A MICROBIAL FUEL CELL USING BIOMINERALIZED MANGANESE OXIDES AS A CATHODIC REACTANT

by

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ABSTRACT

Microbial fuel cells were designed and operated utilizing microorganisms in both the anodic and cathodic compartments. In the cathodic compartment we used *Leptothrix discophora* because of the microorganism’s capability to deposit biomineralized manganese oxides on the electrode. Biomineralized manganese oxides are superior to oxygen when used as a cathodic reactant. In the anodic compartment of most of the fuel cells we oxidized glucose using *Klebsiella pneumoniae*. In one fuel cell we used *Desulfovibrio desulfuricans*, a sulfate reducing bacteria.

Electrons released in the anodic compartment, from the oxidation of glucose or sulfide, were then used in the cathodic compartment to reduce microbi ally deposited manganese oxides. A redox mediator, 2-hydroxy-1,4-napthoquinone (HNQ) was used in the anodic compartment to facilitate electron transfer from the microorganism to the electrode. The fuel cells with glucose oxidizing bacteria were operated for 500 hours and reached an average anodic potential of $-441\pm31$ mV$_{SCE}$ and an average cathodic potential of $+384\pm62$ mV$_{SCE}$. The fuel cells with sulfate reducing bacteria were operated for 136 hours and reached an average anodic potential of $-470\pm44$ mV$_{SCE}$ and an average cathodic potential of $+419\pm59$ mV$_{SCE}$.

Reticulated vitreous carbon or 316L stainless steel was used as the electrode material. The electrode materials did not have a significant effect on the potential of the fuel cell system. The average fuel cell potential for 316L stainless steel was $706.13\pm22$ mV and $759.75\pm73$ mV for reticulated vitreous carbon. When the fuel cells reached steady state we discharged them through a $510\Omega$ resistor and evaluated the available power.
CHAPTER 1

INTRODUCTION

General Fuel Cell

A fuel cell is comparable to an electrolytic cell or battery, where chemicals are oxidized or reduced electrochemically to produce electricity (Smith et al. 2001). Fuel cells contain two electrodes (anode and cathode) that are either separated by an electrolyte, such as a salt bridge, or a membrane. Half reactions take place at the surface of the anode and cathode, and the sum of the two half reactions is the overall reaction. Fuel cells do not contain stored reactants, but rather, the reactants are fed to the cell continuously. Hence, the continuous flow of reagents allows for a continuous supply of electrical current as long as the electrodes and a proton exchange membrane stay intact.

The focus of this study is on small-scale microbial fuel cells. Microbial fuel cells are capable of converting chemical energy into electrical energy (Chang et al. 2004, Chaudhuri et al. 2003). Microorganisms are used to oxidize organic substrates, such as glucose, to create electrical power. The generation of power occurs when the microorganisms utilize substrates to maintain metabolism and reproduction (Madigan et al. 2000). Within the microbe’s cell membrane, the products created from the catabolism of the substrate go through many intermediates that become oxidized and reduced. When oxygen is
absent, electrons can be donated from the microorganism to a redox mediator (Bennetto 1990). The redox mediator is then capable of delivering electrons to an electrode via oxidation of the mediator. Once delivered to the electrode, electrons flow from the substrate oxidizing compartment to a compartment which contains a terminal electron acceptor (cathodic compartment) (Allen and Bennetto 1993).

When a substrate is being anaerobically oxidized in the anodic compartment to produce electrons the potential decreases. While, in the cathodic compartment, a potential increases due to the reduction of a reagent. The difference in potential caused by the oxidation of a substrate at the anode and reduction of a substrate at the cathode allows for current generation. Figure 1-1 gives a general outline of what is occurring in the anodic and cathodic compartments of a microbial fuel cell.
To improve the overall efficiency of the microbial fuel cell many areas have been researched, such as; 1) finding the most efficient anodic reactions (i.e., those producing the highest number of electrons per unit weight of reactant) (Allen and Bennetto 1993; Lee et al. 2002; Logan 2004); 2) finding the most efficient microorganisms (i.e. those that can are able to extract the highest number of electrons from a unit mass of the substrate) (Choi et al. 2001; Chio et al. 2003; Kim et al. 2002; Tsujimura et al. 2001b); 3) selecting effective redox mediators (McKinlay and Zeikus 2004; Park and Zeikus 2000); and 4) selecting effective electrode materials (Katz et al. 1999; Schroder et al. 2003; Gregory et al. 2004;).
Past Microbial Fuel Cell Research

Research on microbial fuel cells started in 1911 by an English scientist, M.C. Potter. Potter discovered that a potential difference was established when one of the fuel cell’s compartments contained metabolizing microbes and the other compartment contained a sterile nutrient salt solution. With the created potential difference, Potter was able to obtain current when a load was applied between the two compartments (Potter 1911).

After M.C. Potter, microbial fuel cell research did not spark interest again until the 1960’s. During this time a realization occurred that power is a precious resource. It became important to study different means of obtaining power, even if the amount of power created was minute. Young et al, started to delve into the theoretical aspects of microbial fuel cells (Young et al. 1966). According to this group, there are three main types of biochemical fuel cells that can be created: 1) a depolarization cell (use organisms that remove an electrochemical product); 2) a product cell (use organisms for the production of an electrochemical product); and 3) a redox cell (use organisms for the conversion of electrochemical products into electrochemical reactants) (Young et al. 1966). Bennetto carried on Young et al.’s work in the 1980s by publishing general concepts of the redox cell (Bennetto et al. 1983a; Bennetto et al. 1983b; Bennetto 1990). Research today uses the same concepts established by these
researchers, including; anaerobic respiration of microorganisms to extract electrons and the two compartment fuel cell design.

**Microorganisms Used in Anodic Compartment**

Many researchers have studied the microbial aspects of the fuel cell, including; microorganisms that cause the highest columbic yield (Allen and Bennetto 1993), microbes that can work at elevated temperatures (Choi et al. 2003), organisms that utilize light to produce energy (Tsujimura et al. 2001b; Karube et al. 1984; de la Garza et al. 2003; Yagishita 1999), heterogeneous mixtures of microbial species (i.e., waste water) (Chang et al. 2004; Lui et al. 2004a; Min and Logan 2004; Kim et al. 2003), and microbes that are capable of direct electron transfer to an electrode (Kim et al. 2002; Gil et al. 2003; Jang et al. 2004).

Microorganisms that do not require a redox mediator to transfer electrons to an electrode are currently sparking interest in the literature. Bond and Lovley (2003) utilized the microorganism *Geobacter sulfurreducens*, based on the concept that electrons produced from the microorganisms can be directly transferred to an electrode due to the cytochromes present on the microbe’s outer membrane (Katz et al. 2003). Some other microbes that are used in mediatorless microbial fuel cell include *Rhodoferax ferrireducens* (Chaudhuri et al. 2003) and *Shewanella putrefaciens* (Kim et al. 1999; Kim et al. 2002).
Environmental isolates, such as sulfate reducing bacteria (SRB), Bacillus sp., and Cyanobacteria have been used in fuel cells as well. SRBs live in ocean sediments and have been used in fuel cells to represent what occurs in the natural environment (Katz et al. 2003; Bond et al. 2002). Bacillus species are thermophilic and alkaliphilic bacteria that are used in fuel cells to study the effect of temperature and pH. This organism allowed researchers to discover that temperature does not have a significant effect on the fuel cell efficiency (between 20-70 degrees Celsius) and that elevated pH (7-9.5) increases the overall efficiency of the fuel cell (Choi et al. 2001). Cyanobacteria create energy through hydrogen production by light excitation of photosystems I and II, instead of anaerobic respiration (Tsujimura et al. 2001a; Tsujimura 2001b). In this system, hydrogen is produced and then deprotonates into protons and electrons at the electrode’s surface. These bacteria can thrive in aerobic conditions while creating energy necessary to power a microbial fuel cell.

The most commonly used microorganisms in the anodic compartment of fuel cells are those that oxidize glucose. There are numerous species of microorganisms that are capable of metabolizing glucose these include: Escherichia coli (Park and Zeikus 2000a; Park et al. 2000), Proteus vulgaris (Bennetto et al. 1983; Thurston et al. 1985; Allen and Bennetto, 1993; Kim et al. 2000a; Kim et al. 2000b), Bacillus subtilis (Choi et al. 2001), Lactobacillus plantarum (Vega et al. 1997),

Knowing that *Klebsiella pneumonia* is capable of metabolizing many organics, including glucose, we decided to use this organism in the anodic compartment of our microbial fuel cell to obtain a decrease in electrode potential and produce current. Gram-negative, *Klebsiella* species are characterized by their non-motile, polysaccharide encapsulated cells. They are also facultative anaerobes who use chemoorganotrophic metabolism (Holt et al. 1994).

**Redox Mediators**

Microorganisms present in the anodic compartment use anaerobic respiration to break down organic substances. During the catabolism of glucose, NADH is formed and is the starting intermediate for the electron transport chain. The electron transport chain consists of the oxidation and reduction of proteins such as cytochrome and quinines (Willner et al. 1998, Ikeda and Kano 2003). When oxygen is absent in the fuel cell compartment, electrons produced by the oxidation of glucose are removed from the electron transport chain via a redox mediator (Lithgow et al. 1986, Roller et al. 1984). Once the redox mediator is reduced, the electrons are shuttled from the microorganism’s membrane to an
electrode. The extraction of electrons from the electron transport chain can be seen in the following diagram (1-2):

![Diagram](image)

Figure 1-2: Schematic of electron transport from glucose to HNQ. Fp-Flavoprotein, Fe-S iron-sulfur protein, Q-ubiquinone, cyt-cytochrome, reduc-reductase. Modified from Madigan et al. 2000.

Some specifications of a good redox mediator include the following:

1) The potential of the redox mediator should be positive enough to provide fast electron transfer from the glucose, but should not be so positive as to affect the potential of the anode (Katz et al. 2003.).  
2) Oxidized state of the redox mediator should be able to penetrate the cell membrane, and the reduced state should easily leave the membrane (Katz et al. 2003).  
3) The redox mediator should not interfere with the metabolism of the cell (Katz et al. 2003).  
4) Adsorption of redox mediator
into the electrode should be kept at a minimum. 5.) The redox mediator should be stable and have soluble properties for long microbial fuel cell run times (Lee et al. 2002, Allen and Bennetto 1993).

There are many different redox mediators that may be used within a fuel cell. Typically, organic dyes can be used, including 2-hydroxy-1,4-naphthoquinone (HNQ), phenazines, phenothiazines, and phenoxyazines (Katz et. al 2003). HNQ was used in the experimental runs in this thesis for its stable properties (Lee et al. 2002, Allen and Bennetto 1993).

Once HNQ is added to the anodic compartment of the fuel cell, the redox mediator is reduced at the surface of the metabolizing microbe’s membrane. Now reduced, the redox mediator can deliver the electrons to the electrode where it is oxidized. This operation can be seen in the figure below (1-3):
In its oxidized form, HNQ contains two double-bonded oxygens. When HNQ is reduced, the oxygen accepts an electron and the double bond are broken, leaving a hydroxyl group on the molecule. The mediator then reforms the double bond when the molecule is reoxidized at the graphite electrode (Figure 1-4).

Figure 1-4: 2-hydroxy-1,4-naphthoquinone (HNQ) in its oxidized and reduced form.
Reactions Occurring In Anodic Compartment

The substrate plays a significant role in the amount of electrons being generated in a fuel cell. Some substrates are capable of producing a higher coulombic yield than some of the more common sugars used. Coulumbic yield is defined as the number of electrons extracted from a substrate and delivered to an electrode (Kim et al. 2000). For example, in a study using Proteus vulgaris, only 50% of the electrons extracted from glucose were delivered to an electrode (Thurston et al. 1985, Allen and Bennetto 1993). However, there was a 63% recovery when galactose was used in the same organism (Kim et al. 2000a). While, sucrose was able to reach a coulombic efficiency approaching 100% the theoretical yield (Thurston et al. 1985, Bennetto et al. 1985) Even though some microbial fuel cells produced a higher efficiency yield, glucose is the most commonly used. When glucose is used, we can theoretically calculate that twenty-four electrons are extracted when glucose is completely metabolized through anaerobic respiration.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{H}^+ + 24\text{e}^- \]

Reactions used in the cathodic compartment

In most microbial fuel cells the cathodic reaction is abiotic, consisting of oxygen and ferricyanide reduction (Chaudhari and Lovley 2003; Lee et al. 2002, Choi et al. 2003; Liu and Logan 2004b):
\[
\begin{align*}
O_2 + 4H^+ + 4e^- & \rightarrow 2H_2O \\
Fe(CN)_6^{3-} + e^- & \rightarrow Fe(CN)_6^{4-}
\end{align*}
\]

Ferricyanide is not practical to use because it often expires quickly and must be changed often (i.e., it is not renewable.) On the other hand, oxygen is known for having notoriously slow reduction kinetics on solid electrodes. To a large extent, research on abiotic cathodes in microbial fuel cells is done to improve kinetics of oxygen reduction on solid electrodes (Willner et al. 1998a; Willner et al. 1998b; Tsujimura et al. 2001a; Palmore and Kim 1999; Lui et al. 2004b, Pham et al. 2004, Barriere et al. 2004, Kang et al. 2003). Hence, if we improve the rate of oxygen reduction occurring on solid electrodes, we can increase the overall efficiency of the fuel cell (Jang et al. 2004; Pham et al. 2004; Palmore and Kim 1999; Tsujimura et al. 2001a; Willner et al. 1998a).

Some researchers have used the idea of incorporating a redox mediator into the cathodic compartment of a fuel cell. For instance, Palmer and Kim (1999) have shown that using a fungal enzyme, laccase, as a catalyst and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) as a redox mediator can create both higher current densities and electrode potentials.
Biomineralized Manganese as an Alternative Cathodic Reactant

Over the past ten years our laboratory has been studying the effects of microbially induced corrosion. When manganese oxidizing bacteria accumulate on a surface (metal or graphite) and deposit manganese oxides, the structure of the surface changes (Gieser et al. 2002). The change in surface often leads to electrochemical changes (i.e., ennoblement) (Braughton et al. 2001). When the manganese oxidizing bacteria deposit manganese oxides, the potential of the electrode increases, leading to a several-hundred millivolt increase in open circuit potential. (Dickinson et al. 1996; Dickinson et al. 1997; Olsen et al. 2000; Braughton et al. 2001; Geiser et al. 2002; Shi et al. 2003).

The increase in potential is due to biomineralized manganese oxide that is in direct electrical contact with the electrode, and the electrode exhibits the equilibrium dissolution potential of the MnO$_2$ by the following half-reactions (Dickinson et al. 1996; Dickinson et al. 1997; Olesen et al. 2000; Geiser et al. 2002; Shi et al. 2003):

\[
\text{MnO}_2(s) + \text{H}^+ + e^- \rightarrow \text{MnOOH}(s) \\
E^0 = +0.81 \text{ V}_{\text{SCE}} \quad E'_{\text{pH}=7.2} = +0.383 \text{ V}_{\text{SCE}}
\]

\[
\text{MnOOH}(s) + 3\text{H}^+ + e^- \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} \\
E^0 = +1.26 \text{ V}_{\text{SCE}} \quad E'_{\text{pH}=7.2} = +0.336 \text{ V}_{\text{SCE}}
\]

The overall reaction is:

\[
\text{MnO}_2(s) + 4\text{H}^+ + 2e^- \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} \\
E^0 = +1.28 \text{ V}_{\text{SCE}} \quad E'_{\text{pH}=7.2} = +0.360 \text{ V}_{\text{SCE}}
\]
The mechanism of microbially modified electrode-surface electrochemistry involves initial deposition of manganese dioxide on the electrode surface and its subsequent reduction to divalent manganese by two electrons from the anode. Since the release of the divalent manganese occurs in close proximity to the manganese oxidizing bacteria-colonized electrode surface, the divalent manganese is immediately reoxidized to manganese dioxide by the MOB and the cycle continues. Figure 1-5 shows this mechanism.

Since microbially deposited Mn$^{2+}$ causes an increase in the electrode potential in the cathodic direction and dissolution of deposited MnO$_2$ requires 2 electrons, Mn$^{2+}$ deposition can be used as a cathodic reactant in a microbial fuel cell and the two electrons can be obtained by the anodic reaction.
We have used biomineralized manganese oxides as the cathodic reactants in microbial fuel cells. Since oxygen is the terminal electron acceptor (microorganisms reduce oxygen to oxidize manganese) manganese oxides can be considered as a redox mediator in the cathodic reaction.

**Manganese Oxidizing Bacteria in Nature**

Manganese oxidizing bacteria are filamentous, gram negative, chemoorganotrophs, which belong to the species beta Proteobacteria (Madigan et al. 2000). The primary characteristic of this microorganism are the sheaths that it creates (Madigan et al. 2000; Holt et al. 1994). When the conditions are right, flagellated swarmers form long filaments leading to multi-cellular sheaths (Madigan et al. 2000). When manganese is present, these sheaths are often covered in manganese oxides (Madigan et al. 2004; Dickenson et al. 1996; Stein et al. 2001). The phenomena of manganese oxides covering the sheaths are due to the microorganisms oxidizing divalent manganese to manganese oxides (Dickinson et al. 1997, Geiser et al. 2002; Stein et al. 2001). It is not known why the microbe contains a protein to oxidize manganese, but, there are two possibilities: 1) the energetic benefits that may occur; 2) manganese oxides may react with humic substances and fulvic acids, causing a release of organic substances (Madigan et al. 2000.)
Figure 1-6: *Leptothrix discophora* plated onto a minimal salt and vitamin with pyruvate (MSVP) media agar plate (modified with Mn$^{2+}$). The brown color that has formed within the colonies is caused by biomineralized manganese onto the agar’s surface.

Manganese oxidizing bacteria can often be found in both freshwater and saltwater ecosystems (Dickinson et al. 1996, Stein et al. 2001, Linhardt 2004). For instance, a group from Green Bay, Wisconsin found the species *Leptothrix*, in freshwater, metal rich sediments (Stein et al. 2001). Our group demonstrated that manganese oxidizing bacteria exist in the freshwater environments of Montana (Braughton et al. 2001). Since manganese oxidizing bacteria are found in many water systems, it causes both problems and benefits.

One of the major problems related to manganese oxidizing bacteria is microbial corrosion. Even on stainless steel, manganese oxidizing bacteria often form cavities, or pitting on the surface of a metal due to the formation of manganese oxides on the surface (Dickinson et al. 1996).
Since microorganisms damage the surface of the metal, there is often structural failure to systems associated with the formation of the manganese oxides on the surfaces (Dickinson et al. 1996). It has been shown that this type of corrosion has led to the downfall of hydroelectric power plants, cooling water lines, heat exchangers, and sewage treatment plants (Geiser et al. 2002).

Nonetheless, manganese oxidizing bacteria have been used for some beneficial reasons. For instance, divalent manganese can be a problem in drinking water systems, due to corrosion and the pink residue that is left behind in the sinks (American Water Association 2004.) Since manganese oxidizing bacteria can oxidize divalent manganese in water, it has been investigated as a possible source for biological removal of manganese from drinking water (Dickinson et al. 1996, Dickinson et al. 1997). Manganese oxides are beneficial in aquatic environments because they serve as a terminal electron acceptor for anaerobic bacteria (Stein et al. 2001). It is essential for anaerobic bacteria to have electron acceptors in order to maintain their metabolism. When anaerobic bacteria are in the environment, they oxidize organic carbon to maintain their necessary lifecycles. Two electrons from the microbe’s metabolic cycle can then be used to reduce certain reactants such as manganese oxides.
Microbial Fuel Cells Using Microorganisms in Both Compartments

Our lab group has studied the effect of manganese deposition on metals for years. We used the knowledge of biomineralization of manganese and used it as a reactant in the cathodic compartment of the microbial fuel cell. The schematic below represents an overview of our proposed microbial fuel cell.

Figure 1-7: Schematic of designed MFC. Glucose was oxidized in the anodic compartment and the electrons were transferred via an electronic conductor to the cathodic compartment where they reduced microbially deposited manganese oxides. To facilitate the electron transfer from glucose to the graphite electrode in the anodic compartment, we used a redox mediator, 2-hydroxy-1,4-napthoquinone (HNQ). In the cathodic compartment manganese was microbially deposited on the graphite electrode as manganese oxides, and was reduced directly, without a redox mediator, by the electrons derived from the anodic reaction.
In the anodic compartment we used the anaerobic respiration of glucose by *Klebsiella pneumoniae*. To facilitate electron transfer to the graphite electrode, we utilized 2-hydroxy-1,4-naphthoquinone (HNQ) in the anodic compartment, following literature findings which suggest that it has high cumbic output (Lee et al. 2002) and is chemically more stable than other redox mediators (Allen and Bennetto 1993). In the cathodic compartment, we used *Lepthothrix discophora* SP-6 and an aerated solution of Mn$^{2+}$. As a result, *L. discophora* SP-6 grew and deposited MnO$_2$ on the electrode surface. By using this design, a decrease in potential in the anode (Allen and Bennetto 1993; Choi et al. 2001; Gregory et al. 2004; Young et al. 1966; Stirling et al. 1983; Park and Zeikus 2003; Gil et al. 2003) and an increase in potential due to the biomineralization of manganese in the cathode occurred (Campbell et al. 2004; Dickinson et al. 1997; Dickinson et al. 1996; Geiser et al. 2002; Shi et al. 2003; Olesen et al. 2000).

**Thermodynamic Calculations**

The Nernst equation is directly related to chemical equilibrium; hence, it is best to understand Gibb’s Free Energy. Change in Gibbs free energy is equal to:

\[ \Delta G = -nF\Delta E \]  

(1-1)
where \( n \) = the moles of electrons formed in the reaction, \( F \) = Faraday’s constant 96485 C/mol (Faraday’s constant represents the charge that each mole of electrons has) and \( E \) = Electrode Potential (Volts).

Under standard conditions (298K, 1 atm), the equation is written as the following.

\[
\Delta G^\circ = -nF \Delta E^\circ
\]  

(1-2)

Gibbs free energy is then directly correlated to the Nernst equation. The reaction quotient (\( Q \)) is represented by the following:

\[
aA + bB = cC + dD
\]

\[
Q = \frac{C^cD^d}{A^aB^b}
\]  

(1-3)

The reagents represented may be in aqueous, liquid, gaseous, or solid form. For the aqueous form we use the molar concentration (\( M \)), for gaseous, we use the partial pressure of the reagent, and for solids and liquids we use the value of one.

In terms of a redox reaction, we would state that:

\[
Q = \frac{C^cD^d}{A^aB^b}
\]

\[
Q = \text{oxidized/reduced}
\]

It has been proven that:

\[
\Delta G = \Delta G^\circ + RT \ln Q
\]  

(1-4)

Therefore,

\[
-nFE = -nFE^\circ + RT \ln Q \text{ where } R = 8.314J/(mol \ast K)
\]  

(1-5)

By substitution, the Nernst equation is formed:
\[ \Delta E = \Delta E^0 - \frac{RT}{nF} \ln Q \]  
\( \text{(1-6)} \)

The Nernst equation takes into account non-equilibrium conditions to calculate potential.

Additionally, power (P) may be calculated by taking the total cell potential \( V_{\text{cell}} = V_{\text{cathode}} - V_{\text{anode}} \) and multiplying it by the amount of current produced (I).

\[ P = I V_{\text{cell}} \]  
\( \text{(1-7)} \)

**Hypothesis**

Our microbial fuel cell can be designed to utilize anaerobic bacteria in the anodic compartment and manganese oxidizing bacteria in the cathodic compartment.

When *Leptothrix discophora* grows onto an electrode surface and deposits manganese oxides onto a surface, the potential of the electrode increases. When *Klebsiella pneumoniae* oxidizes glucose the potential of the electrode decreases.

When the reactions in the anode and cathode have equilibrated; we obtain a potential difference between the electrodes. In our case, the potential of the fuel cell was obtained by the oxidation of glucose in the anodic compartment and reduction of biomineralized manganese oxides in the cathodic compartment. Once a potential difference has been...
achieved, current can be drawn from the fuel cell by applying a load between the two electrodes. Based on this evidence the following hypotheses were formed:

1. When *Leptothrix discophora* oxidized divalent manganese in the cathodic compartment and *Klebsiella pneumoniae* oxidized glucose in the anodic compartment, a potential difference between the anode and cathode would occur. When a load was applied between the two electrodes, the potential difference caused electrons to flow from the anode to the cathode, allowing current to be drawn.

2. Microbially deposited manganese oxides on the fuel cell’s cathode would create higher current and higher electrode potential than when oxygen was the only reactant.
CHAPTER 2
MATERIALS AND METHODS

Microbial Fuel Cell

The microbial fuel cell was constructed following a design proposed by Bennetto (1993), figure 2-1.
Each compartment of the fuel cell was 250 mL in volume. The influent and effluent lines were made of Neoprene® tubing (Cole-Parmer 148441). Flow breakers were used to prevent back flow contamination for the inlet and outlet tubing (figure 2-2).
A polycarbonate shell was used for the microbial fuel cell because of its transparent, inert, and autoclavable properties.

**Electrodes**

The electrodes in the anodic and cathodic compartments were made of Reticulated Vitreous Carbon, RVC, (80 PPI, (The Electrosynthesis Co. 1-716-684-0513) or 316L stainless steel coupons in some experiments. Since current production is proportional to the surface area of the electrode, we used RVC for most of the fuel cell experiments. Reticulated vitreous carbon (figure 2-3) was used for its chemical inertness, high-temperature strength, high surface area to volume ratio, and for its electrical conductivity (Materials and Aerospace Corporation; www.ergaerospace.com/duocel/rvc.htm, 2003).
The RVC electrodes were connected to carbon rods (Thermadyne Arcair® Plain Pointed Electrodes) by direct insertion of the rods into the RVC. The resistance of the connection between RVC and the carbon rods was less than 1 Ω. Potentials were measured against a saturated calomel reference electrode, SCE, (Fisher, cat. # 13-620-51).

Since we could not sterilize the reference electrode, to prevent contamination of the fuel cell we connected the reference electrode with the solution via a salt bridge. A salt bridge was formed using glass tubing with porous glass affixed to the end, as described by Geiser et al. (2002). On the other end of the tubing, a size 00 rubber stopper was slid into place allowing the glass tubing to be placed into the top of the growth chamber of the microbial fuel cell, hole seen in figure 2-1D. The filling solution for the salt bridge consisted of 1g/100 mL of R2A agar (DIFCO
1826-17-1) and 1mL of 0.1 M Na$_2$SO$_4$. The filling solution and syringe apparatus were autoclaved at 121 °C and 1 atm before filling the glass tubing. The filling solution was then added to the glass tubing using the sterile syringe apparatus. The syringe apparatus was constructed using a 3 ml syringe and a 20 cm 3mm tubing attached to the end. Once the filling solution was in place, a connector was added to the glass tubing and the remaining was filled with 1M Na$_2$SO$_4$ solution and the saturated calomel electrode was placed on top.

**Proton Exchange Membrane and J-cloth**

We used a cation exchange membrane: ESC-7000 (The Electrosynthesis Co. 1-716-684-0513) for proton transfer. The proton exchange membrane, Nafion® consists of a synthetic polymer with ionic properties that is resistant to chemicals that disturb the polymer surface.

To utilize the proton exchange membrane in repeated experiments the membrane was rinsed to gently remove any deposits, placed in 1M NaCl for over 24 hours to recharge as described by the vendor, and then carefully inspected to be sure that there were no holes or scratches on the surface. Any defective membranes were not used.

J-cloth (First Brands Corporation.) was used to encapsulate the cation exchange membrane to prevent the electrode from touching the
membrane. The J- cloth also helped minimize microbial growth on cation exchange membrane’s surface.

Growth Medium, Microorganisms in the Microbial Fuel Cell and Inoculum

Anodic Compartment

We used *Klebsiella pneumoniae* (ATCC # 700831) in the anodic compartment of the MFC. The growth medium consisted of tryptone 10g/L; yeast extract 5g/L; NaCl 5g/L; Na₂HPO₄ 1.825g/L; KH₂PO₄ 0.35g/L and glucose 1g/L. One mL of frozen stock culture was used to inoculate 100 mL of the autoclaved growth media in a flask. The flask was placed in a rotary shaker (150 rpm) at room temperature and the microorganisms were grown for ~18hrs prior to experimentation. Later, the microorganisms in the flask were used to inoculate the anodic compartment of the MFC.

Another alternative fuel cell was operated using a sulfate reducing microorganism *Desulfovibrio desulfuricans* in the anodic compartment. The growth medium for this microorganism consisted of sodium lactate 8.5g/L; sodium sulfate 2.23g/L; calcium chloride dehydrated 0.06g/L; PIPES, disodium salt monohydrate, 10.93g/L; ammonium chloride 1g/L; magnesium sulfate 1g/L; yeast extract 50mg/L; tryptone 500mg/L. Prior to autoclaving, the medium was adjusted to a pH of 7.2 using 1N HCl. The
growth culture was inoculated 3 days prior to experimentation, using previously anaerobically grown bacteria (serum bottles).

**Cathodic Compartment**

*Leptothrix discophora* SP-6, grown in MSVP media, was used in the cathodic compartment of the fuel cell. ATTC Culture Medium 1917 MSVP was prepared using the following reagents: (NH₄)₂SO₄ 0.24 g; MgSO₄ · 7H₂O 0.06 g; CaCl₂ · 2H₂O 0.06 g; KH₂PO₄ 0.02 g; Na₂HPO₄ 0.03 g; HEPES 2.383 g; Agar Noble (Difco 0142) 15.0 g (for plates only) was added to 984 mL distilled water. Once all the chemicals dissolved, the pH was adjusted to 7.2 with 6N NaOH. The medium was autoclaved at 121°C for 15 minutes and then cooled to approximately 50°C before aseptically adding filter sterilized: 1.0 mL of vitamin stock solution (see below for reagents); 1.0 ml of 10 mM FeSO₄; 5.0 mL 20% sodium pyruvate solution; and 4.0 mL of .05 M Mn solution. The vitamin stock solution contained (in 1 L of distilled water) Biotin 20.0 mg; Folic acid 20.0 mg; Thiamine HCl 50.0 mg; D-(+)-Calcium pantothenate 50.0 mg; Vitamin B12 1.0 mg; Riboflavin 50.0 mg; Nicotinic acid 50.0 mg; Pyridoxine hydrochloride 100.0 mg; p-Aminobenzoic acid 50.0 mg.

To prepare the inoculum, a MSVP plate (prepared with Agar Noble (Difco 0142)) was streaked with frozen stock culture. The preparation of stock culture is described by Yurt et al. (2002). Once the bacteria formed
colonies, a single colony was transferred to 100 mL of MSVP media in a flask. The flask was placed on a rotary shaker at room temperature operated at 150 rpm. The microorganism grew for ~72 hours and then was used to inoculate the cathodic compartment of the MFC.

**Microbial Fuel Cell Operation**

The microbial fuel cell was operated by following the following protocol:

1. assembled the polycarbonate fuel cell
2. filled the fuel cell with deionized water
3. autoclaved the polycarbonate fuel cell with connected tubing, flow breakers, and rubber stoppers

   The microbial fuel cell was sterilized after complete assembly. The fuel cell, flow breakers, and connected tubing, were autoclaved for 20 minutes with the rubber stoppers off of the top to allow for ventilation. Once done autoclaving, the stoppers were immediately placed in the center hole at the top of the microbial fuel cell (see Figure 2-1D) (where the salt bridge will eventually be located).

4. replaced the deionized water with MSVP medium and inoculated the cathodic compartment with *Leptothrix discophora* SP-6

   The cathodic compartment of the reactor was initially filled with 150 mL of MSVP medium and operated as a batch reactor.
Fifty mL of *L. discophora* inoculum (prepared according to the procedure described above) was then added to the cathodic compartment of the reactor. The cathodic compartment was continuously aerated through port 2 in Figure 2-1B, using air filtered through a 0.2-\(\mu\)m filter (PALL Corporation PN # 4210) at a rate of 2.5 mL/s.

5. operated the cathodic compartment until the cathode reached a steady potential of \(\sim 350\text{mV}_\text{SCE}\)

Once the electrode in the cathodic compartment reached a steady potential of 350\text{mV}_\text{SCE}, fresh sterile MSVP medium was fed at a flow rate of 0.6 ml/hr.

6. replaced the deionized water with the growth LBG medium in the anodic compartment and inoculated it with *Klebsiella pneumoniae* (ATCC #700831) or *Desulfovibrio desulfuricans*

The anodic compartment of the fuel cell was drained to remove the sterile water, filled with \(\sim 150\) mL of growth medium, and inoculated with 1 mL of *K. pneumoniae* or *D. desulfuricans* (depending on experiment). The anodic compartment was purged with nitrogen at a rate of 1.25mL/s to remove oxygen through port 2 in Figure 2-1B; the nitrogen was filtered using a 0.2-\(\mu\)m filter (Corning 431219). After 18 hours of batch growth of *K. pneumoniae*, or three days of growth for *D. desulfuricans*, the
anodic compartment was fed with fresh sterile growth medium at a flow rate of 0.6 ml/hr.

7. Operated the entire fuel cell

Once a constant anodic potential was established and the first current measurement was taken as a control, a redox mediator, 2-hydroxy-1,4-napthoquinone (0.05-mM final concentration in the fuel cell) was aseptically added to the anodic compartment through a filter (0.2-µm filter by Corning, catalog #431219). Both compartments of the fuel cell were continuously fed with medium and bubbled with air or nitrogen. During the entire experiment, potential and current measurements were taken.

**Measurements**

Electrode potentials were measured every 60 minutes using a multimeter (Hewlett Packard data logger, 34970A). To measure the current, we connected the electrodes through a 510 Ω resistor, comparable to that used by Bond and Lovley (2002) and Lee et al. (2002). The current was measured using a Keithley 485 autoranging picoammeter. Once the picoammeter was connected, the peak current was recorded. By definition, the peak current is the maximum amount of current produced immediately after a load (resistor) is applied to the microbial fuel cell. From this measurement, the peak power of the fuel cell
was calculated by multiplying the measured current by the total fuel cell potential (refer to equation 1-8). We realize that the peak values are not representative of the sustainable current or of the sustainable power produced by the fuel cell. However, for the purpose of testing the fuel cell they are sufficient, and the measurement is consistent with other such measurements reported in the literature (Gil et al. 2003; Kim et al. 2002; Lee et al. 2002; Park and Zeikus 2000).

**Manganese Oxides vs. Oxygen as Cathodic Reactants**

Another experiment was performed using an abiotic cathodic compartment with MSVP media including divalent manganese. The anodic compartment consisted of a LBG buffered media and *Klebsiella pneumoniae*. The anodic compartment was bubbled with nitrogen to produce anaerobic conditions and the cathodic compartment was bubbled with air to ensure aerobic conditions. The electrode potential measurements were taken using a saturated calomel electrode (SCE) and peak current was taken using a 510 Ω resister.

**Reagent Analysis**

Daily divalent manganese concentrations were obtained by collecting the effluent liquid from the cathodic compartment. Once obtained, the manganese concentrations were detected using the
methods by Goto et al. 1962. Measurements were obtained by using a mass spectrophotometer (figure 2-4). All of our measurements were below the detection limit.

\[ y = 0.0082x + 0.0035 \]
\[ R^2 = 0.9873 \]

![Figure 2-4: Calibration curve of divalent manganese using a spectrophotometer.](image)

Glucose concentrations were obtained in the anodic compartment and were measured daily using a One Touch Basic Glucose Monitoring System (made by Johnson and Johnson Company).

**Ennoblement Tests on 316L Stainless Steel**

To test how biomineralization of manganese affects the potential of 316L stainless steel, a fermentor was used. In the fermentor, the following parameters were set; pH 7, stir rate 50rpm, MSVP media flow rate (1ml/hr), and air flow rate of 2.5ml/sec. During the experiment, electrode
potentials were observed using a saturated standard calomel reference electrode (SCE) (Fisher, cat. #13-620-51) and a FLUKE 189 true RMS multi-meter.

**Comparison of Cathodic Reactants**

To compare cathodic efficiency of manganese oxides with oxygen, we ran cathodic polarization curves of three cathodic reactants: 1) biomineralized manganese deposited on a graphite electrode, 2) manganese oxides electrochemically deposited on a graphite electrode, and 3) dissolved oxygen reduced on a graphite electrode.

**Microbial Manganese Oxide Deposition**

To deposit biomineralized manganese oxides on the graphite electrode, Aldrich® #496545-60G graphite rods (diameter of 3 mm, with 2 cm of the rod exposed and the rest covered with silicone tubing) were placed into autoclaved MSVP media. The medium was then transferred to a flask and inoculated with a single colony of *Leptothrix discophora* that was grown on a MSVP nutrient agar plate. The flask was placed on a rotating shaker at 150 rpm for four days, during which time the microorganisms biomineralized the divalent manganese and deposited manganese oxides on the graphite rod.
Chemical Manganese Oxide Deposition

To electrochemically plate manganese oxides on the graphite electrodes, we used Aldrich® #496545-60G graphite rods (diameter of 3 mm, with 2 cm of the rod exposed). Each graphite electrode was anodically polarized at 3 mA/cm² for 20 s in a solution of 5mM MnSO₄ and 0.1M Na₂SO₄ at a pH of 6.4 (Dickinson et al. 1996).

Cathodic Polarization

Cathodic polarization of the samples was conducted using the procedure described by Dickinson et al. (1996). A potentiostat/galvanostat (EG&G Princeton Applied Research, model 273A) was used with a graphite auxiliary electrode and a saturated calomel electrode (SCE). To establish the initial potential for the voltage scan, open circuit potentials (OCP) of the samples versus the SCE reference electrode were measured using a handheld multimeter (Wavetek DM23XT with internal resistance 10MΩ). Scans were initiated 50 mV above the OCP, and the samples were scanned at a rate of 0.167 mV/sec. The scan was completed at -800 mVₜₚ. The measurements were conducted in sterile, oxygen-saturated MSVP medium with 0.2mM Mn²⁺ at a pH of 7.2 (Dickinson et al. 1996). Each measurement was repeated at least four times using fresh coupons.
Microbial Fuel Cell Method Development:

The results presented in this thesis were all produced using the same protocol. This protocol was developed through trial and error in the course of running numerous microbial fuel cell experiments, using the following start-up options:

1. Inoculated the anodic and cathodic compartments at the same time.
2. Inoculated the anodic compartment first.
3. Inoculated the cathodic compartment first

We found that inoculating the cathodic compartment first and then allowing the electrode to stabilize worked best. We are uncertain why the fuel cell works best when *Leptothrix discophora* is added first; it was important to follow the protocol that achieved the highest potential difference between the anode and cathode.

Another change that was made during protocol development was the type of electrode material. Initially we used graphite felt (fabric), which worked well because of its high surface area to volume ratio. However, the liquid would soak through the material and would tear when we connected our electrical clips to the fabric. Another material that we tried was 316L stainless steel. We tried 316L stainless steel because it has been used extensively in ennoblement studies and for its inert properties. The limitation with the stainless steel is that it does not have a great deal
of surface area to volume ratio. Hence, it was important to find a material that was durable, easy to use, and has a high surface area to volume ratio. Because of this, we decided to incorporate RVC into our fuel cell as well.

Figure 2-5: Photos A and B represents the two different types of electrodes that we used in the Microbial Fuel Cell. Notice the sponge-like surface that the Reticulated Vitreous Carbon creates. The sponge-like form allows us to achieve a higher surface area to volume ratio. This allows us to create higher amounts of power. (The holes in the 316L stainless steel were for the inlet and outlet ports of the microbial fuel cell.)
CHAPTER 3
RESULTS AND DISCUSSION

Ennoblement Tests on 316L Stainless Steel

Over the past decade, our lab has demonstrated that an electrode’s potential increases when biomineralized manganese was deposited onto the surface of noble metals (Campbell et al. 2004; Dickinson et al. 1997; Dickinson et al. 1996; Geiser et al. 2002; Shi et al. 2003; Olesen et al. 2000; Yurt et al. 2002). We reaffirmed this conclusion by running the same experiment in a fermentor using 316L stainless steel coupons. (Please refer to the ennoblement tests in materials and methods.)

Figure 3-1: Biomineralization of manganese on 316L stainless steel in a fermentor.

When microorganisms deposited manganese oxides on the surface of 316L stainless steel coupons, we monitored an increase in potential. Since there was an increase in potential, we decided to use
biomineralization of manganese as the cathodic reactant in the microbial fuel cell versus the more commonly used cathodic reactants; oxygen or ferricyanide.

**Microbial Fuel Cells**

**Cathode - Manganese Oxidizing Bacteria;**
**Anode – Glucose Oxidizing Bacteria**

The following microbial fuel cell experiments, figure 3-2, used oxidation of glucose in the anodic compartment to extract electrons and reduction of biomineralized manganese oxides in the cathodic compartment. Although we operated the fuel cells several times, the results in figure 3-2 only represent two of the runs.
Figure 3-2: Typical potential variations in anode and cathode by time in our microbial fuel cells. The cathodic compartment increased with time and reached a steady state, while the anodic potentials decreased until they reached a steady state potential.

The fuel cells operated for 500 hours, and the anodic potential reached an average steady state potential of -441.5±31 mV\textsubscript{SCE} while the cathodic potential showed a slow drift in time, and reached an average cathodic potential of 384.5±64mV\textsubscript{SCE}. We have demonstrated that as manganese is deposited onto the surface of the cathode, the electrode potential increases, and as glucose is oxidized in the anodic compartment, the electrode potential decreases. Figure 3-2 also shows that the potential of the microbial fuel cells vary only slightly from one another, hence, the results are reproducible.

The average potential between the anode and cathode was 809.30±19mV\textsubscript{SCE}, and was higher than the potentials reported in literature (Allen and Bennetto, 1993; Park and Zeikus, 2000; Choi et al. 2001; Kim
A higher overall fuel cell potential was achieved because biomineralized manganese was used as the cathodic reactant.

Since the cathode potential depends on the equilibrium between Mn$^{2+}$ and manganese dioxides, we can apply the Nernst equation to predict the cathodic potential in the microbial fuel cell.

Reaction: \[ \text{MnO}_2(s) + 4 \text{H}^+ + 2e^- \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} \quad E^0 = +1.28 \text{ V}_{\text{SCE}} \]

\[
E = E^0 + \frac{RT}{2.3nF} \log \left( \frac{\left[ \text{H}^+ \right]}{[\text{Mn}^{2+}]} \right)
\]

Table 3-1 represents the different theoretical potentials, at a pH of 7.2, on the cathode when biomineralized manganese oxides are being reduced.

<table>
<thead>
<tr>
<th>[Mn$^{2+}$]</th>
<th>Potential (V$_{\text{SCE}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*10$^{-03}$</td>
<td>0.2779</td>
</tr>
<tr>
<td>1*10$^{-06}$</td>
<td>0.3664</td>
</tr>
<tr>
<td>1*10$^{-09}$</td>
<td>0.4549</td>
</tr>
<tr>
<td>1*10$^{-12}$</td>
<td>0.5434</td>
</tr>
<tr>
<td>1*10$^{-15}$</td>
<td>0.6319</td>
</tr>
</tbody>
</table>

Table 3-1: Cathodic potential with varying divalent manganese concentrations. From this table, we can see that the cathode potential changes with respect to changing manganese concentrations.

In the laboratory, our cathode potential stabilized at 384.5 mV and the pH in the cathodic compartment remained at 7.0±0.4. By using the Nernst equation, we can predict that the divalent manganese concentration in the fuel cell was 2.43*10$^{-07}$ M, which is 0.0134ppm. We also used photometric manganese detection, but the divalent manganese
concentration in the effluent of the fuel cell was less than the detection limit of 4ppm.

In the anodic compartment, we obtained the predicted potential drop between -400 to -500mV_{SCE}. In the effluent, the glucose concentration was less than 0.5mg/L versus the inlet concentration of 1g/L, proving that almost all glucose was oxidized in the fuel cell.

We accomplished this drop by glucose oxidation using the microorganism *Klebsiella pneumoniae*. By assuming the following reaction is equilibrating with the anode, we theoretically predicted the following potential using a glucose concentration of .002 M, a partial pressure of 0.2 for carbon dioxide, and a pH of 7.

\[
E = E^o + \frac{RT}{2.3nF} \log \left( \frac{[H^+]^{24} [CO_2]^6}{[C_6H_{12}O_6]} \right)
\]

Where: \(E^0 = -0.0142V\), \([(R*T)/(2.3*F)] = .059\), and \(n=24\)

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

\[E'_{pH=7} = -0.673 \text{ V}_{SCE}\]

Even though we assumed that carbon dioxide was in equilibrium with the electrode, this is often not the case. When microorganisms oxidize glucose, there are numerous side products that are created, including pyruvate, ethanol, lactate, and many others. Since there are these products as well, something other than carbon dioxide and protons may be equilibrating with the electrode. If this was the case, the potential
seen on the electrode would be different than the above calculation and may be one reason why we only see an electrode potential between -400 to -500mV$_{SCE}$ in the anodic compartment.

Figure 3-3 shows the power produced in the two fuel cells. Power was calculated from the total fuel cell potential and was multiplied by the measured peak current (I) ($P=IV$). The average power produced was 0.0176±0.0031 watts and 0.0166±0.0054 watts when a 510Ω resistor was placed between the anode and cathode.

![Figure 3-3](image)

**Figure 3-3:** The variation of power of the microbial fuel cells by time when a 510 Ω resistor was used. The average steady state power was 17.10±4.25 mW. We did not perform any power measurements before 180 hours since the microbial growth and anodic and cathodic potentials were not at steady state.

By introducing HNQ into the anodic compartment, we increased the efficiency of electron transfer from the microorganism to the electrode (please refer to the mechanism in chapter 1: figure 1-3). This can be seen
by the overall fuel cell power production, which increased by 10 times when the redox mediator was added. The current reading before HNQ was only 1.5mA, however, after initial HNQ addition, the current reading was 16.8mA.

During our fuel cell experiments, we quickly realized the importance of adding a redox mediator. However, when the redox mediator was absent, current still could be drawn from the system. We are not sure why this occurred, but some hypotheses include:

1.) The microorganisms were capable of transferring electrons directly from the microbe’s membrane to the electrode as suspected by the following researchers: Bond and Lovley 2003; Chaudhuri and Lovely 2003; Gil et al. 2003; Kim et al. 2003; and Jang et al. 2004.

2.) A microorganism’s cell membrane essentially contains a plethora of potential redox mediators, such as cytochrome. If the cell membrane had been lysed, it was possible that there would be natural redox mediators released into the system.

3.) The nutrient media itself may have contained some redox mediators.

In the cathodic compartment, biomineralized manganese oxides deposited on a graphite electrode were reduced directly without a redox mediator. However, since the ultimate electron acceptor in the cathodic
compartment was oxygen, the manganese/manganese oxides redox couple can be viewed as a redox mediator.

**Manganese Oxides vs. Oxygen as Cathodic Reactants**

In order to show that microbial mineralization of manganese in the cathodic compartment is a better cathodic reaction than the reduction of oxygen alone, a control experiment was performed. The following fuel cell (Figure 3-4) ran for 144 hours utilizing RVC.

![Figure 3-4: Cathode and anode potentials variation by time when oxygen or biomineralized manganese were cathodic reactants.](image)

The cathode reached a potential of 50.6±30mV\textsubscript{SCE} when oxygen was the only reactant, however, the potential increased to 382±58mV\textsubscript{SCE} when there was MOB present in the fuel cell. The power generated was 0.459±0.10mW when oxygen was used as the cathodic reactant and
17.10±4.25 mW when manganese oxides were used. Thus, the power increased by two orders of magnitude when biomineralized manganese was used as a cathodic reactant.

Using thermodynamic calculations, the reduction potential of oxygen is higher than the reduction potential of manganese oxides.

\[
\begin{align*}
\text{MnO}_2(s) + 4 \text{H}^+ + 2e^- & \rightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} \quad E^0 = +1.28 \text{ V}_{\text{SCE}} \quad E'_{\text{pH=7.2}} = +0.360 \text{ V}_{\text{SCE}} \\
\text{O}_2 + 4\text{H}^+ + 4e^- & \rightarrow 2\text{H}_2\text{O} \quad E^0 = +1.23\text{ V} \quad E'_{\text{pH=7.2}} = +0.566 \text{ V}_{\text{SCE}}
\end{align*}
\]

Thermodynamic calculations were derived from reactions occurring on a platinum electrode, whereas, our laboratory reactions occurred on graphite. As we can see from our calculations, we were able to predict the potential of manganese on the electrode; however, the prediction of oxygen was much higher than what occurred in the control experiment. One reason this may occur was that the interaction between the graphite electrode and manganese was greater than the equilibrium between the graphite electrode and oxygen. We can predict the electrode potential when manganese was used since equilibrium occurs between the electrode and manganese. If we were using platinum for our electrode, we could predict the values for any reaction because of the equilibrium properties.
The Effect of Contamination in the Cathodic Compartment

Most of our fuel cell experiments were terminated once the fuel cell became contaminated. We could tell when the fuel cell was contaminated when there was a quick drop in the cathode’s potential (figure 3-5) and by streaking plates.

![Graph showing potential drop due to contamination of fuel cell.](image)

Figure 3-5: Potential drop due to contamination of fuel cell.

One way that the fuel cell may have become contaminated was by microorganisms crossing the proton exchange membrane from the anodic compartment through micro-tears. If this was the case, it may be beneficial to use the organism *Psuedomonas putida* in both compartments of the microbial fuel. *Psuedomonas putida* is unique because it is capable of oxidizing manganese and it is a facultative anaerobe.
Potential Drift

In the fuel cell runs (figure 3-2), we observed a potential drift on the cathode. In the figure 3-6, we can observe that the cathode in fuel cell #1 drifted from $+454\text{mV}_{\text{SCE}}$ to $+278\text{mV}_{\text{SCE}}$ and fuel cell #2 drifted from $+456\text{mV}_{\text{SCE}}$ to $+323\text{mV}_{\text{SCE}}$. This caused a 150mV difference in electrode potential throughout the fuel cell run. Nonetheless, even the lowest electrode potentials were 175mV higher than when oxygen reduction was used as the only cathodic reactant.

![Figure 3-6: Drift of cathodic potentials in the microbial fuel cells.](image)

Throughout the microbial fuel cell experiments, we monitored pH, divalent manganese concentration, flow rate (media and nitrogen), and checked for contamination so we could explain the drift in potential. Throughout the experiments, all of these factors remained relatively constant. However, one factor we did not check was the amount of biomass on the electrode. The more biomass present on the electrode
may cause a decrease of manganese oxides in contact with the electrode.

Nonetheless, potential drift must be further investigated.

**Cathode and Anode Potentials When 316L Stainless Steel or RVC is Used**

Our research was expanded to test how different electrode materials may affect the overall potential of a fuel cell system. In the following experiment, we used 316L stainless steel and reticulated vitreous carbon as electrode materials.

Figure 3-7: Electrode potentials when RVC or 316L Stainless Steel were used in the microbial fuel cell.

We observed that there was a slight difference in potentials when we used different electrode materials. We observed that 316L stainless steel, in the cathode, was able to maintain a potential above $350\text{mV}_{\text{SCE}}$ whereas reticulated vitreous carbon only peaked at $350\text{mV}_{\text{SCE}}$. 
Nonetheless, reticulated vitreous carbon was able to maintain a lower potential in the anode than that of 316L stainless steel. Regardless of these differences, the overall cell potential (anode vs. cathode) was 759.75±73mV_{SCE} for RVC and 706.13±22mV_{SCE} for 316L stainless steel. The current produced in these fuel cells are 0.07±0.01mA on the 316L stainless steel electrodes vs. 0.8±0.2mA on the RVC electrodes. Since RVC has a higher surface area to volume ratio than 316L stainless steel, higher current was drawn.

Sulfate Reducing Bacteria Used in the Anodic Compartment

It has been shown by many researchers that there are sulfate-reducing bacteria in the sediments of open bodies of water (Please see figure A-1 in appendix A). In order to simulate a fuel cell in the natural environment, we decided to run a fuel cell where there were sulfate reducing bacteria used in the anodic compartment of the fuel cell and manganese oxidizing bacteria in the cathodic compartment. Once sulfate is reduced to H$_2$S, which then deprotonates to HS$^-$ in neutral pH, HS$^-$ is then oxidized to extract electrons.

$$\text{HS}^- + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^-$$
Figure 3-8: Microbial Fuel Cell that utilized sulfate reducing bacteria in the anodic compartment and manganese oxidizing bacteria in the cathodic compartment. Representation of what may happen in an environmental microbial fuel cell.

Similar to the other fuel cells, we were able to achieve a drop in potential on the anode and an increase in potential on the cathode. The average potentials in the cell were $-470\pm44 \text{ mV}_{\text{SCE}}$ for the anode and $419\pm59 \text{ mV}_{\text{SCE}}$ for the cathode. The overall cell potential was $883\pm51 \text{ mV}$. If we theoretically calculate the potential using the Nernst equation the following is observed:

$$\text{HS}^- + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \quad E^0 = -0.331\text{V}_{\text{SCE}} \quad E'_{\text{pH}=7}=1.01\text{V}_{\text{SCE}}$$

The Nernst equation shows a lower theoretical value than what was observed in the fuel cell. This may be observed because the oxidation of HS$^-$ does not equilibrate well with the graphite electrode; similar to what happened with the theoretical calculation of oxygen.
Cathodic Polarization

The manganese oxidizing bacteria present in the cathodic compartment of the microbial fuel cell were shown to be more efficient at producing current than oxygen alone. In addition, there is a larger potential difference, allowing for a larger driving force for electrons to flow when a load is applied, thus increasing the current. Dickinson et al. (1996) measured almost identical cathodic polarization curves using 316L stainless steel coupons in 0.01 M Na$_2$SO$_2$ as the supporting electrolyte (Dickinson et al. 1996). Our measurements were done in the MSVP growth medium.

Figure 3-9: Cathodic polarization curves for the following samples; 1) biomineralized manganese on graphite electrode, 2) electrochemically deposited manganese on graphite electrode, 3) clean graphite electrode used to reduce oxygen only.
The cathodic polarization curves show that current density, drawn from reducing manganese oxides, can be up to two orders of magnitude higher than current density drawn from reducing oxygen. Therefore, if we biominerally deposited manganese oxides we will obtain both a higher current and potential in the microbial fuel cell.

**Stability of Peak Current Values**

When we first started running microbial fuel cells, we realized that most of the literature only included studies on peak current (Kim et al. 2002; Lee et al. 2002; Park and Zeikus 2002; Gil et al. 2003). Peak current is defined as the maximum current obtained when a load is applied between the anode and cathode of the fuel cell. In figure 3-10 the maximum current produced occurred at the moment the load was applied between the anode and cathode (12 hours experimental time). Within minutes, the current drifted towards zero as time progressed. Additionally, once the load was applied, there was a decrease in the cathode's potential and an increase in the anode’s potential. Therefore, as current was drawn, the fuel cell’s total potential decreased.
Figure 3-10: Peak current measurements using a 510 ohm resistor.
Conclusions

Based on our fuel cell experiments, we were able to form the following conclusions.

1. When *Leptothrix discophora* oxidized divalent manganese in the cathodic compartment and *Klebsiella pneumoniae* oxidized glucose in the anodic compartment, a potential difference between the anode and cathode would occur. When a load was applied between the two electrodes, the potential difference caused electrons to flow from the anode to the cathode, allowing current to be drawn.

   The potential difference was obtained by manganese depositing onto the cathode, causing a potential increase, and glucose oxidizing in the anodic compartment, causing a potential decrease. When a potential difference occurred, small amounts of current were drawn when a 510Ω resistor was applied between the anode and cathode. Once a redox mediator, HNQ, was added to the anodic compartment, we saw an increase in current by ten times.
2. Microbially deposited manganese oxides on the fuel cell’s cathode created higher current and higher electrode potential than when oxygen was the only reactant.

When divalent manganese was biominerally deposited onto 316L stainless steel or reticulated vitreous carbon, higher electrode potentials and current were obtained than when oxygen was the only reducing agent. We found that there was an increase in potential and a two-fold increase in current when manganese oxidizing bacteria were present in the cathodic compartment than when the system was aerobically abiotic.
References Cited


Shi, X.; Avci, R; Geiser, M; Lewandowski Z.. (2003) Comparative study in chemistry of microbially and electrochemically induced pitting of 316L stainless steel *Corrosion Science, 45*, 2577-2595


APPENDICES
APPENDIX A

FIELD STUDIES
INTRODUCTION

Microbial Fuel Cells in Nature

The microbial fuel cell reactor (figure A-1) may be viewed as a simulation model for what may occur in the nature. In an environment, the fuel cell works similarly to the lab experiments (Reimers et al. 2001, Delong and Chandler 2002).

Figure A-1: An electrode in an anaerobic layer of sediments oxidizes a substrate and donates its electrons to manganese oxides that are biomineralized onto the cathode in an aerobic environment. Modified from Reimers et al. 2001.

Divalent manganese is present in most open bodies of water. If an electrode is deployed into an aerobic water system, there will often be biomineralization of manganese onto the electrode’s surface. The
biomineralization of manganese often leads to a higher electrode potential than when oxygen is the only reducing reactant. Hence, the cathodic reaction in figure 1-7 must work similar to the reaction occurring in figure 1-8.

The anodic reaction that occurs in the environment takes place in anaerobic areas, usually found in sediments. One hypothesis about what may be happening in the sediments is microbial sulfate reduction. Once microorganisms have reduced the solid form of sulfate, the sulfate may be reformed through the oxidation of HS\(^{-}\) (Katz et al. 2003). The process of HS\(^{-}\) oxidation allows us to obtain electrons that are then used to reduce a reactant on the cathode.

\[
\text{HS}^{-} + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^{-}
\]

We can assume that what is happening in nature can also be reproduced in the laboratory.

**MATERIALS AND METHODS**

316 Stainless steel coupons, a flat sheet of metal, and stainless steel wire balls (Please see figure 3-8) were deployed into open bodies of water located near the campus of Montana State University. On a periodic basis, the potential of the deployed samples were measured. The measurements were done using a saturated standard calomel reference electrode (SCE) (Fisher, cat. #13-620-51) and a FLUKE 189 true RMS
multi-meter. During these measurements, it was important that the electrodes connected to the electrometer remain dry and that the coupon was not moved. It is notable that moving the electrode causes a change in the electrochemical interaction that is occurring at the surface, which in turn affects the potential of the coupon.

FIELD EXPERIMENTS

Numerous field studies have already been evaluated within our lab group. The studies involved include; different open water locations (lakes and rivers), different metal surfaces (stainless steel and titanium), and different shapes of electrodes (wire spheres, nickel-sized coupons, and flat 1ft square plates.) The different shapes of electrodes can be seen in figure A-2.

Figure A-2: 316 Stainless steel that was deployed into the environment. The left photo represents a stainless steel plate that was deployed in Hyalite lake. The right photo represents sphere wire coupons that were
deployed into hyalite creek. For the creek study, we wanted a high surface area, so we formed metal spheres made out of 316L stainless steel wire.

When the samples were deployed into the environment, we saw an increase in the electrode potential. The increase in field electrode potential (figure A-3) was similar to that seen in the fermentor experiment (figure 3-1). The difference in these two figures is the amount of time it took the electrode potential to increase. We noticed that it did not matter what material type (316L stainless steel or titanium), shape (sphere or plate), or location (Roskie or Hyalite); we always saw an increase in electrode potential. We are not sure how temperature effects how fast the metal ennobles, but we are assuming that it is slower in cooler temperatures because the metabolism of the manganese oxidizing bacteria will be lower.

Figure A-3: 316L Stainless Steel sphere with high surface area was deployed in Roskie creek
FUTURE FIELD WORK

Identification of Manganese Oxidizers in the Field

For years, our lab has concentrated on the biomineralization of manganese onto 316L stainless steel surfaces in the field environment. Even though we have been studying the biomineralization of manganese, we are not sure which organisms are causing the deposition. In the future, it may be beneficial to run DNA analysis using the PCR (polymerase chain reaction) or DGGE (denaturing gradient gel electrophoresis). These two microbiological techniques can be used to identify both the ecology and the individual species found in the water environment.

Anodic Reactions in Montana

Additionally, we have only started to conduct field studies on the anodic electrode. We have initiated the anodic studies by deploying a 316L stainless steel coupon into the duck pond located on Montana State University’s campus. We have decided that the duck pond would be a good choice for this study because the water is assumed to be anaerobic due to the numerous ducks visiting the area. In this environment, potential of the 316L stainless steel coupon was -295mV$_\text{sce}$ during the summer. Even though this result was preliminary, we know that there was a drop in
electrode potential when the environment was anaerobic. We have not implemented many anode studies because it was often difficult to find or access areas that were anaerobic. If we could obtain access, some ideal places to continue anode electrode studies would be in a wastewater treatment plant or in the sediments of a lake or river. In the future, we hope to form an environmental fuel cell where the cathode is located in an aerobic environment and the anode is located in an anaerobic environment.
APPENDIX B

ORIGINAL TEXT SUBMITTED TO ENVIRONMENTAL SCIENCE AND TECHNOLOGY
A MICROBIAL FUEL CELL USING ANAEROBIC RESPIRATION AS AN ANODIC REACTION AND BIOMINERALIZED MANGANESE AS A CATHODIC REACTANT

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Abstract

We have operated a microbial fuel cell in which glucose was oxidized in the anodic compartment and the electrons derived from this reaction were transferred via an electronic conductor to the cathodic compartment, where they reduced biomineralized manganese oxides deposited on a graphite electrode. In the anodic compartment, to facilitate the electron transfer from glucose to the graphite electrode, we used a redox mediator, 2-hydroxy-1,4-napthoquinone. In the cathodic compartment, biomineralized manganese oxides deposited on a graphite electrode were reduced directly without a redox mediator. However, since the ultimate electron acceptor in the cathodic compartment was oxygen, the manganese/manganese oxides redox couple can be viewed as a redox mediator. As a result of this study, we conclude that biomineralized manganese oxides are superior to oxygen when used as cathodic reactants in microbial fuel cells. In our setup, the manganese oxides supply a current density almost two orders of magnitude higher than that drawn from the same fuel cell reducing oxygen directly. To obtain reproducible results, we operated several fuel cells. They were all operated for 500 hours, reaching anodic potentials of \(-441.5\pm31\) mV\(_{\text{SCE}}\) and cathodic potentials of \(384.5\pm64\) mV\(_{\text{SCE}}\). The peak power of 17.10\(\pm4.25\) mW was monitored when the electrodes were connected by a 510Ω resistor.

Keywords: Microbial fuel cell, manganese oxidation, *Leptothrix discophora*, *Klebsiella pneumoniae*
INTRODUCTION

Most researchers of microbial fuel cells focus on the anodic part of the fuel cell. Typically, they attempt to 1) determine the most efficient anodic reactions, those producing the highest number of electrons per unit weight of the reactant (Allen and Bennetto 1993; Lee et al. 2002; Logan 2004); 2) determine the most efficient microorganisms, those that can offer the highest reaction rate of oxidation or are able to extract the highest number of electrons from a unit mass of the substrate (Choi et al. 2001; Kim et al. 2002; Tsujimura et al. 2001b); 3) study the effectiveness of redox mediators (McKinlay and Zeikus 2004; Park and Zeikus 2000); and 4) select more effective electrode materials (Gregory et al. 2004; Katz et al. 1999; Schroder et al. 2003).

The cathodic compartments of microbial fuel cells are less studied, and in most microbial fuel cells the cathodic reaction is abiotic, typically the reduction of oxygen or ferricyanide (Chaudhuri and Lovley 2003; Choi et al. 2003; Lee et al. 2002; Liu and Logan 2004):

\[
\text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O}
\]  

(1)

\[
\text{Fe(CN)}_6^{3-} + \text{e}^- \rightarrow \text{Fe(CN)}_6^{4+}
\]  

(2)

Ferricyanide is a good choice of cathodic reactant because its concentration is not limited by solubility, as is oxygen concentration. However, it is toxic to microorganisms and difficult to use in microbial fuel cells. On the other hand, oxygen is known for having notoriously slow reduction kinetics on solid
electrodes and quite low solubility in water (Jang et al. 2004; Pham et al. 2004).

Since oxygen is a universal oxidant, much research on abiotic fuel cells is done to improve the kinetics of oxygen reduction on solid electrodes (Palmore and Kim 1999; Tsujimura et al. 2001a; Willner et al. 1998). Improving oxygen reduction efficiency on solid electrodes would be beneficial to microbial fuel cell applications. Having in mind the difficulties with using oxygen as the cathodic reactant, we attempted to use biomineralized manganese oxides instead. Since oxygen remains the terminal electron acceptor (microorganisms reduce oxygen to oxidize manganese) the redox couple composed of manganese ions and manganese oxides can be considered a redox mediator in the cathodic reaction.

Previously, our research group showed that biomineralized manganese oxides can be efficient cathodic reactants in microbially influenced corrosion (Campbell et al. 2004; Dickinson et al. 1996; Dickinson et al. 1997; Geiser et al. 2002; Olesen et al. 2000; Shi et al. 2003). To use manganese oxides as cathodic reactants in microbial fuel cells, biofilms of manganese-oxidizing microorganisms were grown on the surfaces of solid state cathodic electrodes. These oxides were then reduced by the electrons derived from the anodic reactant and delivered via an electronic conductor. We expected that biomineralized manganese oxides might be superior cathodic reactants for two reasons: (1) manganese oxides are solid state and do not change activity when the reactant is consumed, which bypasses the difficulty caused by the limited solubility of oxygen in water and (2) manganese oxides are attached to the electrode surface, which bypasses the
problem with the mass transport resistance of dissolved cathodic reactants. The
goal of this study was to evaluate the feasibility of using biomineralized
manganese oxides as cathodic reactants in microbial fuel cells, and we tested their
cathodic efficiency in many arrangements.

Manganese-oxidizing bacteria (MOB) colonizing noble metals immersed
in natural waters deposit biomineralized manganese oxides on their surfaces and
increase their open circuit potential (Braughton et al. 2001; Dexter and Gao
1988; Dickinson et al. 1997; Geiser et al. 2002; Mattila et al. 1997). Because the
biomineralized manganese oxides are in direct electrical contact with the
electrode, the electrode exhibits the equilibrium dissolution potential of the MnO₂
through the following half-reactions:

\[
\begin{align*}
\text{MnO}_2(s) + H^+ + e^- &\rightarrow \text{MnOOH}(s) \quad E^0 = +0.81 \text{ V}_{SCE} \quad E'_{pH=7.2} = +0.383 \text{ V}_{SCE} \quad (3) \\
\text{MnOOH}(s) + 3H^+ + e^- &\rightarrow \text{Mn}^{2+} + 2H_2O \quad E^0 = +1.26 \text{ V}_{SCE} \quad E'_{pH=7.2} = +0.336 \text{ V}_{SCE} \quad (4)
\end{align*}
\]

The overall reaction is:

\[
\text{MnO}_2(s) + 4H^+ + 2e^- \rightarrow \text{Mn}^{2+} + 2H_2O \quad E^0 = +1.28 \text{ V}_{SCE} \quad E'_{pH=7.2} = +0.360 \text{ V}_{SCE} \quad (5)
\]

The mechanism of the cathodic reaction involves an initial deposition of
manganese dioxide on the electrode surface and its subsequent reduction by two
electrons from the anodic reaction, which results in the release of manganese ions.
Since the release of the divalent manganese occurs in close proximity to the
MOB-colonized electrode surface, the divalent manganese is immediately
reoxidized to manganese dioxide by the MOB and the cycle continues (Figure 1).
Figure 1. Schematic diagram of manganese deposition and reoxidation used as the cathodic reaction (Olesen et al. 2000; Shi et al. 2003).

To demonstrate that biomineralized manganese oxides can serve as efficient cathodic reactants in microbial fuel cells (MFC) we constructed and operated MFC with anodic compartments utilizing anaerobic respiration by *Klebsiella pneumoniae* (ATCC #700831) and cathodic compartments using biomineralized manganese oxides deposited by *Leptothrix discophora* SP-6 (Figure 2).
Figure 2. Schematic of designed MFC. Glucose was oxidized in the anodic compartment and the electrons were transferred via an electronic conductor to the cathodic compartment, where they reduced microbially deposited manganese oxides. To facilitate the electron transfer from the glucose to the graphite electrode in the anodic compartment, we used a redox mediator, 2-hydroxy-1,4-napthoquinone. In the cathodic compartment manganese was microbially deposited on the graphite electrode as manganese oxides and reduced directly, without a redox mediator, by the electrons derived from the anodic reaction.

In the anodic compartment we used the anaerobic respiration of glucose by *Klebsiella pneumoniae*. To facilitate electron transfer to the graphite electrode, we used 2-hydroxy-1,4-napthoquinone (HNQ) in the anodic compartment, following literature findings which suggest that it has high columbic output (Lee et al. 2002) and is chemically more stable than other redox mediators (Allen and Bennetto 1993). In the cathodic compartment, we used *Leptothrix discophora*
SP-6 and an aerated solution of Mn$^{2+}$. As a result, *L. discophora* SP-6 grew and deposited MnO$_2$ on the electrode surface.

**MATERIALS AND METHODS**

The body of the fuel cell was made of polycarbonate (Figure 3) following the construction proposed by (Allen and Bennetto 1993). Each compartment was 250 mL in volume. The influent and effluent lines were made of Neoprene$^\text{®}$ tubing (Cole-Parmer 148441). Flow breakers were used to prevent back flow and contamination from the inlet or outlet tubing.
Both electrodes in the anodic and cathodic compartments were made of Reticulated Vitreous Carbon, RVC which are porous plates made of graphite (80 PPI, (The Electrosynthesis Co. 1-716-684-0513). The RVC electrodes were connected to carbon rods (Thermadyne Arcair® Plain Pointed Electrodes) by direct insertion of the rods into the RVC; the electrical resistance of the
connection was less than 1 Ω. Potentials were measured against the standard calomel reference electrode (SCE) (Fisher, cat. #13-620-51.)

To separate the compartments, we used a proton exchange membrane: ESC-7000 (The Electrosynthesis Co. 1-716-684-0513). To prevent mechanical damage to the membrane, we used J-cloth (First Brands Corporation) between the membrane and the RVC electrode. It was cut to the size of the cation exchange membrane and glued to the surface of a rubber gasket using silicone sealant. Additionally, J-cloth helped minimized microbial growth on the cation exchange membrane surface. We recycled the cation exchange membranes and used them more than once. After each run, the MFC were taken apart, the proton membranes were rinsed gently to remove surface deposits, and then the membranes were placed in 1M NaCl for 24 hours to recharge, as recommended by the vendor. Before installing in the fuel cells, we carefully inspected the membranes for mechanical damage; defective membranes were discarded.

**Growth medium, microorganisms and inoculum**

In the anodic compartment of the fuel cell, we grew *Klebsiella pneumoniae* (ATCC #700831). The growth medium was composed of tryptone, 10g/L; yeast extract, 5g/L; NaCl, 5g/L; Na₂HPO₄, 1.825g/L; KH₂PO₄, 0.35g/L and glucose, 1g/L, known as a Luria-Bertani medium with glucose (LBG). As inoculum, we used 1mL of frozen stock culture per 100 mL of the autoclaved growth medium in a 250 mL flask. The flask was placed in a rotary shaker at
room temperature, operated at 150 rpm, and the microorganisms were grown for 
~18hrs prior to inoculating the anodic compartment.

In the cathodic compartment we grew *Leptothrix discophora* SP-6 using
the ATTC Culture Medium 1917 MSVP. The medium was prepared by mixing
together: 0.24g of (NH₄)₂SO₄, 0.06 g of MgSO₄.7H₂O, 0.06 g of CaCl₂.2 H₂O,
0.02 g of KH₂PO₄, 0.03 g of Na₂HPO₄, 2.383 g of HEPES, and 15.0 g of Agar
Noble (Difco 0142) (for plates only). It was added to 984 mL distilled water, and
the pH was adjusted to 7.2 using a 6N NaOH. The medium was autoclaved at
121°C for 15 minutes and then cooled to approximately 50°C before aseptically
adding the following filter-sterilized solutions: 1mL vitamin solution, 1.0 mL
from 10 mM FeSO₄, 5.0 mL from 20% sodium pyruvate, 4.0mL from 0.05 M
manganese stock solution. The vitamin solution contained (in 1 L of distilled
water) 20.0 mg of biotin, 20.0 mg of folic acid, 50.0 mg of thiamine HCl, 50.0 mg
of D-(+)-calcium pantothenate, 1.0 mg of vitamin B12, 50.0 mg of riboflavin,
50.0 mg of nicotinic acid, 100.0 mg of pyridoxine hydrochloride, 50.0 mg of p-
aminobenzoic acid.

To prepare the inoculum, a MSVP plate (prepared with Agar Noble (Difco
0142)) was streaked with frozen stock culture of *Leptothrix discophora* SP-6 as
described by Yurt et al. (Yurt et al. 2002). Once the bacteria formed colonies, a
single colony aseptically transferred to 100 mL of MSVP medium in a flask. The
flask was placed on a rotary shaker at room temperature operated at 150 rpm. The
microorganisms grew for ~72 hours and then the solution was used to inoculate the cathodic compartment of the MFC.

After assembling the MFC, two rubber stoppers (size 00) were placed in the center hole at the top of the MFC (see Figure 3D and E; Port 4) (where the salt bridge would eventually be located). Both the anode and cathode compartments were filled with deionized water. The MFC and all components (tubing, flow breakers, rubber stoppers etc.) were autoclaved for 20 min with the rubber stoppers off the top to allow for ventilation. Once autoclaved, the stoppers were immediately placed in port 4.

**Microbial fuel cell operation**

To start operating the microbial fuel cell, we followed the steps:

1. assembled the polycarbonate fuel cell
2. filled the fuel cell with deionized water
3. autoclaved the polycarbonate fuel cell with connected tubing, flow breakers, and rubber stoppers
4. replaced the deionized water with MSVP medium and inoculated the cathodic compartment with *Leptothrix discophora* SP-6
5. operated the cathodic compartment until the cathode reached a steady potential of ~350mV\textsubscript{SCE}
6. replaced the deionized water with the growth LBG medium in the anodic compartment and inoculated it with *Klebsiella pneumoniae* (ATCC #700831)
7. operated the entire fuel cell

Since we could not sterilize the reference electrode, to prevent contamination of the fuel cell we connected the reference electrode with the solution via a salt bridge, which was made of glass tubing with porous glass affixed to one end, as described by Geiser et al. (Geiser et al. 2002). The other end of the tubing was equipped with a rubber stopper and was fixed to the top of the MFC. The autoclave-sterilized tubing was connected to the fuel cell. The filling solution for the salt bridge was made of 1g/100 mL of R2A agar (DIFCO 1826-17-1) and 1mL of 0.1 M Na$_2$SO$_4$. The filling solution and syringe apparatus were autoclaved at 121°C and 1 atm before filling the glass tubing. The filling solution was then added to the glass tubing using a syringe. Once the filling solution was in place, a connector was added to the glass tubing and the remainder was filled with 1 N Na$_2$SO$_4$ solution.

**Start-up procedure**

The cathodic compartment of the reactor was initially filled with 150 mL of MSVP medium and operated as a batch reactor. Fifty mL of *L. discophora* inoculum (prepared according to the procedure described above) was then added to the cathodic compartment of the reactor. The cathodic compartment was continuously aerated through port 2 in Figure 3B, using air filtered through a 0.2-$\mu$m filter (PALL Corporation PN # 4210) at a rate of 2.5 mL/s. Once the electrode in the cathodic compartment reached a steady potential of 350mV$_{SCE}$, fresh sterile MSVP medium was fed at a flow rate of 0.6 ml/hr. Then, the anodic
compartment of the fuel cell was drained to remove the sterile water, filled with ~150 mL of growth medium, and inoculated with 1 mL of *K. pneumoniae*. It was purged with nitrogen at a rate of 1.25mL/s to remove oxygen through port 2 in Figure 3B; the nitrogen was filtered using a 0.2-µm filter (Corning 431219). After 18 hours of batch growth of *K. pneumoniae*, the anodic compartment was fed with fresh sterile growth medium at a flow rate of 0.6 ml/hr. Once a constant anodic potential was established and the first current measurement was taken as a control, 0.05-mM (final concentration in the fuel cell) 2-hydroxy-1,4-napthoquinone (the redox mediator) was aseptically added to the sterile growth medium through a filter (0.2-µm filter by Corning, catalog #431219).
Measurements

Electrode potentials were measured every 60 minutes using a multimeter (Hewlett Packard data logger, 34970A). To measure the current, we connected the electrodes through a 510 Ω resistor, comparable to that used by Bond and Lovley (2002) and Lee et al. (2002) when operating microbial fuel cell (Bond and Lovley 2003; Lee et al. 2002). The current was measured using a Keithley 485 autoranging picoammeter. Once the picoammeter was connected, the peak current was recorded. By definition, the peak current is the maximum amount of current produced immediately after a load (resistor) is applied to the microbial fuel cell. From this measurement, the peak power of the fuel cell is calculated by multiplying the measured current by the cell potential. We realize that the peak values are not representative of the sustainable current or of the sustainable power delivered by the fuel cell. However, for the purpose of testing the fuel cell they are sufficient, and the measurement is consistent with other such measurements reported in the literature (Gil et al. 2003; Kim et al. 2002; Lee et al. 2002; Park and Zeikus 2000).

Chemical analysis

In the cathodic department, concentrations of divalent manganese ions were evaluated daily by sampling the effluent, and measured using the methods of Goto et al. 1962 with the detection limit of 4 ppm. In the anodic compartment, glucose concentration was measured daily using the One Touch Basic Glucose Monitoring System made by Lifescan, a Johnson and Johnson company.
Cathodic Polarization

To compare cathodic efficiency of manganese oxides with oxygen, we measured cathodic polarization curves of three cathodic reactants: 1) biomineralized manganese deposited on a graphite electrode, 2) manganese oxides electrochemically deposited on a graphite electrode, and 3) dissolved oxygen reduced on a graphite electrode.

To deposit biomineralized manganese oxides on the graphite electrode, Aldrich® #496545-60G graphite rods (diameter of 3 mm, with 2 cm of the rod exposed and the rest covered with silicone tubing) were placed into MSVP growth medium, autoclaved, and filtered with nutrients and manganese added to the medium. The medium was then transferred to a flask and inoculated with a single colony of *Leptothrix discophora* that was grown on a MSVP nutrient agar plate. The flask was placed on a rotating shaker at 150 rpm for four days, during which time the microorganisms biomineralized the divalent manganese and deposited manganese oxides on the graphite rod.

To electrochemically plate manganese oxides on the graphite electrodes, we used Aldrich® #496545-60G graphite rods (diameter of 3 mm, with 2 cm of the rod exposed). Each graphite electrode was anodically polarized at 3 mA/cm² for 20 s in a solution of 5mM MnSO₄ and 0.1M Na₂SO₄ at a pH of 6.4 (Dickinson *et al.* 1996).

Cathodic polarization of the samples was conducted using a potentiostat/galvanostat (EG&G Princeton Applied Research, model 273A) with a graphite
auxiliary electrode and a saturated calomel electrode (SCE). To establish the
initial potential for the voltage scan, open circuit potentials (OCP) of the samples
versus the SCE reference electrode were measured using a handheld multimeter
(Wavetek DM23XT with internal resistance 10MΩ). Scans were initiated 50 mV
above the OCP, and the samples were scanned at a rate of 0.167 mV/sec. The scan
was completed at -800 mV_{SCE}. The measurements were conducted in sterile,
oxygen-saturated MSVP medium with 0.2mM Mn²⁺ at a pH of 7.2 (Dickinson et
al. 1996). Each measurement was repeated at least four times using fresh
coupons.

**Comparing effectiveness of biomineralized manganese oxides and oxygen as
cathodic reactants**

To compare the cathodic efficiency of biomineralized manganese oxides
with that of oxygen, we operated two microbial fuel cells. In the anodic
compartments of both reactors, glucose was anaerobically oxidized by *Klebsiella
pneumoniae* (ATCC #700831). We used the reduction of biomineralized
manganese oxides deposited by *Leptothrix discophora* SP-6 in the cathodic
compartment of the first fuel cell (Figure 2, Equation 5), and the reduction of
oxygen in the second fuel cell (Equation 1).
RESULTS AND DISCUSSION

Microbial fuel cell operation: cell potentials and currents

Even though we operated the fuel cells several times, in this paper we present only two representative results (Figure 4). The anodic potential reached a steady state of -441.5±31 mV_{SCE}, while the cathodic potential showed a slow drift in time and reached, after 500 h operation, 384.5±64 mV_{SCE}. The variation in the cathodic potential may have been caused by the mixed potential of the electrode resulting from the presence of two cathodic reactants, manganese oxides and oxygen. As time progressed, and the biofilm accumulated on the electrode, oxygen concentration in the proximity of the electrode decreased because of microbial activity, and the potential drifted to that predicted from the thermodynamic calculations for manganese oxides alone, 360 mV (Equation 5).

Once the cell potential stabilized, the Mn^{2+} concentrations in the effluent decreased below our detection limit (4 mg/L). In the effluent from the anodic compartment, the glucose concentration was less than 0.5 mg/L versus the inlet concentration of 1g/L. However, glucose does not equilibrate with the electrode directly, and this did not have any effect on the electrode potential.

The average potential difference between the electrodes was 809.30±19 mV_{SCE}, somewhat higher than the potentials reported in the literature (Allen and Bennetto 1993; Choi et al. 2001; Kim et al. 2002; Park and Zeikus...
2000; Reimers et al. 2001) because the biomineralized manganese oxides in the cathodic compartment produced a higher potential than oxygen did.

Figure 4. Typical temporal variations in the anode and cathode potentials in our microbial fuel cells.

![Graph showing temporal variations in potentials](image)

Figure 5 shows the power generation in the microbial fuel cells. The average power generated was $0.176\pm0.0031$ watts when a $510\Omega$ resistor was placed between the anode and the cathode.
Figure 5. Temporal variation of power generated by the microbial fuel cell when we used a 510 Ω resistor: the average steady state power was 17.10±4.25 mW. We did not measure power before 180 hours because the microbial growth and the anodic and cathodic potentials were not at a steady state.

It is important to note that after HNQ addition, power production increased by ten times; the current reading taken prior to HNQ addition was 1.5 mA, and that taken after HNQ addition was 16.8 mA. Hence, the addition of a redox mediator increased the transport efficiency of electrons between the microorganisms and the electrode, as expected.

Comparing the cathodic efficiency of biomineralized manganese and oxygen

The cathodic polarization curves measured for our samples are shown in Figure 6. As expected, the chemically deposited manganese oxides and microbially deposited manganese oxides had similar cathodic polarization
curves. However, the cathodic polarization curves measured for oxygen are very different from the cathodic polarization curves measured for manganese oxides: manganese oxides can deliver up to two orders of magnitude higher current density than that delivered reducing oxygen. Dickinson et al. (1996) measured almost identical cathodic polarization curves using 316L stainless steel coupons in 0.01 M Na₂SO₂ as the supporting electrolyte (Dickinson et al. 1996). Our measurements were done in the growth medium.

![Cathodic polarization curves](image)

**Current density log(i), A/cm2**

**Figure 6.** Cathodic polarization curves for the following samples: 1) biomineralized manganese on a graphite electrode, 2) electrochemically deposited manganese on a graphite electrode, 3) oxygen reduction on a graphite electrode.

Figure 7 shows that when oxygen is the cathodic reactant the equilibrium potential of graphite electrodes is 50.6±30mV, and that when biomineralized manganese oxides are used as the cathodic reactants, the equilibrium potential increases to 382±58mV SCE. Biomineralized manganese oxides increase the cell
potential by approximately 300 mV, as compared to the equilibrium potential reached in the presence of oxygen. The power generated was 0.459±0.10 mW when oxygen was used as the cathodic reactant and 17.10±4.25 mW when manganese oxides were used; the biomineralized manganese increased the power generated by the fuel cell by two orders of magnitude.

Figure 7. Cathode and anode potential variation by time when oxygen or biomineralized manganese was the cathodic reactant.

Figures 6 and 7 show the results and demonstrate that using biomineralized manganese oxide instead of oxygen as the cathodic reactant in microbial fuel cells increases the cathodic current and the cell potential.

CONCLUSIONS
1. We designed and operated microbial fuels cell using *Klebsiella pneumoniae* oxidizing glucose in the anodic compartment and *Leptothrix discophora* depositing biomineralized manganese oxides in the cathodic compartment.

2. The fuel cells operated for 500 hours and reached an average anodic potential of -441.5±31 mV_{SCE}, and an average cathodic potential 384.5±64 mV_{SCE}. The power produced by the fuel cells was 17.10±4.25 mW when a 510Ω resistor was used.

3. Microbially deposited manganese oxides are more effective as cathodic reactants than oxygen is.

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**References**


