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Internal Resistance of Microfluidic Microbial Fuel Cell: Challenges and Potential Opportunities

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Abstract

The efficiency of microbial fuel cells (MFCs) is affected by several factors such as activation overpotentials, ohmic losses and concentration polarization. These factors are handled in micro-sized MFCs using special electrodes with physically or chemically modified surfaces constructed with specified materials. Most of the existing µL-scale MFCs show great potential in rapid screening of electrochemically-active microbes and electrode performance; although they generate significantly lower volumetric power density compared with their mL counterparts because of their high internal resistance. This review presents the development of microfluidic MFCs, with summarization of their advantages and challenges, and focuses on the efforts done to minimize the adverse effects of internal resistance (ohmic and non-ohmic) on their performance.

Running title: Complications of micro-scale microbial fuel cells

Keywords: Microfluidic microbial fuel cell; internal resistance; microelectromechanical systems
1. Definitions and General Remarks of MFC

Microbial fuel cells (MFCs) are bio-electrochemical systems that use bacterial metabolism to generate electrical current from a variety of organic substrates (Pant et al., 2012). The use of microorganisms eliminates the separation of individual enzymes, thus providing economical biocatalysts for biological fuel cells.

Classic MFCs are designed with cathode and anode chambers separated by a proton exchange membrane (PEM) (Fig. 1). Organic substrates are oxidized at the anode via bio-catalyzed reactions in which the released electrons are transferred to the cathode, to form the current, via an external electrical circuit that connects both electrodes. Suitable electrocatalysts
(including some of biological origin) complete reduction reactions at the cathode, where the protons transported via the PEM are combined with electrons and oxygen to form water. It is now evidenced that electricity can be produced from any biodegradable substrate, ranging from pure fuels such as glucose, acetate, ethanol, cysteine and bovine serum albumin to complex mixtures of organic matter including domestic, animal, meat-packing and food-processing wastewaters (Pant et al., 2010). The driving force of a typical MFC using glucose as fuel can be articulated at anode and cathode, respectively, as follows (Grzebyk and Pozniak, 2005):

\[
C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \\
6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O
\]

Interaction between electrodes and microbes can occur via direct electron transfer (DET) or mediated electron transfer (MET). In DET, microbial enzymes are set in such a way that electronic states in the surface material and enzyme active center (or other conductive structures) overlap, increasing the probability of electron transfer across the interface. In MET, natural or artificial electron transferring agents can readily participate in redox reactions of biological components. Accessibility, suitable redox potentials, electrostatic interactions, pH and ionic strength are major factors that play a role in facilitating MET (Dominguez-Benetton et al., 2012).

MFCs have several advantages over the conventional technologies currently used for producing energy from organic matter. First, high conversion efficiency is enabled from the direct transformation of substrate energy (contained in chemical bonds) to electricity (Logan, 2007). Second, MFCs take advantage of general biochemical reaction, based on the physiology of microbial communities, without any extra energy input for aeration on the cathode. Third, an MFC does not require gas treatment because the off-gases of MFCs are mainly composed by carbon dioxide which does not have any useful energy content (as compared to e.g. methane from anaerobic biodigestion). Furthermore the redox products are usually CO₂ and H₂O, which
are not considered contaminants. Fourth, MFCs can use a huge range of resources as their fuel because they do not need to be high quality. Wastes and wastewaters apply since they are generally full of organic compounds of which most are biodegradable (Rabaey and Verstraete, 2005). Fifth, MFCs have the potential for general application in sites lacking electrical infrastructures and also to inflate the diversity of fuels we use to fulfill our energy requirements (Logan, 2007).

2. Overpotentials: Bottlenecks of MFC

There are several bottlenecks which limit the performance of large scale implementation of MFC technology, i.e. overpotential associated with preceding chemical or biochemical reactions (Bard and Faulkner, 2001). However, the direct flow of electrons from the bacteria to the electrode is hindered mainly by the transfer resistances which are known as overpotentials (Larminie and Dicks, 2000). These overpotentials minimize the potential achieved from the MFC and therefore decrease the energy efficiency. The losses can be mainly classified as activation overpotentials, concentration polarization and ohmic losses.

Electrochemical reactions at MFC electrode surfaces require certain activation energy, for charge transfer to either complete substrate oxidation at the anode (or for preceding biochemical electron transfer from a reducing equivalent) or reduce oxygen at the cathode (Larminie and Dicks, 2000). The activation overpotential could be decreased by increasing the operation temperature, up till certain extents, only for indirect systems. Also, it could be decreased by the addition of a catalyst to the electrode (Schroder et al., 2003). Increasing the roughness and specific surface of the electrode decrease the current density and hence the activation losses.

Concentration polarization occurs when compounds are being oxidized faster at the anode than they can be transported to the surface, and this could be due to the large oxidative
force of the anode (Rabaey et al., 2005b). Concentration polarization would be a problem, in cases where diffusion is seriously hindered by, for example, a thick non-conductive biofilm, hydrodynamics and geometrical aspects of the cell design.

Ohmic losses are due to electrical resistances of the electrodes, membrane and electrolyte. However, the resistance over the MFC can increase rapidly by suboptimal contacts or limited conductivity and turbulence of the electrolyte. The structure of the anode should support free flow of influent and effluent, growth of a biofilm, conductivity and sufficient turbulence for adequate proton diffusion towards the membrane and cathode. The cathode resistance could be inclined by using effective materials or microorganisms as catalyst, increasing the specific surface area and the mass transport rate, or adapting the electrolyte solution (Sleutels et al., 2012). Furthermore, it has been observed that cathode resistance could be significantly reduced by increasing the size of the cathode compared to the anode (Fan et al., 2008).

The selection of a separating membrane between anode and cathode represents a choice between two disparate interests: high selectivity for protons and high stability in a bacterial colloidal and nutrient rich environment. Nafion™ has been widely used as proton exchange membrane (PEM) for fuel cells and MFCs (Bond and Lovley, 2003), and has the large advantage of being very selective for protons. This membrane type scores high for selectivity but low for stability. The limitation for applying membranes lies in the development of a pH gradient between anode and cathode which involves an additional energy loss. Alternatively, a high pH value at the cathode enables a lower cathodic resistance, in which some strategies were tested to decrease this pH gradient, i.e. CO₂ addition to the cathode (Sleutels et al., 2012). A second approach is the use of a more general cation exchange membrane (CEM), such as Ultrex™ (Rabaey et al., 2003). This type of membrane has a larger resistance and is less selective but
generally shows larger stability. These membranes have been reported to perform adequately for over three months (Rabaey et al., 2005a). Several studies commonly reported MFC challenges caused by the membrane, which are pH gradient in the anode chamber, O\textsubscript{2} diffusion, H\textsubscript{2} loss, fluxes of carboxylates, and concentration overpotential across the membrane (Torres et al., 2008a).

On the other side, losses occur in the cathode compartment due to overpotentials. To decrease the activation overpotential, catalysts need to be added to the electrode, or a suitable mediator is needed to transfer the electrons from the cathode to oxygen. Generally, Pt is used as a catalyst in the electrode (Schroder et al., 2003), at concentrations up to 45% w/w, entailing a considerable cost. However, activated carbon (AC) air-cathodes are inexpensive and useful alternatives to Pt-catalyzed electrodes in MFCs in terms of cathode performance and cost (Zhang et al., 2011). Recently gas diffusion electrodes based on activated carbon have been proposed as an alternative to low-cost cathodes for MFCs (Pant et al., 2011).

3. Microfluidic MFC Integrated Research

The mL-scale devices serve as convenient tools for exploring the interplay between device architecture and electrochemically-active microbes, as well as fundamental problems in electron transfer at the microbial/anode interface. Indeed, owing to the flexibility in adopting favorable configurations for reducing internal resistance and improving mass transport, the best results to date have been achieved from mL-scale MFCs. The appreciable power output and small volume of milliliter to liter MFCs suggest their potential use as portable power supplies, especially if connected in series to achieve increased voltage and power (Dekker et al., 2009). There is a growing interest in the development of even smaller MFCs, in which the effective chamber volumes are reduced to the (sub) microliter regime. This desire is motivated by the fact that we still do not have an in-depth understanding of the extracellular electron transfer processes
associated with MFC operation, especially for a small colony of bacterial cells. µMFCs can enable crucial studies of these processes in a smaller group of cells with excellent control over the microenvironment, thus serving as a versatile platform for fundamental MFC studies.

The advantages of µMFCs originate from their unique structural features and scales. Prepared by microfabrication techniques, µMFCs typically feature chambers with well-defined thicknesses (tens to hundreds of micrometers) and high surface area/volume ratios. As an immediate result, the ratio of microbial cells coupled to the solid electrodes relative to the cells that remain in suspension increases proportionally with the surface area/volume ratio. In this case the system must operate in continuous mode, since the volume of electrolyte containing the substrates of interest would not be enough to sustain growth for long time. Potential operation in continuous mode brings in other problems, such as how to fix an appropriate hydraulic retention time, which ultimately will represent a dilution factor and more ultimately will directly affect the growth rate. Besides, as electrode size becomes smaller, the “border effect” becomes larger, in which the electrical field lines at the borders of an electrode are not normal with respect to the horizontal plane of the electrode. This is relevant because when the electrical field distribution lines are normal to the equipotentials, then the electrical field can be considered uniform. At the borders, the lines are not normal and hence uniformities in the electrical field are present. This will also affect other parameters, such as diffusion, especially for non-planar micro- and ultra-micro- electrodes (Mignano, 1995). Conversely, because the current generation in an MFC is associated with local electron transfer at the microbial/electrode interface, the number of electrically addressable cells should scale down with electrode area. In addition, µL-MFC arrays that are individually addressable are emerging as a versatile platform for high throughput identification and characterization of electrochemically-active microbes (Choi and Chae, 2012a). Compared with conventional H-shaped, two-chamber MFCs, µL-MFC arrays consume less
materials and reagents, enable parallel, reproducible and cost-effective analysis of electrogenic strains, and thus can greatly accelerate MFC research. Although there have been many reports on microfluidic investigations of microorganisms (Weibel et al., 2007), only a few have thus far described investigations of µMFCs. This is probably because the typical MFC reactor requires a relatively complicated dual-chamber configuration that is separated by an appropriate ion exchange membrane, and contains anode, cathode and well-separated anolyte and catholyte. In addition, the bulky carbon-based electrodes in conventional MFCs are not compatible with the microfabrication processes.

4. Advantages and Challenges of Microfluidic MFC

Assuming the overall reaction rate is mass transfer limited; constructing MFCs in smaller scales provides possible higher surface mass flux. For the transport of substrates from bulk to the electrode (biofilm) surface, most of the mass transfer resistance is presented in a thin stagnant film outside the solid surface (Dominguez-Benett on et al., 2012). The mass transfer coefficient increases when the characteristic length of electrode ($L_E$) decreases. Therefore, comparing macro-scale and micro-sized MFCs having similar electrode shapes, flow conditions and bulk substrate concentrations, the later ones offer higher mass flux density per unit area and significant “border effect”, respectively. Additionally, stirring is not necessary in micro-sized MFCs since competitive mass flux density can be achieved with low fluid velocity (Wang et al., 2011). Large surface area/volume ratio is another advantage of micro-sized MFCs. The large surface area/volume ratio represents more efficient usage of substrates per unit volume due to the merit of increased surface area for mass transport and reactions. Anodes with microstructures or surface coatings not only increase the surface area for current collecting but also improve the coupling ability of microbes to the electrode surface (Crittenden et al., 2006; Pocaznoi et al., 2012; Siu and Mu, 2008). The micro-/nano-topology affects bacterial adhesion and the
production of extracellular- polymeric materials, which are important in the formation of biofilm (Park et al., 2008). Microstructures having dimensions comparable to biological cell sizes result in the strongest cell-surface adhesion (Whitehead et al., 2006). Last but not the least, the fabrication cost and time of micro-sized devices have been decreased dramatically since the development of soft lithography (Duffy et al., 1998), which is beneficial for developing cost-effective micro-sized MFCs.

The internal resistance of a MFC consists of two parts: non-ohmic and ohmic resistances (Fan et al., 2008). Non-ohmic resistance includes charge-transfer resistance and diffusion resistance (Larminie and Dicks, 2000) and these can be decreased by methods such as increasing the projected surface area of anodic and cathodic electrodes (Fig. 2A) and selecting electrodes with good catalytic abilities. The ohmic loss contributes a considerable part of the power deprivation in MFCs. The ohmic resistance of MFCs can be reduced by enlarging the geometric area between cathode and anode (usually the size of exposed PEM) (Fig. 2B), arranging the electrodes closely (Fig. 2C) and using solutions with high conductivity (Fig. 2D) (Benetton et al., 2010). The voltage across an ion exchange membrane is defined as the potential difference between the anode and cathode electrodes and ohmic resistance can be written as:

\[ \Delta V_\Omega = \frac{(d \times I)}{(K \times A)} \]  

(1)

where \( d \) is the distance between electrodes, \( I \) is the current, \( K \) is the conductivity of solutions, \( A \) is the cross-sectional area (cm\(^2\)) through which ionic conduction occurs. The ohmic resistance is directly proportional to the distance between electrodes and is inversely proportional to the anode area. Therefore, the ohmic resistance can be most efficiently reduced by decreasing the ratio \( d/A \) (Torres et al., 2008a). An important aspect of achieving high volumetric power density is having a small energy loss, as described by equation (2) in terms of maximum available output voltage.
\[ E_{\text{MFC}} = \text{OCV} - IR_{\text{int}} = \text{OCV} - I \times (R_{\text{ohm}} + R_{\text{ac}} + R_{\text{cc}} + R_{\text{sa}} + R_{\text{ca}} + R_{\text{mem}} + R_{\text{c/b}}) \]  \quad (2)

where \( R_{\text{int}} \) is internal resistance; and \( R_{\text{ohm}} \) is electrolyte resistance; \( R_{\text{ac}} \) is anode concentration polarization; \( R_{\text{cc}} \) is cathode concentration polarization; \( R_{\text{sa}} \) is anode activation overpotentials; \( R_{\text{ca}} \) is cathode activation overpotentials; \( R_{\text{mem}} \) is resistance of membrane and \( R_{\text{c/b}} \) is resistance of chemical or biochemical reactions preceding the electrochemical ones (Dominguez-Benetton et al., 2012). For a given current density, energy losses in a fuel cell should be minimized to maximize OCV, consequently improving power density. While \( R_{\text{ac}}, R_{\text{cc}}, R_{\text{sa}}, R_{\text{ca}} \) and \( R_{\text{mem}} \) depend on material properties, microelectromechanical systems (MEMS) technology can reduce electrolyte resistance (\( R_{\text{ohm}} \)) by reducing the distance between electrodes. \( R_{\text{ohm}} \) can be expressed by:

\[ R_{\text{ohm}} = 1 / (A \times K) \]  \quad (3)

where \( I \) is electrode distance (cm) and \( A \) is the cross-sectional area (cm\(^2\)) through which ionic conduction occurs, and \( K \) is the specific conductivity (\( \Omega^{-1} \text{ cm}^{-1} \)) of the electrolyte (Fan et al., 2008).

5. Microfluidic MFC State of Art

5.1 Fabrication Methods

\( \mu \text{MFC} \) device could be divided into two main compartments separated by PEM. The first compartment is the anode chamber and is fabricated as illustrated in Fig. 3A by SU-8 negative photoresist on gold film in which Ti/Au films are evaporated onto the polished side of a silicon wafer. The wafer is then spin coated with SU-8 2050 negative photoresist. The photoresist is prebaked prior to near-ultraviolet (UV) exposure through a negative photomask containing the anode flow channel design and then the wafer is baked and agitated in Nano SU-8 developer (Qian et al., 2009). The gold surface in the channel functions as the anode. Alternatively, a thin layer of titanium and gold could be sputter deposited on a glass substrate in which the thin layer
of titanium is used as the adhesion between the glass substrate and the gold layer (Chen et al., 2011). Diffusional mixing along the length of the channel has the impact of reducing the current. Flow rate also affects the power output by affecting Reynolds number, normally used to characterize different flow regimes such as laminar or turbulent flow, and therefore expected mixing conditions and diffusion in the channel. This deleterious effect may be minimized through an application of specific optimization of the channel length and Reynolds number.

The second compartment is the cathode chamber which is fabricated from PDMS in a silicon mold using standard soft-lithography techniques (Fig. 3B) (Whitesides et al., 2001). Silicon wafer is first patterned with positive photoresist, followed by UV exposure and dry etching to create the mold. To replicate PDMS stamps, a mixture of elastomer and curing agent was poured onto the silicon mold and cured, then peeled off. Holes for insertion of polyethylene (PE) tubing are punched through the PEM and PDMS stamp at the end positions of the anode and cathode flow channels. Conductive carbon cloth is cut to approximately 10 mm × 12 mm to serve as the cathode. Furthermore PDMS could be used for the fabrication of both electrodes and its structure was modified by the microfabrication of PDMS MFC with micropillars embedded on the electrode surfaces in order to improve electricity output (Qian et al., 2009). Commercial Nafion® 117 or CMI 7000 membranes are usually used as PEM. Nafion membrane is pretreated by sequentially boiling in H\textsubscript{2}O\textsubscript{2} and water, followed by soaking in sulfuric acid solution and then water.

To assemble the dual-chamber µMFC, the anode chip is first sterilized, then blown dry with nitrogen. The PEM, carbon cloth cathode and PDMS stamp are manually stacked in sequence while carefully aligning the tubing holes for the flow channels. A thin carbon cloth wire and an electrical wire are used to extend the anode and cathode, respectively, to the copper pins on the chip carrier. An external load connected to the pins completed the circuit. For liquid
transport, four sterile PE tubes are plugged into the holes in the PDMS stamp to form two independent routes for liquid.

5.2 Approaches to Overcome Internal Resistance

Microfluidic approaches using small channels and wells typically fabricated in PMMA and PDMS have been implemented to generate an inexpensive solution for µMFC. Table (1) highlights the main specifications of currently fabricated µMFCs aiming to reproduce mL scale microbial batch processes and achieve reasonable current and power levels, while Fig. 4 illustrates the pictorial chronological milestones of µMFCs progress. In the following paragraphs µMFCs are described based on their contribution to overcome internal resistance, as well as on their level of integrated components for a better control of the microbial microenvironment.

5.2.1 Distance between Electrodes

The shortened diffusion distance enables the electrode to be more responsive to the electrochemical change in the micro-chamber (Qian et al., 2011). Qian et al. (2009) tried to lower electrolyte resistance and enhance power output by setting up a vertically stacked µMFC with the shortest proton diffusion length (100 µm), and the resulted power density was 1.5 mW/m². Also, in two different studies, µMFCs were fabricated using the same gold electrodes and applying the same microorganism (Saccharomyces cerevisiae), but with a different proton diffusion distances. In the first study the distance between electrodes was 383 µm (Siu and Mu, 2008), whereas, in the second one it was 160 µm (Chiao et al., 2006). The power density obtained in both studies was inversely related to the distance between electrodes, while it was 4.012 mW/m² from the first setup, in the second one, only 0.023 mW/m² could be obtained.

Higher internal resistance is expected for micro-scale electrochemical devices due to substantial increases in contact and electrolyte resistances associated with small contact area, although a reduced proton diffusion length can partially counteract this effect.
5.2.2 Electrodes Surface Area/Volume Ratio (SAV)

A good configuration for bio-electrodes must provide a large surface area for bacterial adhesion and ensure efficient current collection. One of the challenges in miniaturization is to increase current and power density within a limited electrode surface area and restricted compartment cavity size (Lovley, 2006). In comparison with the silicon micromachined MFCs, the PDMS MFC has a 4.5 times increase in electrode surface-area-to-volume ratio and 40.5 times increase in power density. This was confirmed when (Choi et al., 2011) fabricated a PDMS dual chamber µMFC with high specific surface area to volume (500 cm$^{-1}$) to lower the internal resistance. The output power and current density were 47 mW/m$^2$ and 330 mA/m$^2$, respectively. One main contributor for high volumetric power density was the high specific surface area (500 cm$^{-1}$), compared to other MFCs (Hou et al., 2009; Qian et al., 2009; Siu and Mu, 2008). Having high specific surface area allows more anode respiring bacteria (ARB) in a given volume of MFCs, leading to high volumetric power density. In addition, the high specific surface area leads to lower electrolyte resistance, which increases the power output. MEMS MFCs having a specific surface area (510 cm$^{-1}$) only produced 2.3 mW/cm$^3$ (Chiao et al., 2006).

5.2.3 PEM Area

PEMs physically separate the anode and cathode compartments while allowing protons to pass to the cathode in order to sustain an electrical current. Power generation in MFCs is a function of the surface areas of the PEM. This was illustrated by Oh and Logan (2006) when they tested a PEM with three different fixed anode and cathode surface areas (3.5, 6.2 and 30.6 cm$^2$) in two-chambered MFCs. The power density increased with the PEM size in the order 45, 68 and 190 mW/m$^2$, respectively. When the surface area of the PEM is smaller than that of the electrodes, it limits power output due to an increase in internal resistance. The same positive relation was confirmed when MFC was scaled down to the micro size in two separate
investigations. Choi et al. (2011) enlarged the size of the PEM to 2.25 cm$^2$ and got a relatively high power density (47 mW/m$^2$) compared with Qian et al. (2009) who used smaller PEM (0.15 cm$^2$) and achieved power density of only 1.5 mW/m$^2$.

5.2.4 Electrolyte Conductivity

In MFCs, addition of salts increases the conductivity and reduces the internal resistance, being beneficial to electricity generation (Rousseau et al., 2013). Oxygen or ferricyanide are commonly used as electron acceptors in conventional MFCs, where either reagent is continuously or periodically pumped into the cathode chamber (Hou et al., 2012). In μMFCs, ferricyanide is commonly loaded into cathode chambers due to the high concentration of readily available electron acceptors since it is difficult to pump oxygen into small-scale chambers. When using ferricyanide as an electron acceptor, Fe (III) accepts one electron from the cathode, forming Fe (II) as the final product (Logan et al., 2006). The amount of electron acceptors that can be utilized in these devices could be restricted if the chamber volume is limited, thus Fe (III) (electron acceptor) becomes depleted during normal operation. Also, the electrolyte pH is crucial to the MFCs power output. Generally, the commonly used neutrophilic bacteria require a pH close to neutral for their optimal growth; while oxygen reduction on the cathode electrode results in an alkaline pH (Rozendal et al., 2006). A traditional MFC can maintain two different pH conditions to optimize the anodic and cathodic reactions. It is, however, impossible to do so in air-cathode MFCs, because only one electrolyte is present. Recently, Wang and Su (2013) compared the activity of electrochemically-active and -inactive mixed culture anolytes in a membraneless μMFC. The highest open circuit voltages (OCVs) produced by inactive and active microflora were 131 and 246 mV, respectively. The difference in OCVs output between these two identical anolyte solutions indicates the electrochemical activity of the microflora.
5.2.5 Microbial Electrogenicity

Not all ARBs are able to generate high current density. Bacteria requiring soluble mediators for extracellular electron transfer (EET) (e.g., *Saccharomyces cerevisiae*, *Pseudomonas aeruginosa*, and *S. oneidensis* MR-1) cannot produce high current/power density, because the diffusion rates of the mediators significantly limit the rate of EET by diffusion (Chiao et al., 2006; Hou et al., 2009; Qian et al., 2009; Siu and Mu, 2008; Torres et al., 2010). Moreover, *S. cerevisiae* are unable to produce shuttling compounds; thus, they must be supplied expensive and often toxic exogenous mediators (Lovley, 2008). *Shewanella* and *Pseudomonas* sp. can excrete mediators, but they produce a small current density, in a range of 1 to 2500 mA/m$^2$ (Marsili et al., 2008). In contrast, *Geobacter* sp. form a conductive biofilm matrix for fast EET, resulting in high current densities, in the order of $10^5$ mA/m$^2$, or $10^4$ mA/m$^2$ (Torres et al., 2008b).

Recently, metal-reducing geobacteria, such as *Geobacter metallireducens*, *Rhodoferax ferrireducens*, and *Shewanella putrefaciens*, have been used as biocatalysts (Chaudhuri and Lovley, 2003; Lovley, 2006). These species have conductive enzymes, cytochromes, in their outer cellulosic membrane that provide an electron conduction path from the intercellular cell body to the electrode surface without needing a soluble electron mediator (Schroder, 2007). Although mediator-less MFCs have a coulombic efficiency over 80%, some of these microorganisms can catalyze glucose only when metallic ions such as Fe$^{3+}$ ions are present in the anodic medium as the primary metabolic substrate (Chaudhuri and Lovley, 2003).

The µMFC approach is possible because members of the family Geobacteraceae conserve energy for growth through respiration of the anode electrode in a fuel cell with nearly stoichiometric recovery of electrons as electricity (Lovley, 2006). They grow as attached biofilm on electrodes and without required contributions by planktonic cells to the current (Bond and
Lovley, 2003). An understanding of how Geobacteraceae externalize electrons generated in central metabolism during electron donor oxidation is continually evolving (Lovley and Nevin, 2008), however, anode respiration likely involves multiple c-type cytochromes (Kim et al., 2008) and conductive pili (Reguera et al., 2005); both of which are required for optimal current production. The conductive pili of Geobacteraceae are necessary for biofilm formation (Reguera et al., 2006) and are hypothesized to enable respiration of the anode by cells which are not in direct contact with the anode. Similar mechanisms of electricity production by Geobacteraceae biofilms likely occur in the µMFC with the sole difference of an additional requirement of diffusion-limited conditions between the anolyte and catholyte in order to maintain the potential gradient between the electrodes. Like Geobacteraceae, members of the family Shewanellaceae may conserve energy for growth during dissimilatory iron reduction and may also produce conductive pili structures (Gorby et al., 2006). However, Shewanellaceae may grow aerobically and exhibit a distinctly alternate mechanism from Geobacteraceae for externalizing electrons to electrode surfaces which involves planktonic cells as well as attached biofilm cells (Lanthier et al., 2008). Marsili et al. (2008) identified flavin compounds produced by S. oneidensis which mediate electron transfer to electrodes and may enable planktonic cells of S. oneidensis to respiration when not in contact with the biofilm or electrode.

Li et al. (2011) compared the performance of G. sulfurreducens and S. oneidensis in a membrane-less µMFC using gold electrodes and isotonic phosphate buffer as catholyte. They found that the current density of Shewanella (25.42 mA/m²) was superior to that of the Geobacter (18.40 mA/m²). In the µMFC flow-through system, planktonic cells would not be expected to contribute significantly to current production, but the electron mediating flavin compounds may attach to the electrode and facilitate electron transfer (Marsili et al., 2008). Further lowering oxygen intrusion and increasing the accumulation of ARB are the steps needed
to bring the performance of μMFCs up to the level of macro-MFCs. MEMS hermetic-wafer bonding technology via eutectic/anodic compounds could be used to reduce oxygen intrusion into an anode chamber (Choa, 2009). To increase ARB accumulation, it is possible to manufacture a thicker spacer, still using MEMS technology, so that the ARB biofilm reaches 50–100 mm. However, as the spacing is enlarged, the possible leak paths of oxygen from outside to the anode chamber may increase. Future work remains to find alternative materials for the spacer which have low oxygen permeability and are compatible with MEMS technology.

5.2.6 Electrode Materials

The selection of anode materials could exert substantial influence on cell attachment and electron coupling. The best electrodes used in macroscopic MFCs have been carbon-based materials, such as graphite felts, graphite rods, carbon cloth, etc, due to their large surface area and functional organic groups favoring cell vitality as well as easy handling (Richter et al., 2008). As a result, the best output and stability have been reported from MFCs with carbon-based anodes (Logan, 2008; Richter et al., 2008). Graphite, in the form of carbon cloth or graphite felt, has typically been the material of choice for the construction of MFC anodes, and conductive elements such as manganese, iron, quinines, and neutral red have been incorporated in graphite electrodes to significantly increase power output (Lowy and Tender, 2008). However, graphite is not suitable for microfabricated MFC array systems (Richter et al., 2008). The surfaces of graphite electrodes are non-uniform and difficult to pattern in small-scale devices. This non-consistency frustrates efforts to compare performances between individual miniaturized MFCs. Recently, gold has been identified as a potential material for MFC anode development (Richter et al., 2008). Gold is highly conductive, can be vapor deposited, and is compatible with a wide array of conventional microfabrication modalities (Ringelstein et al., 2006). Thus, gold is a very attractive anode candidate for the development of an MFC screening platform.
Most reported µMFCs employed gold as an anode material, although they were prone to high internal resistances, relatively low power densities, and high fabrication costs (Chiao et al., 2006; Crittenden et al., 2006; Qian et al., 2009; Siu and Mu, 2008). Another drawback of using gold surfaces is its possible denaturing effect on the key redox proteins of certain bacterial strains, leading to the loss of electron transport capabilities (Richter et al., 2008). Many studies in the mL-to-L regime of MFC reactors have suggested that high-surface area carbon electrodes can enhance the rate of extracellular electron transfer by microbes, and therefore increase power generation (Logan et al., 2006). In contrast, the use of carbon-based electrodes in sub-100 µL MFCs has not been reported, most likely because traditional carbon electrodes are bulky. Qian et al. (2011) tried to overcome this limitation by designing a µMFC to incorporate thin carbon cloth electrodes into a sub-5 µL chamber embedded in a thin polydimethylsiloxane (PDMS) frame. The use of the carbon cloth anode led to a reduced internal resistance and more power production compared to a flat gold anode in the µMFCs of similar configuration (Qian et al., 2009). The carbon cloth produced more than 10-fold power (250 nW) than the gold (23 nW), despite only a triple increase of the micro-chamber volume. Therefore, it is predicted that by employing porous carbon electrodes with optimized pore sizes to accommodate more bacterial cells, an increase of energy output in µMFCs would result. Most µMFCs used gold as an electrode material as gold is one of the most popular materials used in microfabrication processes. The power density of µMFC is expected to improve substantially if alternative electrode materials, meeting microfabrication process requirements and providing better surface characteristics for bacterial biofilm formation, could be discovered.

5.2.7 Anodic Structure

A higher internal resistance is usually expected for µMFCs due to its smaller contact area and the higher resistance of PEM and air cathode, compared with large-scale MFCs. This high
internal resistance could limit the maximum power output and adversely affect microbial respiration. Fabrication of nano-structured anodes with a high surface area and the resulting enhanced biochemical reactivity might offer an effective solution to this problem (Qian et al., 2009). Photolithography can be employed to prepare shape-designed anodic microelectrodes easily and precisely. This is useful to investigate the effect of electrode shape on the electricity generation capacity of µMFCs, and whether the shape of the anodic electrode should be precisely controlled or not. The first trial to increase the electrodes surface area was performed by Siu and Mu (2008) by microfabricating a PDMS MFC that uses an embedded micropillar structure to increase electrical output. The micropillars have typical cross-sectional dimensions of 40 μm × 40 μm and a height of 8 μm. The effective electrode surface area is increased 1.8 times by the micropillars, changed from 1.2 cm² flat geometric surface to 2.16 cm² effective electrode area, and positively generate a power density of 4.012 mW/m².

5.2.8 Operation Mode

MFCs could be operated in three different modes; batch, fed-batch and continuous mode. The batch mode applies the repeated cycles of anolyte and catholyte replacement. The fed-batch process is based on feeding of a growth limiting nutrient substrate to the MFC, while in continuous mode the same volume of anolyte or catholyte is continuously added and removed to keep the volume constant. The same three modes were used upon miniaturization to the scale of µMFC. Hou et al. (2012) described MFC array device that incorporates microfluidic technology to enable continuous long-term analysis of MFC performance at high throughput utilizing periodic anolyte/catholyte replenishment with twelve of the cathode chambers periodically replenished with ferricyanide and the other twelve unreplenished. Similar maximum power densities were observed during early time points after inoculation with (14.9 mW/m²) or without (13.8 mW/m²). After 18 hours, the maximum power density of the MFC units without catholyte
replenishment showed 234% lower power of 5.9 mW/m$^2$, whereas units with catholyte replenishment did not show significant change in maximum power output (15.9 mW/m$^2$). Therefore, catholyte replenished units provided a 270% higher power output than units without replenishment. The initial short-term study comparing batch-mode and catholyte replenished MFCs readily revealed that batch-mode µMFCs were not suitable for long-term MFC studies due to power drops caused by electron acceptor depletion in the catholyte. If the long-term power generating capacities of µMFCs are compromised by the depletion of catholyte, then replenishment of this essential component should revive operations. Also, Choi et al. (2011) noticed the increase in current from 0.5 to 49 mA upon the supplementation of both anolyte and catholyte solutions continuously to the chambers at 1.5 mL/min and this increase was approximately 5 h after the semi-continuous feed of anolyte/catholyte. Moreover, a PDMS-based, 5 µL MFC device batch-fed with lactate-based minimal medium was demonstrated (Qian et al., 2011). Using *S. oneidensis* MR-1, the batch-fed µMFC yielded reproducible current peaks up to six feeding cycles and in total sustained for more than 100 h and generated an enhanced power density of 62.5 W/m$^3$.

**5.2.9 µMFC Configuration**

Various MFC platforms, including miniature MFC devices that enable parallel comparison of electricity generation in MFCs, are emerging (Siu and Mu, 2008). However, state of the art microfabrication and highly integrated parallel measurement approaches have not yet been exploited to construct an MFC array with highly consistent architecture and performance (Weibel et al., 2007). The first µMFC array that supports long-term operation and analysis was reported by Hou et al. (2009) who applied the anolytes and catholytes replenishment in each of the reaction chambers. The microfabricated 24-chamber system applies integrated microfluidic channels and microvalves to periodically or continuously reload the anolyte or catholyte of the
integrated µMFC units. This system overcame the limitations of previously developed batch-mode µMFC arrays through the use of microvalve-controlled microfluidic channel networks that enable both periodic and continuous catholyte and anolyte replenishment for long-term MFC characterization. These new capabilities allow miniaturized MFC arrays to be used for conducting the same kinds of experiments that have to otherwise be conducted with conventional MFCs, albeit at much higher throughput and significantly lower reagent consumption.

Single MFC units can be assembled in series to produce higher power output and operating voltage. In macro-sized or mL-scale MFCs, Wilkinson (2000) demonstrated a 6-cell stacked MFC and Aelterman et al. (2006) reported a stack in series increasing the output voltage to 2.02 V and power to 0.228 mW/cm$^3$. The same structure was scaled down in an array of three µMFCs connected in series reported by Choi and Chae (2012a). The total output power increased by a factor of 10 when compared to the maximum values (47 mW/m$^2$) of previously reported µMFCs (Choi et al., 2011). Also, the power density and operating voltage of the MFC array were significantly improved over the maximum values in the previously reported µMFCs.

One of the most important considerations in the µMFC configuration is the anode chamber depth due to the inverse relationship between it and the internal resistance. Since the total internal resistance consists of anodic, membrane, cathodic, and electrolyte resistance (Torres et al., 2010), and the anodic resistance is the main bottleneck in the micro-sized MFCs (Choi et al., 2011), contrary to larger-scale MFCs where the cathode is the limiting component (Sleutels et al., 2012). These results suggest that the internal resistance increases probably due to high electrode energy losses from the small population of bacteria attached to the surface. The feasibility of optimal biofilm formation on power density of MFC was demonstrated by constructing a µMFC using 254 µm-thick PTEE (polytetrafluoroethylene) spacer. This spacer is five times lower in oxygen permeability than that of PDMS, which is expected to help the power
density of MFC substantially. In addition to the spacer, PEEK (polyetheretherketone) fluidic tubings that show fifty times lower oxygen permeability than that of fluorinated ethylene propylene tubing were used (Choi et al., 2011).

6. Summary and Future Prospects

The µMFCs offer unique features such as large SAV ratio, short electrode distance, fast response time, and low Reynolds number, providing alternative choices in constructing MFCs. The existing µMFCs are still limited by relatively low volumetric power density and coulombic efficiency, probably due to their high internal resistances. However, they show great potential in the rapid screening of electrode materials (Crittenden et al., 2006) and electrochemically active microbes (Hou et al., 2009). Compared with mL-scale MFCs using the same microbes (Ringeisen et al., 2006), µL-scale MFCs generated similar volumetric current density but significantly lower volumetric power density (Qian et al., 2009). This points out that the internal resistances of µMFCs were around 40 fold higher than that in the mL-scale MFCs. The results obtained by Chiao et al. (2006) and Siu and Chiao (2008) suggested that electrode distance contributed a small part of the internal resistance in their systems. Since the development of the first µMFC in 2006, the volumetric power density and coulombic efficiency have been increased over 500% with improved electrode topology and SAV ratio. However, high internal resistances in µMFCs limit their power output (<32 W/m³) and the more promising applications for current µMFCs are fast responding analyses.

Carbon-based anodes are known for high SAV ratio and easy adaptation of microorganism and they are widely used in mL-scale MFCs; however, the conventional carbon-based materials are incompatible with microfabrication methods. The recent studies using carbon nanotubes (CNT) as electrodes (Qiao et al., 2007) provide promising solutions for fabricating carbon-based anodes in µMFCs. The CNT-based electrodes show great improvement in the
electricity generation (Sharma et al., 2008) and offer good biocompatibility (Morozan et al., 2007). Except for electrodes with low resistance, reducing membrane resistance can also decrease the total internal resistance of µMFCs. Microfluidic systems offer a potential solution for diminishing resistance caused by the PEM. For example, microfluidic fuel cells using defined chemicals have taken the advantages of laminar flow to keep the fuel (anolyte) and oxidant (catholyte) well-separated without PEM (Chang et al., 2006; Chen et al., 2007). Since the thickness of PEM remains unchanged as the device size decreases, it is expected to contribute a larger portion of the internal resistance in a two-chamber µMFC. Using a membrane-less configuration provides a promising way to decrease the internal resistance. For improving performance, significant research activities on immobilizing microbes are expected in microfluidic applications. The use of mediator molecules would be combined for higher efficiencies and immobilizing both microbes and mediators will be explored in parallel.

Generating multiple small-scale microfluidic-based units in parallel within a confined area has been demonstrated previously (Whitesides, 2006); using this unit approach with microbes in microfluidics is a direct extension of these approaches. Furthermore, building these systems in three dimensions would also realize further increases in energy production for the same area on a single device thus increasing the energy return on the battery size, which is a tremendous limitation in many current applications. In this manner, the natural and engineered metabolic diversity of microorganisms may be harnessed to enable the design of µMFC which serve as independent energy sources, self-powered sensing devices in a variety of deployments. Moreover, the small size of the µMFC offers much greater flexibility by limiting potential disturbances to the sensed or powered environment and offering high-throughput screening via whole cell lab-on-a-chip technology.
Despite their relatively low performance, MFCs can still be an attractive alternative power source for a host of low-power, long-term applications when integrated on-chip in a microfluidic device. Implantable medical devices such as heart pacemaker and glucose sensor can be potential customers of microfluidic MFCs since they require longevities of years for in vivo operations at a compact size. *S. cerevisiae* converts chemical energy stored in glucose in the human blood stream into electrical energy (Siu and Mu, 2008) and would be readily applicable to implantable power generation with “infinite” reactant supply. During in vivo operation, the devices must be capable of existing in the physiological environment without unacceptable biofouling occurring over time, which otherwise would lead to failure of the devices and potentially to physiological harm to the human body (Davis and Higson, 2007). Maluf and Williams (2000) claimed that preliminary medical evidence indicates that silicon is benign in the human body. However, there are a lot of scientific arguments around the biocompatibility of the commonly used materials in microfabrication, including silicon, which may require further investigations (Madou, 2002). Integrated µMFCs equipped with electroosmotic pumps, fabricated from silica nanospheres, can use laminar flow to separate the fuel and its oxidant to control the interaction of the two fluids without a physical barrier as would be required in conventional fuel cells. As a compact and green energy source, biofuel cells in microfluidic architecture can be an effective solution for small-scale power source applications such as biological sensors, implantable medical devices, and portable electronics. Significant research advancements, however, must be made to witness them in realistic and practical applications. Establishment of future guidelines to develop µMFC depends on understanding the biocatalytic activities in parallel with scientific efforts to enhance the device configurations.
References


Fig. 1. Schematic diagram of typical two-chamber microbial fuel cell producing electricity through electron transfer to the anode.
Fig. 3. Schematic of μMFC fabrication process. A: Photolithography and metal etching techniques employed for fabrication of the gold microelectrodes. B: Conventional soft lithography used to fabricate the microstructure channel (Image reproduced, with permission, from (Li et al., 2011)).
Table 1: Overview of fabricated μMFC devices and their main characteristics.

<table>
<thead>
<tr>
<th>μMFC Description</th>
<th>Fabricated Material</th>
<th>Anode</th>
<th>Cathode</th>
<th>Electrode SAV (cm²)</th>
<th>Anode Volume (µL)</th>
<th>Catholyte</th>
<th>Microbe</th>
<th>PEM¹ Area (cm²)</th>
<th>Electrode Distance (µm)</th>
<th>P_max (mW/m²)</th>
<th>I_max (mA/m²)</th>
<th>Hours of Operation and Mode</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual chamber</td>
<td>Si</td>
<td>Au</td>
<td>Au</td>
<td>32</td>
<td>16</td>
<td>FeCN</td>
<td>S. cerevisiae</td>
<td>1</td>
<td>160</td>
<td>0.023</td>
<td>150</td>
<td>Bat.</td>
<td>(Chiao et al., 2006)</td>
</tr>
<tr>
<td>Dual chamber with μpillar electrodes</td>
<td>PDMS</td>
<td>Au</td>
<td>Au</td>
<td>144</td>
<td>15</td>
<td>FeCN</td>
<td>S. cerevisiae</td>
<td>1.2</td>
<td>383</td>
<td>4.012</td>
<td>3.02</td>
<td>Bat.</td>
<td>(Siu and Mu, 2008)</td>
</tr>
<tr>
<td>Array (24)</td>
<td>PDMS</td>
<td>Au</td>
<td>Pt/C cloth</td>
<td>N/A</td>
<td>N/A</td>
<td>FeCN</td>
<td>S. oneidensis MR-1</td>
<td>0.49/ well</td>
<td>N/A</td>
<td>1.15</td>
<td>37</td>
<td>17 Bat.</td>
<td>(Qian et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Si/PDMS⁵</td>
<td>Au⁶</td>
<td>C cloth</td>
<td>100</td>
<td>1.5</td>
<td>FeCN⁴</td>
<td>S. oneidensis MR-1</td>
<td>0.15</td>
<td>100</td>
<td>1.5</td>
<td>130</td>
<td>&gt;180 Bat.</td>
<td>(Qian et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>PDMS/PC⁷</td>
<td>C³ cloth</td>
<td>C cloth</td>
<td>100</td>
<td>5</td>
<td>FeCN⁴</td>
<td>S. oneidensis MR-1</td>
<td>0.4</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
<td>&gt;100 Cont.</td>
<td>(Chen et al., 2011)</td>
</tr>
<tr>
<td>Air cathode array (8)</td>
<td>Glass/ PDMS⁸</td>
<td>Au</td>
<td>Pt/C</td>
<td>N/A</td>
<td>25</td>
<td>Air</td>
<td>S. oneidensis MR-1</td>
<td>0.05</td>
<td>N/A</td>
<td>29</td>
<td>2148</td>
<td>900 Cont.</td>
<td>(Choi et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Glass/ PMMA</td>
<td>Au</td>
<td>Au</td>
<td>N/A</td>
<td>0.3 phosphate buffer</td>
<td>-</td>
<td>G. sulfurreducens</td>
<td>-</td>
<td>200</td>
<td>N/A</td>
<td>18.40</td>
<td>Bat. 15 Cont.</td>
<td>(Li et al., 2011)</td>
</tr>
<tr>
<td>Membrane-less dual chamber Array (3)</td>
<td>Glass/ PDMS</td>
<td>Au</td>
<td>Au</td>
<td>N/A</td>
<td>50</td>
<td>FeCN</td>
<td>S. oneidensis MR-1</td>
<td>1</td>
<td>N/A</td>
<td>330</td>
<td>N/A</td>
<td>80 Cont.</td>
<td>(Choi and Chae, 2012a)</td>
</tr>
<tr>
<td></td>
<td>Glass/ PDMS/ PTEE⁹</td>
<td>Au</td>
<td>Au</td>
<td>64.5</td>
<td>15.5</td>
<td>FeCN</td>
<td>G. sulfurreducens</td>
<td>1</td>
<td>N/A</td>
<td>44</td>
<td>260</td>
<td>100 Cont.</td>
<td>(Choi and Chae, 2012b)</td>
</tr>
<tr>
<td>Dual chamber with PDMS spacers Array (24)</td>
<td>Glass/ PDMS/ PTEE⁹</td>
<td>Au</td>
<td>Au</td>
<td>N/A</td>
<td>435</td>
<td>FeCN</td>
<td>S. oneidensis MR-1 and 7Ca</td>
<td>N/A</td>
<td>N/A</td>
<td>15.9</td>
<td>150</td>
<td>18.3 Cont.</td>
<td>(Hou et al., 2012)</td>
</tr>
</tbody>
</table>

¹ PEM: Proton exchange membrane; ² SAV: Surface area to volume ratio; ³ PDMS: Polydimethylsiloxane; ⁴ PC: Polycarbonate; ⁵ PMMA: Poly(methyl methacrylate); ⁶ PTEE: Polytetrafluoroethylene; ⁷ Au: Gold; ⁸ C: Carbon; ⁹ Pt: Platinum; ¹⁰ Bat.: Batch.
Fig. 4. Pictorial collection of the main milestones of microfluidic microbial fuel cell development; (A): Image reproduced, with permission, from (Hou et al., 2011), (B): Image reproduced, with permission, from (Chen et al., 2011), (C): Image reproduced, with permission, from (Choi and Chae, 2012a), (D): Image reproduced, with permission, from (Choi and Chae, 2012b).