MFC and fed it with wastewater from the same source. Initially the MFC generated a current of 20 μA with an external load of 10 Ω. When the anode solution was replaced with a more nutritious wastewater from a different source, the current increased with concomitant COD (Chemical Oxygen Demand) reduction. After repeated replenishments of the wastewater the current output eventually reached 1.2 mA (Chang *et al.*, 2006). Fig. 4 shows the process of enrichment and selection of electricity-producing bacteria.

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**Fig. 4.** Enrichment of electricity-producing bacteria. (Figure drawn with modifications after Chang *et al.*, 2006).
Fig. 4A shows that in the initial stage of enrichment, there are only planktonic cells. No electricity is generated because there are no sessile cells on the anode to transfer electrons to the anode or mediators secreted by these organisms. When sessile cells start to appear on the anode, electron transfer starts (Fig. 4B). It is believed that electrode reducing is an energy conserving microbial respiration process (Bond et al., 2002), the electron donating bacteria can be enriched during MFC operation (Chang et al., 2006). When electrons are removed from the cytoplasm, beneficial organic carbon oxidation proceeds forward. This means that when there are no other electron acceptors in the medium as in the case of MFC operation, the electricity-producing bacteria tend to attach to the surface of anode and transfer electrons to the anode. Electricity production will increase during the enrichment process. At the end of the enrichment process, a variety of electricity-producing bacteria attach to the anodic surface and form a biofilm and the current output of the MFC reaches a maximum value and becomes stabilized (Fig. 4C).

Nutrients can be introduced to facilitate the enrichment process in the MFC. Copiotrophic cultures can be enriched with artificial wastewater containing acetate, propionate or even glucose and glutamate, and oligotrophic cultures with artificial wastewater or river water with added nutrients (Kang et al., 2004; Phung et al., 2004). Introducing fermentable substrates promoted a more diverse microbial population in the MFC than non-fermentable substrates such as acetate. Electrochemically inactive bacteria may also be promoted together with EAB in the same biofilm consortium. Their metabolism may play a critical role in assisting EAB during electricity production (Chang et al., 2006).

Genetically engineered “super-bugs” to reduce or eliminate electron transfer resistance: Roles of cytochromes, polysaccharide and type IV pili

Preface. The study of specific factors in bacteria that are critical for optimal electrogenesis has really only just begun in the past few years. Harnessing maximal electrogenic properties in bacteria requires organisms that are genetically tractable and possessing properties that include (i) the ability to metabolize a wide variety of carbon-rich substrates, (ii) the ability to form surface-associated communities on anodic surfaces known as biofilms, and (iii) the capacity to synthesize and secrete mediators. Below is a brief synopsis of three factors that are known to be involved in optimal MFC current generation. Future advances in this area present a true exciting possibility for using genetically engineered super-bugs to maximize MFC power output beyond any other existing MFC optimization methods could achieve.

Cytochromes. The first genome-wide screen to date for the assessment of the role of different bacteria gene products in electrogenesis was performed in G. sulfurreducens (Kim et al., 2008). Bacteria lacking OmcF, an outer membrane c-type cytochrome, possessed significantly reduced overall current production (~0.3 relative to 0.75 mA over 200 hr). The reduced current generated by the omcF mutant was not dependent upon iron reduction, as an omcB mutant was shown to generate near wild-type current. DNA microarray analysis conducted on the omcF mutant versus wild-type bacteria revealed decreased transcription of genes known to be upregulated when bacteria were on the anodic surface. Among these were genes encoding metal efflux and/or
type I secretion proteins, outer membrane cytochromes, OmcS and OmcE and several hypothetical proteins. More recently, Krushkal and co-workers showed that an \textit{omcB} mutant, lacking the ability to reduce soluble or insoluble iron, were able to adapt and grow on soluble iron with time. It as then speculated that multiple regulatory elements played a role in the adaptive behavior (Krushkal \textit{et al.}, 2009).

\textit{Polysaccharide.} In \textit{S. oneidensis} MR-1, Kouzuma and co-workers (Kouzuma \textit{et al.}, 2010) used a transposon mutagenesis approach to identify a mutant in the SO3177 gene, encoding a putative formyltransferase involved in cell surface polysaccharide biosynthesis. This mutant possessed a cell surface that was more hydrophobic than wild-type organisms, an ability to adhere better to graphite felt anodes, and a 50\% better current generation capacity, respectively. Similar to \textit{Geobacter} species, the extended respiratory chain of \textit{S. oneidensis} MR-1 is also involved in the process of dissimilatory iron-reduction. The outer membrane cytochromes MtrC and MtrF were found to be powerful reductases of chelated ferric iron, birnessite, and a carbon anode in an MFC (Bucking \textit{et al.}, 2010).

![Fig. 5. Fundamental 3-dimensional structure of the pilus “nanowire” of \textit{P. aeruginosa} strain PAK and \textit{Geobacter} sp. indicating the insulating properties of this unique appendage (Randall T. Irvin, U. of Alberta, personal communication with DJH).](image)

\textit{Type IV pili: involvement in attachment to anodic surfaces and electron conduction.} A major means by which bacteria can conduct electrons to anodic surfaces is via the synthesis of type IV pili, which emanate from the surface of many bacterial genera, including many of those listed in Table 1. These “nanowires” have been demonstrated and characterized on electrogenic \textit{Shewanella} and \textit{Geobacter} species (Reguera \textit{et al.}, 2005; Reguera \textit{et al.}, 2006).

Conduction atomic force microscopy has clearly established that these type IV pili are highly conductive under modest voltage biases. The conducting pili of \textit{Shewanella} and \textit{Geobacter} are 3-start helical assemblages of a short type IV pilin that display a high homology to the N-terminal region of the classical type IV pilin of \textit{P. aeruginosa} (Fig. 5). Recently, researchers have established that the \textit{P. aeruginosa} pilin structural protein possess a binding domain that mediates direct contact and exceptionally strong contact with stainless steel (Giltner \textit{et al.}, 2006). The pilin binding domain de-localizes electrons from the stainless steel and effectively
increases the electron work function (EWF) of the stainless steel surface substantially while a truncated monomeric form of pilin bound to the surface has a substantially lower EWF (i.e., it readily releases electrons when exposed to a voltage bias). This establishes that the *P. aeruginosa* pilin structural protein, like those of *Shewanella* and *Geobacter*, is a functional “semi-conductor.”

In *G. sulfurreducens*, the conductive pili are essential for Fe(III) oxide reduction and optimal current production in MFC’s (Reguera *et al.*, 2005; Reguera *et al.*, 2006). In fact, the bacteria that utilize insoluble iron or manganese specifically synthesize both flagella and type IV pili so that they may undergo a motile process toward these electron sources known as chemotaxis (Childers *et al.*, 2002). PilR is a recently discovered transcription factor that is required for the genetic activation of *pilA*, encoding the pilus structural subunit. Surprisingly, PilR is required for both soluble and insoluble iron reduction (Juarez *et al.*, 2009). More recently, PilR DNA-binding motifs were assessed in a genome-wide screen. More recently, using Pattern Location software, Krushkal and co-workers probed the *G. sulfurreducens* genome for candidate PilR-binding domains and identified 523 putative sequence elements (Krushkal *et al.*, 2010). These included 328 category I tandem repeat patterns 5′-[(N)₄₋₆STGTC]r-3′ and 5′-[(N)₅₋₇TGTC]r-3′, with r = 2 or 3. Many of these motifs resided immediately upstream of genes involved in pilus and flagellum, secretion, and cell wall synthesis.

**Fig. 6.** Wild-type *P. aeruginosa* 24 and 96 hr biofilms vs. those of an isogenic pilT mutant (bottom panels). Note the dramatically more robust biofilms formed by the *pilT* mutant [Reprinted from Chiang and Burrows, 2003]

**What genetic modifications should we engineer to create better electrogens?** To create a far better electrogenic bacterium, there are many means to facilitate this goal. The first would be to mutate the organism to generate more polar, conductive pili, such as *pilT* mutations in *P. aeruginosa* (Fig. 6). It is well known that both pili and flagella are known to be required for biofilm formation in electrogenic *P. aeruginosa*. Fig. 7 demonstrates that
both the type IV pilus and the single flagellum are required for optimal biofilm formation in *P. aeruginosa* (Yoon et al., 2002). Second, we would construct mutants that are fully capable of forming biofilms, yet unable to disperse from the biofilm. Third, we would limit production of polysaccharides so that nutrient or feedstock flow to electrogenic organisms would be maximized to the bacteria and not impaired matrix components such as polysaccharide DNA, lipid or protein. Fourth, we would restrict anaerobic respiratory metabolism in denitrifying bacteria such that electrons will only flow to the anode and not to another anaerobic terminal electron acceptor such as nitrate, sulfate or sulfur. Fifth, we would control cell division, as rapid cell growth could clog the anodic compartment. Sixth, we would increase the ability of certain organisms to generate mediators. Thus, such bacteria would not only generate electricity via biofilm (e.g., pilus) mediate conductivity, but also that facilitated through mediators. Finally, we would entertain the possibility of cloning in an uncoupling protein (e.g., the UCP class in eukaryotes) that would increase the rate of oxidation of substrates, thereby increasing electron flow to the anode. These are testable hypotheses currently being investigated in our research collaborations.

![Wild-type, fliC, pilA](image)

**Fig. 7.** Confocal microscopic examination of biofilm formation by *P. aeruginosa* wild-type, flagellum-deficient (*fliC*), and pilus-deficient (*pilA*) bacteria grown under anaerobic conditions. A, top view; B, sagittal view 22 mm into the biofilm. [Reprinted from Yoon et al., 2002 with permission]

### MFC Reactor Designs

As mentioned above, a typical MFC consists of an anode and a cathode separated by a proton exchange membrane to prevent direct oxidation. In fact, any reactor that can provide the anode an anaerobic environment and the cathode an aerobic one as well as a pathway for charge exchange is possible for MFC operations. For MFCs with a biocathode, the cathodic chamber can either be aerobic or anaerobic depending on whether aerobes
or anaerobes are used in the chamber (Lefebvre et al., 2008). Various MFCs were designed for different purposes.

Two-chamber MFCs

Many different configurations are possible for two-chamber MFCs. The most commonly used design is a two-chamber MFC built in a classic “H” shape (Fig. 8A), consisting of two bottles connected by a tube with a proton exchange membrane in the middle (Logan et al., 2006). The key point to this design is the use of a small membrane separating the two chambers. However, these MFCs typically have a high internal resistance because of the long distance between the two electrodes and the small surface area of the membrane, hence limiting power density output. Two-chamber MFCs are typically run in batch mode. They are especially suitable for laboratory research, such as examining power production using new substrates, electrode materials, membranes or types of microbial communities that arise during the degradation of specific compounds, or for MFC based sensors.

![Fig. 8. Different two-chamber MFCs.](image)

In order to reduce the internal resistance, MFCs with a larger membrane area and a shorter electrode distance, such as cube-type MFCs and flat-type MFCs, reduced the internal resistance substantially, thereby increasing the power generation. Kim and coworkers constructed a cube-type MFC from two Plexiglas cylindrical chambers each 2 cm long and 3 cm in diameter separated by the membrane (Fig. 8B). The reactor was utilized to study the effect of different membranes on internal resistance (Kim et al., 2007). Min and Logan (2004) designed a Flat Plate MFC (FPMFC) with only a single electrode/PEM assembly (Fig. 8C). A carbon-cloth cathode that was hot pressed to a Nafion PEM is in contact with a single sheet of carbon paper that serves as an anode to form an electrode/PEM assembly. The anodic chamber can be fed with wastewater or other organic
Biomass and dry air can be pumped through the cathodic chamber without any liquid catholyte, both in a continuous flow mode (Min and Logan, 2004). The smallest MFC for power generation reported so far was built by Ringeisen et al. (2006), which had a diameter of about 2 cm with a high volume power density. This type of miniature MFC reactor has great potential to be used as power source for autonomous sensors in remote area for long-term operations. Recently, Hou et al. (2009) described a micro-array of 24 MFCs through microfabrication for the purpose of fast screening of electrogenic microbes.

The above two-chamber MFCs, however, are not attractive in scaling up because of their complexity and high costs. He and coworkers used a tubular upflow MFC that was made from Plexiglas (He et al., 2005). The cathode chamber (9 cm tall, 250 cm³ in wet volume) was located on the top of the anode chamber (20 cm tall, 520 cm³ in wet volume), and both of them were packed with reticulated vitreous carbon (RVC) (Fig. 8E). CEM (cation exchange membrane) was installed between two chambers with a 15° angle to the horizontal plane, which prevented accumulation of gas bubbles. The reactor produced up to 170 mW/m² membrane area when fed a sucrose solution in anode and ferricyanide solution in cathode, with Coulombic efficiencies ($C_E$) ranging from 0.7% to 8.1%. The reactor had an internal resistance of 84 Ω, which limited power production and likely contributed to the low $C_E$.

**Single-chamber MFCs**

It is not essential to have a cathode filled with liquid catholyte or in a separate chamber when oxygen was used at the cathode. Therefore, the cathode can be in direct contact with air either in the presence or the absence of a membrane (Logan et al., 2006). This type of MFC was referred to as a single-chamber MFC. Single-chamber MFCs have many inherent advantages over two-chamber MFCs, such as simpler designs, cost savings, and no need for aeration in the cathode chamber. Fig. 9 shows the schematic of a typical single chamber MFC.

![Fig. 9. Schematic of a typical single chamber MFCs](image)

Several variants have emerged in an effort to increase power density or allow continuous flow through the anode chamber. Liu and Logan (2004) designed a cube-type air-cathode reactor, which consists of an anode placed inside a plastic cylindrical chamber (28 ml) and a cathode placed outside (Fig. 10A). The anode was constructed of carbon paper without wet proofing, while the cathode was either a carbon electrode/PEM assembly or a standalone rigid carbon paper without PEM. The power produced with glucose in the original tests was 494
mW/m² in the absence of the CEM, which is 232 mW/m² higher than that with the CEM. A side-arm bottle MFC was developed and tested using both pure and mixed cultures (Logan et al., 2007). Actually, it resembles an H-type MFC without the cathode bottle. A graphite brush was used as the anode and carbon paper with Pt-coated on the bottle facing side served as the cathode (Fig. 10C). The reactor produced a maximum power density (with glucose as substrate) of 1430 mW/m² (2.3 W/m³, CE=23%).

Fig. 10. Different single-chamber MFC designs. (A) Cube-type MFC. [Reprinted from Liu and Logan, 2004, with permission] (B) Horizontal tube-type MFC. [Reprinted from Liu et al., 2004, with permission.] (C) Side-arm bottle MFC. [Reprinted from Logan et al., 2004, with permission] (D) Upflow-type MFC. [Reprinted from You et al., 2007, with permission.]

Among single-chamber MFC designs, tubular MFC systems are gaining popularity again because of their scalable characteristics. Open-air cathodes can be put either outside or inside the anode chamber. Liu and coworkers constructed a horizontal tubular single-chamber reactor with cathode inserted in the center of an acrylic tube (Fig. 10B). The anode consisted of eight graphite rods surrounding the cathode. The cathode was made by hot pressing a CEM (Nafion) to carbon cloth, and wrapping the cloth around a tube drilled with holes to allow oxygen transfer to the cathode surface. The reactor removed 80% of the COD and generated a power density of 26 mW/m² (CE < 12%) (Liu et al., 2004). You et al., (2007) reported a tubular MFC system with an outer cathode and an inner anode using carbon granules (Fig. 10D). The anode chamber was a 3-cm in diameter, 13.5-cm high and 20-mm thick cylindrical Plexiglas tube with small holes (2.0 mm in diameter) on the wall for proton transport from the anode to the cathode. The cathode was made of a piece of flexible Pt-coated (0.8 mg/cm²) carbon cloth tightly bonded around the outside wall of the tube. This reactor has an internal resistance of 28 Ω and produced a maximum power density of 50.2 W/m³ (You et al., 2007). Flexible materials are often used for the air cathode of tubular MFCs so that the tubular MFCs are easily scaled up. However, with increasing volume, mechanical strength of the cathode material becomes a concern, because hydrostatic pressure on the cathode may cause its deformation and water leakage.
**Stacked MFCs**

Though MFCs can directly harvest electricity from biodegradable organic matters, the electricity produced from a single MFC is limited due to the low voltage and current density of a single cell unit. Stacked MFCs either in series or parallel or their combination seems to be a simple and easily used means to solve this problem. Only a select studies in the literature have reported stacked MFCs. The results of these studies demonstrated that enhanced voltage or current output can be achieved by stacked MFCs (Aelterman et al., 2006), but voltage reversal remains a large obstacle (Oh and Logan, 2007).

Aelterman and coworkers designed a 6-cell stack and tested the performance of the stack with acetate as the substrate and ferricyanide as the catholyte (Fig. 11A). The individual MFC cells were separated by rubber sheets and connected using copper wires. The anode and cathode chambers (each 156-ml total volume, 60-ml liquid volume) contained graphite rods in the beds of graphite granules. The reactor produced a power density of 59 W/m$^3$ in stack (parallel) mode and 51 W/m$^3$ in series mode based on a total volume of 1.9 L. The average $C_F$ for the cells was 12% when cells were arranged in series. It increased to 78% when operated in parallel. Voltages from the cells were found to be unequal when cells were connected in series. Some cells even yielded negative voltages that adversely affected power generation (Aelterman et al., 2006). Oh and Logan (2007) showed that substrate depletion could drive a cell into voltage reversal. Logan (2008) suggested that chemical fuel cells can be easily matched in power output to avoid voltage reversal while stacked MFCs often suffer voltage reversal due to fluctuations in biological systems. A possible solution to alleviate this problem may be the use of diodes.

Fig. 11B shows a bipolar-plate type of stacked MFC with five cells used by Shin et al. (2006). The reactor used thionin as a mediator for electron transfer by *Proteus vulgaris* grown on glucose. They reported a power density
of 1300 mW/m² with ferricyanide as the catholyte and 230 mW/m² with pure oxygen gas. Liu et al. (2008) used a novel design consisting of stacked MFCs bridged internally through an extra cation exchange membrane (Fig. 11C). The MFC stack, assembled from two single MFCs, doubled voltage output and halved the optimal internal resistance. The COD removal rate increased from 32.4% to 54.5%. The performance improvement could be attributed to the smaller internal resistance and enhanced cation transfer. Their investigation of a half-cell study further confirmed the important role of the extra CEM. This study proved that it was beneficial in terms of increased voltage output and reduced cell resistance when the anode and cathode were sandwiched between two CEMs (Liu et al., 2008).

Materials Used in Constructing MFCs

Anode materials
In an MFC, the anode is where electricity-producing bacteria form a biofilm and it is the receptor of electrons. Therefore, an ideal anode materials should be highly conductive, non-corrosive, possessing a high specific surface area, non-fouling, inexpensive and easily made Logan (2008). A variety of materials have been used as anodic electrodes, including carbon materials, graphite materials, conductive polymers, and metals. Table 2 lists the commonly used electrode materials and their properties.

Table 2. Commonly used electrode materials in MFCs

<table>
<thead>
<tr>
<th>Materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon paper</td>
<td>High conductivity</td>
<td>Brittle, low specific surface area, expensive</td>
</tr>
<tr>
<td>Carbon cloth</td>
<td>High conductivity, flexible, high specific surface area</td>
<td>Expensive</td>
</tr>
<tr>
<td>Reticulated vitreous carbon</td>
<td>High conductivity, high porosity, large specific surface area</td>
<td>Brittle</td>
</tr>
<tr>
<td>Graphite rod</td>
<td>High conductivity, defined surface area</td>
<td>Low specific surface area, expensive</td>
</tr>
<tr>
<td>Graphite felt</td>
<td>High conductivity, high porosity, large specific surface area, flexible</td>
<td>Low strength</td>
</tr>
<tr>
<td>Graphite granules bed</td>
<td>Low cost, high porosity, high surface area</td>
<td>High contact resistance</td>
</tr>
<tr>
<td>Graphite fiber brush</td>
<td>High conductivity, high porosity, large specific surface area, flexible</td>
<td>Expensive</td>
</tr>
<tr>
<td>Conductive polymers</td>
<td>Large surface area, flexible</td>
<td>Low conductivity</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>High conductivity, Low cost</td>
<td>Poor bacteria attachment, low power production</td>
</tr>
</tbody>
</table>

In order to boost the performance of an anode, several treatment methods were reported, such as ammonia gas treatment, electrochemical treatment, heat treatment, and addition of mediators. Cheng and Logan (2007) treated carbon cloth using 5% NH₃ gas in a helium carrier gas at 700°C for 60 minutes. This reduced the start up time of MFC by 50% and increased power density from 1640 mW/m² to 1970 mW/m². Their analysis suggested that this could be attributed to the increase of the positive surface charge of the cloth from 0.38 to 3.99 meq/m². Wang and coworker found that heat treatment of carbon mesh at 450°C for 30 min decreased atomic O/C ratio and removed contaminants that interfered with charge transfer, thereby enhancing the performance of anode
(Wang et al., 2009a). Park and Zeikus (2002) bound neutral red (NR) to a woven graphite electrode and increased power to 9.1 mW/m² compared to only 0.02 mW/m² without NR, using a pure culture of *S. putrefaciens* and lactate as fuel. Tang and coworkers electrochemically treated graphite felt with a constant current density (30 mA/cm² based on the projected area of anode) for 12 hours, and this produced a current of 1.13 mA that was 39.5\% higher than untreated anodes. Their analysis showed that the newly generated carboxyl groups were responsible for the enhanced electron transfer, due to their strong hydrogen bonding with peptide bonds in bacterial cytochromes (Tang et al., 2010).

**Cathode materials**

The same aforementioned anode materials can also be used for cathodes. If ferricyanide or MnO₂ is used as the final electron acceptors, no catalyst is needed for cathodic reactions (Logan, 2008). But if oxygen is used as the final electron acceptor, a catalyst must be employed because graphite and carbon materials are poor catalysts for oxygen reduction (e.g. cathodic reaction). Platinum or a metal plated with platinum is typically used as the cathode catalyst due to its excellent catalytic ability, but platinum’s high cost makes its large-scale applications such as wastewater treatment prohibitive. Therefore, a series of metals and their complexes have been investigated as replacements for platinum in the cathode in MFC, such as Fe(III) (Park and Zeikus 2002, 2003; ter Heijne et al., 2006), cobalt complexes (Cheng et al., 2006; Zhao et al., 2005), manganese oxide (Mao et al., 2003; Rhoads et al., 2005), lead dioxide (Morris et al., 2007), and manganese dioxide (Li et al., 2010). Some of these non-precious catalysts could produce a power density at a level comparable to those achieved with Pt-based cathode, but the longevity of such materials is not well studied.

In order to avoid using expensive metal catalysts, bacteria have been used as biocatalysts in the cathode chamber. This is known as a biocathode (He and Angenent 2006; Huang et al., 2011). A biocathode can be either aerobic or anaerobic. Aerobic biocathodes rely on aerobes to catalyze the oxidation of transition-metal compounds, such as Mn(II) or Fe(II) by oxygen. Anaerobic biocathodes utilize anaerobes or facultative anaerobes capable of reducing non-oxygen oxidants such as nitrate, nitrite, sulfate, iron, manganese, selenate, arsenate, urinate, fumarate and carbon dioxide (Lefebvre et al., 2008). These non-oxygen oxidants serve as terminal electron acceptors.

**Membranes**

The majority of MFC designs require the separation of the anode and the cathode compartments by a membrane. The purpose of the membrane is to keep anode and cathode solutions separated while allowing ion transfer. This prevents direct oxidation of organic matters that happens when oxidants cross into the anode chamber from the cathode chamber. Nafion is the most commonly used CEM to allow the passage for ion exchange while partitioning the anode chamber and the cathode chamber. Besides CEM, AEM, bipolar membrane (BPM), charge mosaic membrane (CMM), ultrafiltration membrane (UFM) may play the role of facilitating the transport of ions through the membrane in order to maintain electroneutrality in MFC systems. Fig. 12 shows the theoretical working principle of membrane charge transport of four different types of ion exchange membranes.
Laboratory MFCs often use membranes to partition the anode chamber and the cathode chamber. A major disadvantage of using membranes is their high costs and fouling. Membranes also present significant internal resistances that lead to reduced power production (Logan, 2008). There is a growing consensus among MFC researchers that it is not essential to use membranes in MFC to separate the biological anode from the cathode reactions. Salt bridge (Min et al. 2005a), porcelain septum (Park and Zeikus, 2003), microporous filter (Biffinger et al., 2007) and physical barriers (Jang et al., 2004) are alternative ion exchange systems. In fact, it was observed that the anode could even be kept anaerobic due to diffusional resistance between the anode and the cathode without any physical partitioning (Bond et al. 2002). For example, in a sediment MFC, nothing but the aqueous solution separates the anode and from the cathode. The distance between the two electrodes serves as the barrier for oxygen diffusion although it is not 100% efficient.

**MFC Applications**

**Power supply**

MFCs are able to convert the chemical energy stored in the chemical bonds of organic compounds into electricity instead of producing heat from their direct oxidation that is limited by the Carnot cycle thermal efficiency. Therefore, just like chemical fuel cells, MFCs possess much higher conversion efficiency (>70%) (Du et al., 2007). However, So far, the power produced by MFCs is still too low to be useful in most applications. And it is likely that even with major advances in the future, MFCs will not contribute to the power grid. After all, MFCs do not use high density fuels such as pure hydrogen used in a hydrogen fuel cell. But MFCs are especially suitable for powering small telemetry systems and wireless sensors that are not energy intensive in remote area (Ieropoulos et al., 2005a; Shantaram et al., 2005).

Ieropoulos et al. (2005) developed the robot EcoBot-II and used MFCs as the onboard energy supply. EcoBot-II was able to perform sensing, information processing, communication and actuation when fed with flies (substrate). A microbial fuel cell consisted of a sacrificial anode combined with the reduction of biomineralized...
manganese oxides was designed to power electrochemical sensors and small telemetry systems to transmit the data acquired by the sensors to remote receivers. In order to ensure enough power was supplied, energy produced by the MFC was stored in a capacitor and used in short bursts when needed (Shantaram et al., 2005). A Benthic Unattended Generator (BUG) MFC was also tested to power a data buoy that monitored air temperature, pressure, and humidity as well as water temperature. The system radioed data every 5 min to a shore-based receiver (Tender et al., 2008). MFCs for a long-term space flight such as a mission to Mars are attractive since they can generate electricity while degrading organic wastes generated onboard a spaceship. It is conceivable in the future that a miniature MFC inside a human body fueled by the nutrients inside the body can be used to power an implanted medical device for long-term uses (Chiao et al., 2007).

**Wastewater treatment**

MFCs are regarded as a promising future technology for wastewater treatment for the several reasons. Firstly, MFCs are able to harvest energy from organic matters and treat wastewaters at the same time. The amount of power generated by MFCs in the wastewater treatment process reduces the power input needed in the aerobic treatment stage (Du et al., 2007). Secondly, MFCs yield 50 - 90% less solids to be disposed of (Holzman, 2005), because organic molecules such as acetate, propionate, butyrate can be broken down to CO₂ and H₂O. Thus, using MFCs at a wastewater treatment plant could substantially reduce the operating costs for solids handling. Thirdly, a wide range of organic wastewaters can be treated by MFCs, if proper electricity-producing bacteria were enriched in the anode. Wastewaters that are rich in organic matters are all great biomass sources for MFCs, such as sanitary wastes, food processing wastewater, swine wastewater and corn stover (Suzuki et al., 1978; Liu et al., 2004; Oh and Logan, 2005; Min et al., 2005b; Zuo et al., 2006). Fourthly, unlike the expensive pure hydrogen used in a hydrogen fuel cell, these wastes are practically free fuels for MFCs. Finally, MFCs with biocathodes use wastewater that contains oxidants other than oxygen as terminal electron acceptors, allowing treatment of two different wastewater streams at the same time with concomitant electricity generation (Virdis et al., 2010; Huang et al., 2010). MFCs also hold promises in some niche applications such as reduced power demand in treating black or grey wastewaters at military camps where fuel costs are up to 10 times higher than normal (such as some US military camps in current war-torn Afghanistan.)

The current densities generated by laboratory MFCs almost approach levels that required for practical applications. However, so far, those MFCs with high power density that required for practical applications are typically operated on small scales, varying from just a few milliliters to several liters (Rozendal et al., 2008). What’s more, the performance of MFCs with real organic wastewater as the electrolyte and substrate was far lower than artificial wastewaters (Pant et al., 2010). Therefore, scaling up of the MFCs for wastewater treatment is not straightforward because of certain microbiological, technological and economic challenges. More work is required using real wastewater streams. It is also imperative to reduce the capital costs for large-scale MFC devices if they are to be used for wastewater treatment (Rozendal et al., 2008).
Biohydrogen production

The MFC can be modified for hydrogen production by adding a small voltage to the system and omitting oxygen from the cathode, and the MFC-like reactor is referred to as microbial electrolysis cell (MEC) (Liu et al., 2005; Rozendal, 2005). Fig. 13 shows the fundamental principle of MEC. In an MEC, exoelectrogens oxidize organic matters, generating CO$_2$, electrons and protons in the anode chamber. Electrons and protons are transported to cathode via external circuit and the PEM, respectively. An external voltage is added to the circuit, allowing hydrogen production at the cathode. Typically voltages of 0.3V or larger are needed to overcome electrode overpotentials when acetate is degraded, which is much less than the voltages required for water electrolysis which is typically 1.8 - 2.0 V (Rozendal et al., 2006a). If a more energetic organic carbon such as lactate that has a more negative standard reduction potential than acetate, the external voltage requirement would be substantially less or even eliminated.

![Fig. 13. Schematic diagram of a typical two-chamber MEC.](image)

Since both the anodic chamber and the cathodic chamber are anaerobic in a MEC, membrane (e.g., PEM) is not necessary for the system. It has been proven that removing the membrane from MECs can not only reduce the capital costs and simplify the reactor design, but also lower the internal resistance, thus eliminating the pH gradient across the membrane, and enhancing the hydrogen production rate (Call et al., 2008; Hu et al., 2008). Hence, single-chamber MECs become more attractive for scale-up. However, without a membrane, hydrogen consumption by methanogens reduces the hydrogen recovery and purity, and hydrogen reoxidization by exoelectrogens increases energy losses (Wang et al., 2009b; Clauwaert et al., 2009).

While MECs were invented only a few years ago, performances of MECs have improved significantly in terms of hydrogen production rate, conversion efficiency of substrate and energy recovery (Logan et al., 2008). MECs are able to convert a wide range of low-grade organic matters into hydrogen (Cheng et al., 2007; Ditzig et al., 2007), and the energy input is many times smaller than that of water electrolysis. Therefore, MEC is a promising new technology for renewable and sustainable biohydrogen production. However, further research work is needed to reduce construction costs, enhance hydrogen production rate, and inhibit methane generation in MECs.
Challenges and Prospects

While MFCs hold great potentials for various applications, major challenges remain for MFCs to be practical. Firstly, the power densities of MFCs must be augmented because they are too low for most envisioned application. One key bottleneck for MFCs is the electron transfer rate limitation in microbial biofilms. A breakthrough may be achieved by fundamental observations, or through transposon (or other) mutagenesis or hypothesis-driven genetically engineering new super-bugs that possess large number of pili per cell and/or secrete efficient electron mediators at sufficiently high local concentrations. These may be achieved by several microbes working together in a synergistic biofilm consortium, where one organism provides a by-product of normal metabolism that another organism can use to continue an electrogenic cycle of events. Conceivably, such a breakthrough can greatly reduce or eliminate the electron transfer bottleneck. Using the powerful traits of the voraciously metabolic bacteria, \textit{P. aeruginosa}, a number of viable strategies were outlined earlier to improve electron transfer. Using such avirulent organisms, and coupled with other bacteria that could offer synergistic properties to the MFC, exciting new breakthroughs in MFC research are ahead.

Another bottleneck in MFCs is the high internal resistance, resulted from slow proton diffusion from anode to cathode and slow oxygen reduction kinetics at the cathode (Kim \textit{et al.}, 2007). In MFCs, catalyzed cathodic reaction consumes more protons at the cathode than the membrane can deliver. This causes a pH gradient across the membrane and therefore increases the internal resistance. Proton transfer in an aqueous solution is seriously hampered by other cations, such as Na$^+$, K$^+$, NH$_4^+$ and Mg$^{2+}$, whose concentrations are typically 10$^5$ times greater than that of protons at neutral pH (Rozendal \textit{et al.}, 2006b; Zhao \textit{et al.}, 2006). Development of proton-specific membrane may alleviate this problem (Kim \textit{et al.}, 2007). Another effective approach is through optimized reactor design, such as shortening the distance between the anode and the cathode (Logan \textit{et al.}, 2006; Du \textit{et al.}, 2007). Using platinum as the catalyst for oxygen reduction could reduce the cathodic overpotential, achieving a maximum current three to four times higher (Pham \textit{et al.}, 2004). However, the cost of using platinum is prohibitive for large-scale applications such as wastewater treatment.

Secondly, the construction cost of MFCs must be reduced. Currently, expensive proton exchange membrane (such as Nafion) and precious metals (such as platinum) are generally used in lab-scale MFCs. They account for more than 80% of the construction cost. The high cost of these materials makes MFCs uncompetitive against energy production from wind and solar and biofuels. MFCs also need to compete with inexpensive methane digesters that are popularly use in some third world countries to produce methane for household cooking and lighting (Verstraete \textit{et al.}, 2005). If expensive laboratory materials are used, the capital costs of full-scale MFCs will be orders of magnitude higher than those of conventional wastewater treatment systems (Rozendal \textit{et al.}, 2008). Low cost materials are desired. Researchers have demonstrated that Nafion membranes can be substituted with relatively inexpensive ion exchange membranes (Kim \textit{et al.}, 2007b) and some designs do not even require membranes (Liu and Logan, 2004). To avoid using expensive platinum for catalysis of oxygen reduction, various metals and their complexes have been investigated as materials for cathodes in MFCs, such as...
Fe(III) (Park and Zeikus, 2002; 2003; Ter Heijne et al., 2006), cobalt complexes (Cheng et al., 2006; Zhao et al., 2005), and manganese oxide (Mao et al., 2003; Rhoads et al., 2005) have also been studied. These materials cost much less than platinum although they tend to be less effective (Kim et al., 2007). Zhao et al., (2005) claimed that Iron(II) phthalocyanine and cobalt tetramethoxyphenylporphyrin catalyzed oxygen reduction in some tests with comparable performance as platinum. Biocathodes do not need expensive metal catalysts because they use biocatalysis to reduce non-oxygen oxidant. This presents a different approach to the reduction of MFC costs. Biocathodes also have the added ability to process a second wastewater stream in the cathode chamber (Huang et al., 2010).

Thirdly, there is still a lack of understanding of electricity-producing bacteria’s electron transfer mechanisms, especially in microbial communities involving electrochemically inactive bacteria. Researchers found that in MFCs with electrochemically active iron-reducing bacteria such as Shewanella and Geobacter species, there were more non iron-reducing bacteria in the biofilm communities (Logan and Regan, 2006). These electrochemically inactive bacteria enhanced electricity generation by the electrochemically active bacteria because MFCs with pure cultures generated 2-3 orders of magnitude less power (Rabaey et al., 2004). It is possible that some of the electrochemically inactive bacteria secreted electron mediators that accelerated electron transfer by the electrochemically active bacteria. It is also possible that there are still undiscovered electrochemical mechanisms in synergistic biofilm communities that can be exploited to improve MFC performances. With a better understanding of how biofilm communities function electrochemically new super-bug communities can be generically engineered to improve MFC performances beyond any conventional approaches could achieve.

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