Usage of Microbial Fuel Cell Technology to Prevent Iron Release nearby Landfills in Northwest Florida

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Report #

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ABSTRACT (1 page only)
Landfill leachate is being blamed for elevated levels of iron observations in the groundwater from monitoring wells downgradient of landfills. The geomicrobial iron reduction processes are believed to be responsible for the iron release in the groundwater. Low cost technologies are in urgent need to prevent iron release nearby landfills. The objective of this study is to investigate the feasibility of the usage of microbial fuel cell (MFC) technology for landfill leachate decomposition, iron release prevention and possible power generation. For MFC applications nearby landfills, multiple physical, chemical and biological factors play important roles in determining MFC performances, which complicates the design of the MFC systems. For this research, landfill leachate collected from landfills located in Northwest Florida was tested in a laboratory scale MFC to provide evidence that landfill leachate can be decomposed and electrons released from leachate decomposition in the anodic chamber can be transported and consumed in the cathodic chamber and consequently, electricity can be generated and iron release can be prevented. Based on the results of laboratory MFC research, iron release prevention was tested to mimic real case scenarios nearby landfills. The proposed MFC technology is of great benefit to landfills located in remote territories in terms of energy generation and environmental protection.

EXECUTIVE SUMMARY

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Objective:
This study investigates the feasibility of the usage of MFC technology for landfill leachate decomposition, iron release prevention and power generation. In this research, we design experimental scale MFCs where *Shewanella putrefaciens* utilizes landfill leachate as the energy source. The electrons released from landfill leachate decomposition flow from the anode to the cathode, where they are accepted by selected electron acceptors instead of iron oxides to generate electricity. Since electrons released during landfill leachate decomposition are transited and consumed by designated electron acceptors, iron release is consequently prevented.

Methodology:
Two sets of MFCs, i.e., static MFC and continuous MFC were tested for landfill leachate decomposition and power generation in this research. Based on the experimental results, a custom-made experimental setup was developed to simulate iron release prevention nearby landfills. The activities of *Shewanella putrefaciens* as well as the impact factors on MFC performance were investigated.

Results:
Among the landfill leachate collected from the four counties, landfill leachate collected from Okaloosa County generated the most power, followed by Leon County, Gadsden County and Santa Rosa County. It was discovered that the power generation was as high as 25 mW/m^2 for landfill leachate. Without the application of MFC technology, landfill leachate may trigger iron release as high as 150 mg/l. With MFC technology application, 75 to 80% of the iron release can be prevented.

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1. Introduction

Landfill leachate is being blamed for elevated levels of iron and arsenic, especially iron observations in the groundwater from monitoring wells downgradient of landfills. The geomicrobial iron reduction processes are believed to be responsible for the iron release in the groundwater (Williams et al. 2009). In Florida, soils typically consist of poorly drained sandy soils of Myakka, which are an acid soil characterized by a subsurface accumulation of humus and Al and Fe oxides (Carlisle 1995). Although Myakka soil series is widely extensive in the state of Florida, it can hardly be seen in any other states. In northwest Florida, iron content in the soil is even higher than the rest of the state. Researchers from Florida State University have demonstrated that a pure culture of *Shewanella oneidensis* strain MR-1 and several *Geobacter* spp. are capable of conserving energy for growth with the structure Fe (III) bound in smectite clay as the sole electron acceptor (Kostka et al. 2002). This is a very important discovery since most of the iron on earth exists in the form of silicate minerals or iron oxides. When conditions permit, microbial mediated iron reduction and release is the mechanism for elevated iron observations in groundwater nearby landfills (Williams et al. 2009). Specifically, when organic rich landfill leachate is leaked to the subsurface soil, organic compounds in the leachate are oxidized by intrinsic microorganisms to carbon dioxide and water, and electrons are freed, which are picked up by iron oxides. Consequently, ferrous iron is released. To prevent ferrous iron release, the freed electrons must be consumed by other electron acceptors instead of iron oxides. Microbial fuel cells (MFCs) provide an excellent solution for this problem by totally separating electron consumption from organic carbon oxidation (Watanabe 2008). Currently, there are many types of available MFC reactors throughout the world. But all MFC reactors share the same operating principles: an anode electrode and a cathode electrode are connected by an external circuit and the difference in voltage between the anode and cathode, along with the electron flow in the circuit, generates electrical power (Lovley 2008).

Microorganisms play the key role in MFC reactors. Under anaerobic conditions, organic substrates are oxidized by microorganisms to produce carbon dioxide, protons and electrons as described below (Bennetto et al. 1983):

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^- \quad (1)$$

If the microorganisms are electrochemically inactive, the electron transfer from the microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, neutral red and so on (Takagi et al. 1998). MFCs use the mediators to shuttle the electrons to cross the outer cell lipid membranes and plasma wall to liberate electrons to the anode (negatively charged electrode). After the release of the electrons, the mediators return to their original oxidized state and are ready to repeat the process. It is important to note that this process can only happen under anaerobic conditions. Since oxygen has greater electronegativity than the mediators, oxygen would accept the liberated electrons if oxygen is present (Davila et al. 2008). It should also be noted that most of the mediators available are expensive and toxic. Therefore, mediator-less MFCs have been developed. A mediator-less MFC does not require a mediator but uses electrochemically active bacteria to transfer electrons to the anode, i.e., electrons are carried directly from the bacterial respiratory enzyme to the anode. Electrochemically active bacteria typically have electrochemically active redox enzymes such as cytochromes on their outer membranes that can transfer electrons to external materials (Kim et al. 2005a). The electrochemically active bacteria include *Shewanella putrefaciens* (Schaetzle et al. 2008), *Aeromonas hydrophila* (Kim et al. 2006), and others. Some bacteria, which have pili on their external membranes, are also able to transfer their electron production via these pili. The same holds true for the bacterial family of *Geobacteraceae*, which has been reported to form a biofilm on the anode surface in MFCs and to transfer electrons with high efficiency (Bond and Lovley 2003). In addition, *Rhodoferax* species isolated from anoxic sediments has also been found to efficiently transfer electrons to a graphite anode using glucose as the sole carbon source (Chaudhuri and Lovley 2003). Remarkably, this bacterium is the first reported strain that can

completely mineralize glucose to carbon dioxide while concomitantly generating electricity at 90% efficiency.

During MFC operations, the anode is the electron acceptor recognized by the bacteria. Therefore, the microbial activity is strongly dependent on the redox potential of the anode (Manohar and Mansfeld 2009). The cathode in the separate chamber of the MFCs is positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain, which can also be external to the MFCs (Jadhav and Ghangrekar 2008). Oxygen is usually used as the electron accepter at the cathodic chamber; however, there are concerns that large volumes of circulating gas are required. Another convenient option is to use a solution of a solid oxidizing agent. For electricity generation, the anode and cathode are connected by a wire (or other electrically conductive path including electrically powered devices such as a light bulb) and the two chambers are connected by a salt bridge or ion-exchange membrane, which allows the produced protons to pass from the anodic chamber to the cathodic chamber, to complete the circuit.

MFCs have a number of potential uses. The first and most obvious one is to harvest electricity (Rabaey and Verstraete 2005). MFCs are a particularly promising power source for long-term underwater applications based on their demonstrated ability to generate current by utilizing indigenous nutrients or carbon sources. Research on MFCs at present attracts dramatic attention since MFCs can directly convert a large diversity of organic compounds into electricity. The most exciting discoveries in the past few years in MFC research are the development of MFCs that can harvest electricity from the organic matter in aquatic sediments (Holmes et al. 2004). These systems are now known as Benthic Unattended Generators or BUGs. BUGs are being designed for powering electronic devices in remote locations, such as the bottom of the ocean, where it would be expensive and technically difficult to routinely exchange traditional batteries. BUGs consist of an anode buried in anoxic marine sediments connected to a cathode suspended in the overlying aerobic water. Similar designs can potentially power electronic devices in remote terrestrial locations and can even eventually be modified to harvest electricity from other sources such as compost piles, septic tanks and waste lagoons. It should be

noted that the power generated by MFCs is low, but techniques do exist to convert it to useful power levels. Most importantly, very dilute organic wastes that cannot serve as substrates in other energy production systems can be used an energy source for MFCs. For MFC applications nearby landfills, not only can energy be generated, iron release can also be prevented since the released electrons can transit the iron rich soil and be consumed by other provided electron acceptors. At the same time, leaked leachate can be simultaneously bioremediated. MFCs have been demonstrated to be able to use landfill leachate as the power source (Greenman et al. 2009).

Objectives

The objective of this study is to investigate the feasibility of the usage of MFC technology for landfill leachate decomposition, iron release prevention and possible power generation. For MFC applications nearby landfills, multiple physical, chemical and biological factors play important roles in determining MFC performance, which complicates the design of a MFC system. We hypothesize that the redox potential of the anode designed for this research ensures the activities of *Shewanella putrefaciens* to utilize landfill leachate as the energy source. We further hypothesize that the electrons released from landfill leachate decomposition will flow from the anode to the cathode, where they can be accepted by selected electron acceptors instead of iron oxides. The Specific objectives of this research project include:

1. Landfill leachate collection and *S. putrefaciens* culturing: Leachate from sixteen landfills located in Northwest Florida is to be collected and electrochemically active bacterium of *Shewanella putrefaciens* is to be screened and cultured.

2. Laboratory scale MFC experiments: Landfill leachate collected from landfills located in Northwest Florida is to be tested in a laboratory scale MFC to provide evidence that landfill leachate can be decomposed and electrons released in the anodic chamber can be transported and consumed in the cathodic chamber and consequently, electricity can be generated.

3. Iron release prevention experiments: Based on the results of MFC experiments, custom-designed iron prevention setup is to be tested with iron rich soil to mimic real case scenarios nearby landfills. In addition to providing evidence that landfill leachate can be decomposed and electricity can be generated, prevention of ferrous iron release is to be demonstrated.

2. Background

2.1 Iron Release nearby Landfills in Northwest Florida

It is suspected that geochemical and geomicrobial iron reduction/oxidation processes are responsible for iron release in the groundwater. Iron-reducing bacteria reduce iron oxides to ferrous iron and release it to the groundwater when landfill leachate contacts the soil (Williams et al. 2009). Researchers have demonstrated that *Shewanella oneidensis* is capable of conserving energy for growth with the structure Fe (III) bound in smectite clay as the sole electron acceptor (Dong et al. 2003; Kim et al. 2005b; Lee et al. 2006) :

$$CH_2O + 2Fe_2O_3 + 3H_2O = CO_2 + 4Fe^{2+} + 8OH^- \qquad (2)$$

This is a very important discovery since most of the iron on earth exists in the form of silicate minerals or iron oxides (Perez-Gonzalez et al. 2010). When conditions permit, microbial mediated iron reduction and release may be the mechanism for elevated iron observations in groundwater. Laboratory iron reduction experiments were conducted in our laboratory using soil samples collected from landfill sites reacting with the corresponding leachate under chemistry and biology conditions similar to the concerned site (Williams et al. 2009). These experiments were conducted in a sealed glass reaction vessel in the anaerobic chamber to mimic the situations in the subsurface where landfill leachate interacted with the soils. The results confirmed that iron reducing bacteria were present in the growth chambers and iron was consequently released. Iron concentrations were observed to be as high as 450 mg/L and 420 mg/L within 55 days for soil samples collected from Jackson County (Spring Hill South Landfill) and Walton County reacting with the corresponding landfill leachate. Soil collected from the other landfill sites in Northwest Florida produced less than 200 mg/L of iron (Williams et al. 2009). According to Equation (2), low pH favored the iron reduction process. By monitoring the pH variation, we found that the pH of the leachate was very low for Jackson County and Walton County. Consequently, higher iron release was observed for their reactions.

The iron-reducing bacteria were isolated and identified. It was discovered that *Shewanella putrefaciens* was one of the major species (Figure 1). *Shewanella putrefaciens* is one of very few isolated microorganisms that are able to use iron(III) as an electron acceptor. Thus it plays an important role in iron transformation in the environment. In our prior research, we also found that the released ferrous iron may adsorb on *Shewanella putrefaciens* surfaces. Based on the laboratory observation, it seemed that sorption of ferrous iron on *Shewanella putrefaciens* increased linearly with reaction time until around 100 minutes. After 100 minutes, sorption of ferrous iron on *Shewanella putrefaciens* became moderate. The effect of pH on ferrous iron equilibrium adsorption on *Shewanella putrefaciens* was further investigated. Based on the speciation analysis, ferrous iron does not precipitate in the pH range of 3 to 9. Therefore, the effect of precipitation on ferrous iron adsorption on *Shewanella putrefaciens* was minimal. Ferrous iron had linear isotherms on *Shewanella putrefaciens* under the pH range investigated for this research. From isotherm experiments, the average partition coefficient was found to be 0.073 L/g, 0.059 L/g, 0.050 L/g, 0.039 L/g, 0.035 L/g, 0.033 L/G and 0.026 L/g for pH of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 respectively (Subramaniam et al. 2010).

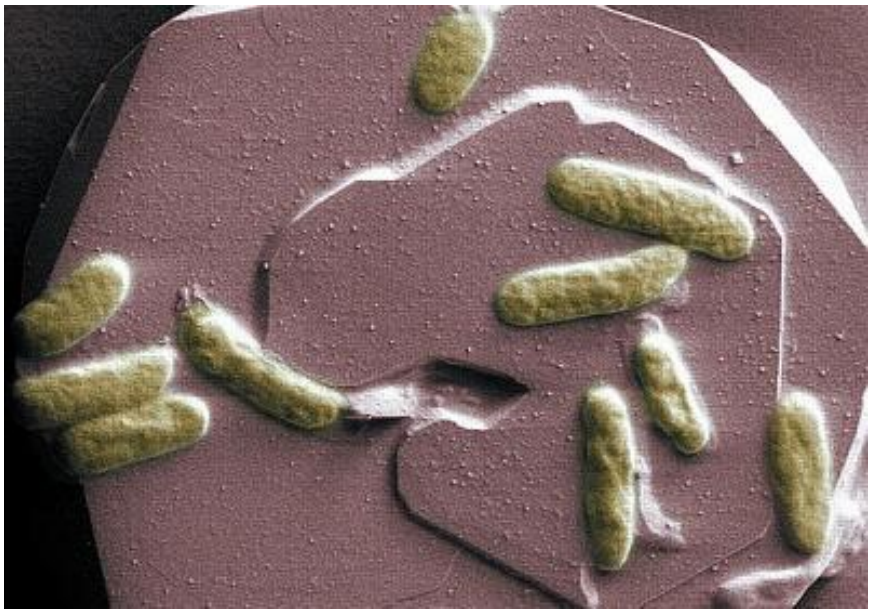

Figure 1. *Shewanella putrefaciens* under Electron Scanning Microscope

Besides above reasons, *Shewanella putrefaciens* species are of interest in this research because of their novel electron transfer capabilities, which makes it possible for us to harvest electricity from waste organic matter. In the subsurface soil, *Shewanella putrefaciens* is able to oxidize organic compounds to carbon dioxide with iron oxides as the electron acceptor. In other words, *Shewanella putrefaciens* gains its energy by using iron oxides.

2.2 Microbial Fuel Cell (MFC)

2.2.1 General Description

The development of processes to generate biofuels and bioenergy has been of special interest lately. Among these, MFCs have received increasing attention (Luckarift et al. 2010). This process, which collects the electricity generated by microbes when they metabolize substrates, is considered to be one of the most efficient energy sources because no burning is required to produce energy. MFCs convert chemical energy, available in a bio-convertible substrate, directly into electricity. MFCs consist of an anode, a cathode, a proton or cation exchange membrane and an electrical circuit (Figure 2). In the MFCs, microorganisms catalyze the anaerobic oxidation of diverse organic substances (such as wastewater pollutants, organic waste, and organic matter in soils or sediments) to carbon dioxide, protons and electrons in the anode chamber (Wang et al. 2010; You et al. 2010). In the absence of oxygen, bacteria need to switch from their natural electron acceptor to an insoluble acceptor, i.e., the MFC anode. Due to the ability of bacteria to transfer electrons to the insoluble electron acceptor, the electrons originating from the microbial metabolism can be collected. The electron transfer can occur either via membrane-associated components, soluble electron shuttles or an electrical circuit with a load or a resistor to the cathode. The potential difference between the anode and the cathode, together with the flow of electrons results in the generation of electrical power (Borole et al. 2010; Feng et al. 2010; Fornero et al. 2010). At the same time, the protons flow through the proton or cation exchange membrane to the cathode. This membrane allows the flow of hydrogen ions from one chamber to the other but will not allow the passage of either electrons or gasses present in the chamber. At the cathode,

an electron acceptor is chemically reduced. Ideally, oxygen is reduced to water. To obtain a sufficient oxygen reduction reaction (ORR) rate, a platinum-catalyst is usually used. However, many researchers have tried to use other non-noble metal catalysts (Alzate-Gaviria et al. 2008).

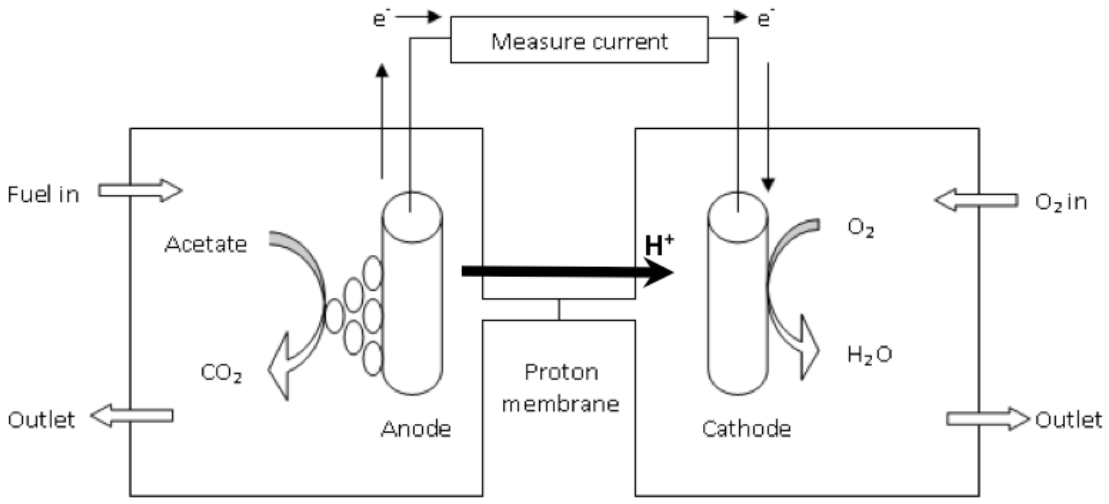

Table 2. Sketch of MFC

To date, many organic substrates have been investigated as possible energy sources to generate electricity using MFCs. The substrates used in MFCs range from carbohydrates (glucose, sucrose, cellulose, starch), volatile fatty acids (formate, acetate, butyrate), alcohols (ethanol, methanol), amino acids, proteins and even inorganic components such as sulfides or acid mine drainages (Donovan et al. 2008; Fornero et al. 2008; Freguia et al. 2008; He et al. 2008; Ishii et al. 2008; Jadhav and Ghangrekar 2009; Rezaei et al. 2008; Sharma et al. 2008; Strik et al. 2008; Thygesen et al. 2009; Yang et al. 2009). In order to benchmark new MFC components, reactor designs or operational conditions, acetate is commonly used as a substrate because of its inertness towards alternative microbial conversions (fermentations and methanogensis) at room temperature. This results in high coulombic efficiencies of up to 98% (Freguia et al. 2008; Rabaey et al. 2003; Read et al. 2010) and high power outputs of up to 115 $W.m^3$ for mixed anodophylic cultures (Cheng and Logan 2007).

The microbial conversion of substrates is a key process to generate electricity in a MFC. The electrical power produced by an MFC is based on the rate of electrons moving through the circuit and electrochemical potential difference across the electrodes. Many factors affect current production, including substrate concentration, bacterial substrate oxidation rate, presence of alternative electron acceptors, and microbial growth (Rabaey and Keller 2008; Virdis et al. 2009). Electrochemical potential, on the other hand, depends on the redox couple between the bacterial respiratory enzyme or electron carrier and the potential at the anode, which is determined by the terminal electron acceptor in the cathode and any system losses. Due to the positive potential difference (ΔE) between the poles of the MFC, the flow of electrons (I) generates a useful power (P) according to:

$$P = I \times \Delta E \quad (3)$$

The ratio between the voltage and the current is determined by the external resistance (R_{ext}) according to Ohms law:

$$\Delta E = I \times R_{ext} \quad (4)$$

When the external resistance is infinite (open circuit conditions), no current flows and the open circuit voltage is obtained. Conversely, when the R_{ext} is zero (short circuit conditions; ΔE = zero), the short circuit current is generated.

2.2.2 Electrochemically Active Bacteria

Unlike natural environmental systems, the anode compartment of a MFC is an engineered environment in which the availability of soluble electron acceptors is limited. The microbial electricity generation relies on the drive of bacteria to acquire maximum energy (Fedorovich et al. 2009; Hou et al. 2009; Park et al. 2001; Pham et al. 2003). The main electron acceptor present in a MFC, enabling bacteria to use respiratory processes, is a solid conductive electrode. The higher amount of metabolic energy released by transferring electrons to the electrode compared to the use of other electron acceptors is their drive to colonize the electrode and develop electron transfer strategies. Bacteria transfer electrons to anodes either directly or via mediated mechanisms. In direct electron

transfer, bacteria require physical contact with the electrode for current production (Biffinger et al. 2009; Chang et al. 2006; Yoon et al. 2007). The contact point between the bacteria and the anode surface requires outer membrane-bound cytochromes or putatively conductive pili called nanowires. Although direct contact of an outermembrane cytochrome to an anodic surface would require microorganisms to be situated upon the electrode itself, direct electron transfer mechanisms are not limited to short-range interactions, as nanowires produced by *Geobacter sulfurreducens* have been implicated in electron conduction through anode biofilms more than 50 um thick (Ishii et al. 2008; Trinh et al. 2009). In mediated electron transfer mechanisms, bacteria either produce or take advantage of indigenous soluble redox compounds such as quinones and flavins to shuttle electrons between the terminal respiratory enzyme and the anode surface.

Electrochemically active bacteria can achieve electron transfer without the presence of carriers. The electrochemically active bacteria including *Shewanella putrefaciens*, *Aeromonas hydrophila*, and others have been examined in MFCs (Ishii et al. 2008; Trinh et al. 2009). Some other bacteria, which have pili on their external membranes, are also able to transfer their electron production via these pili. The same holds true for the bacterial family of *Geobacteraceae*, which has been reported to form a biofilm on the anode surface in MFCs and to transfer electrons with high efficiency (Bond and Lovley 2003). In addition, *Rhodoferax* species isolated from anoxic sediments have also been found to efficiently transfer electrons to a graphite anode using glucose as the sole carbon source (Chaudhuri and Lovley 2003). Remarkably, this bacterium is the first reported strain that can completely mineralize glucose to carbon dioxide while concomitantly generating electricity at 90% efficiency. Among above-mentioned electrochemically active bacteria that can be used in MFCs, *Shewanella* is also an iron-reducing bacterium, which makes its activities most important in the iron-rich Northwest Florida soil (Baron et al. 2009; Marsili et al. 2008).

In present technology, the current production from the MFCs is limited to powering small electronic devices for short periods. The highest power densities reported were approximately 50 Watts per cubic meter of fuel cell volume (Cheng et al. 2006b). The

only MFC application to date is powering monitoring devices in remote locations. However, MFCs have the potential to be developed for a wider range of applications and are thought to be the future technology for energy-efficient wastewater treatment. Scientists are exploring various configurations of microbial fuel cells: microbial electrolysis cells (for hydrogen production), microbial desalination fuel cells, and benthos/sediment microbial fuel cells (Guo et al. 2010; Manuel et al. 2010; Teng et al. 2010).

2.2.3 Respiration versus Fermentation

Microorganisms survive and grow due to the energy they generate by transferring electrons. Respiring bacteria gain energy by the transfer of electrons to external acceptors. During respiration, microorganisms liberate electrons from an electron rich substrate at a low redox potential and transfer these electrons through a number of electron transport complexes through the cell membrane where a final electron acceptor is reduced (Noguchi et al. 2004; Prado et al. 2004; van Maris et al. 2001; Zabalza et al. 2009). Microorganisms do not use the energy produced by the flow of electrons in a direct way, instead, the flow of electrons is used to create a proton gradient across the cell membrane (Wright and Bishop 1962). The energy released by the inward flux of the protons through a membrane complex (ATP synthase) is used to regenerate energy carrier molecules, such as adenosine triphosphate (ATP). By creating this proton gradient, the potential difference between the electron donor (i.e. the substrate at low potential) and the electron acceptor is translated into a process for the generation of energy (Barford 1985). The higher the potential difference between the electron donor and electron acceptor, the higher the proton driven potential difference and the higher the potential amount of ATP that can be refueled. Respiring microorganisms can use a large variety of different electron acceptors, ranging from oxygen, nitrate, iron and manganese oxides to sulfate, but their ability to use the acceptor with the highest redox potential will increase their energy for growth (Madigan et al. 2000) and is their incentive to explore alternative electron acceptors.

In many environments, the availability of electron acceptors is limited, which impedes microorganisms from using the respiratory pathway. In these cases, which are abundant in many environmental conditions, fermenting organisms are likely to establish themselves. Fermenting bacteria generate energy by the internal recirculation of electrons. Fermentation is an ATP-regenerating metabolic process in which degradation products or organic substrates serve as electron donor as well as electron acceptor (Schlegel 1992). The advantage of this pathway is that fermenting organisms are able to grow in numerous environments which are non-supportive for organisms that only use the respiratory pathway because suitable electron acceptors are lacking (Goddard 1945; Scott 1945). Fermenting organisms are important within the overall microbial processes in nature for their ability to degrade polymeric compounds into readily degradable monomers. However, fermentation is energetically far less efficient compared to respiration as only 1 to 4 moles of ATP are formed during the fermentation of glucose where 26 to 38 moles of ATP are formed during the aerobic degradation of glucose (Schlegel 1992). This is also reflected in the Gibbs free energy value, which is a factor of 7 lower for the fermentation of glucose compared to the aerobic respiration. The remainder of the Gibbs free energy is not lost but is conserved within the excreted fermentation products such as volatile fatty acids, hydrogen, alcohols and many more. The tradeoff between their low energetic yield, and their ability to colonize niches devoid of readily available electron donors and acceptors, determines the success of fermenting organisms in many ecosystems (Goddard 1945; Scott 1945).

2.2.4 Future Work in MFCs

There are still many obstacles that need to be overcome before this technology can be put to use. Currently the voltage and amperage generated by microbial fuel cells is so low that it has no useful applications. In order to develop solutions to these problems, research is being done to engineer more efficient hardware for the fuel cells and to understand how different microbes interact with the anodes/cathodes when transporting electrons. MFC research endeavors are increasing each year. Much attention is dedicated to optimizing power generation. Improvements in MFC design and materials have significantly improved reactor performance by 10,000-fold since 1999. Despite this

advance, a further increase of 10- to 100- fold is required for MFCs to be considered for practical applications.

Identifying the more efficient electrochemically active bacteria is one of the means. One of the microorganisms which is being studied in depth for its application in microbial fuel cells is *Geobacter sulfurreducens*. This bacterium is of special interest because it is the most abundant species on anode surfaces in microbial fuel cells grown with more than one bacterial species. This *Geobacter* species, which generates electricity by oxidizing compounds and reducing the anode, has been shown to generate substantial amounts of energy due to multiple mechanisms of transporting electrons to extracellular sources through either pili or c-type cytochromes (Kline et al. 2010; Richter et al. 2010). Another appealing characteristic of *Geobacter sulfurreducens* is its ability to form thick biofilms around the surfaces which it uses as an electron acceptor (Ishii et al. 2008; Trinh et al. 2009). The formation of biofilms on the anodes allows a higher current production because all the cells are involved in electron transport to the anode. The biofilm allows all the grouped cells to be actively involved in the transfers of electrons to the anode. This bacterium has been observed to grow biofilms as thick as 50 μm around the anode in a MFC. The formation of biofilms is possible thanks to outer membrane structures of pili. In the case of *Geobacter sulfurreducens* the gene that allows the formation of the type IV pili is *pilA* (Ishii et al. 2008; Trinh et al. 2009). Wild type cells that express the gene are able to form biofilms on almost any surface. Mutants that have a *pilA* deletion can adhere to different surfaces but are not able to either express pili or form thick biofilms. Complemented *pilA* mutants (having a *pilA* gene reinserted) are once again able to express pili and form biofilms. It has been shown that *Geobacter sulfurreducens* MFCs that are grown with wild type and *pilA* complemented strains generate much more electricity than MFCs grown with *pilA* deficient strains (Richter et al. 2008).

The materials used for the anode also have a big effect on how efficiently the power can be generated. Currently, graphite is the most commonly used material for MFC anodes (Hasanaly 2010; Li et al. 2010). Graphite has a rough surface which provides more surface area for the cells to attach to. It has been proven that materials that have surfaces

with rough areas similar to the diameter of the cells will have more microorganisms bound to them. In the case of *Geobacter sulfurreducens* MFCs, graphite provides a rough surface not only for individual cells to bind directly to the anode but it also allows these cells to anchor firmly to the surface by means of pili. The biofilms that are formed will be tightly bound to the anode and will most likely not separate from the electron acceptor even when the medium in which the organisms are grown is constantly being mixed.

2.2.5 MFC Applications in Iron Release Prevention

Besides generating energy, MFCs are powerful research tools. With electrical current a proxy for bacterial activity, MFCs are controlled systems for addressing a range of questions. MFC-based research continues to expand this knowledge into a diversity of engineering applications. MFC technology has the potential unique applications in iron release prevention nearby landfills. Nearby the landfills, when organic rich landfill leachate is leaked to the subsurface soil, organic compounds in the leachate are oxidized by intrinsic microorganisms to carbon dioxide and water, and electrons are freed and picked up by iron oxides in the soil (Williams et al. 2009). Consequently, ferrous iron is released. To prevent ferrous iron release, MFCs provide a potential solution by totally separating electron consumption from organic carbon oxidation. Electrons are passed onto a terminal electron acceptor such as oxygen in the cathode region to prevent iron reduction in the anode region. Differences in redox potentials make it possible for iron release prevention.

The type of substrate fed to a MFC potentially has an impact on the structure and composition of the microbial community. The more reduced the substrate is, the more energy there is available to divide across the community. This may lead to an increase of the possible interactions and niches. Until recently, no clear image of the effect of the type of substrate on the microbial community is available.

3. Materials and Methods

3.1 Landfill Leachate and Soil Sample Collection

Landfill leachate was collected from leachate sumps located at sixteen landfills in Northwest Florida, including Steelfield Landfill (Bay County), Calhoun County Landfill, Perdido Landfill (Escambia County), Franklin County Landfill, Quincy-Byrd Landfill (Gadsden County), Five Points Landfill (Gulf County), Holmes County Landfill, Springhill Landfill (Jackson County), US 27 South Landfill (Leon County), Liberty County Landfill, Baker Landfill (Okaloosa County), Santa Rosa Central Landfill (Santa Rosa County), Santa Rosa Holley Landfill (Santa Rosa County), Lower Bridge Landfill (Wakulla County), Walton County Central Landfill, and Mudhill Landfill (Washington County) (Figure 3). After collection, the leachate was stored in temperature-controlled containers at $4^{o}C$ and immediately transported to the laboratory. The leachate was stored under refrigeration at $4^{o}C$. Based on the results of our previous research, the landfill leachate had a composition of COD up to 20,000 $mg \cdot L^{-1}$, NH_4^+-N up to 500 $mg \cdot L^{-1}$, and phosphorus up to 200 $mg \cdot L^{-1}$. Soil samples that were used for this research were collected from the referenced landfill sites. Specifically, soil samples were collected 1 to 3 feet below the surface, 100 to 300 feet away from the landfills (Figure 4). The collected soil samples were immediately placed in either a Ziploc bag or a Styrofoam cooler and sealed. All the soil samples were immediately delivered to the laboratory and placed under refrigeration at $4^{o}C$ until used in the experiments.

3.2 *Shewanella putrefaciens* Culturing

Mediator-less MFCs depend on the electrochemically active bacteria to transfer electrons to the anode. Electrochemically active bacteria use the anode in their metabolism, thus they strategically position themselves on the anode surface to form a biofilm. Bacteria in the biofilm produce a matrix of material so that they stick to the anode. The electrochemically active redox enzymes such as cytochromes on their outer membrane potentially transport electrons. Recently, some metal reducing bacteria have been reported to directly transfer electrons to the anode, which are commonly found in

sediments, especially in the iron rich Northwest Florida subsurface soil. For instance, specific cytochromes at the outside of the cell membrane of *Shewanella putrefaciens*

Figure 3. Landfill Leachate Collection Site at Santa Rosa Central Landfill

make these strains electrochemically active in case they are grown under anaerobic conditions. For this research, we cultured electrochemically active *Shewanella putrefaciens* using collected soil samples as the inocula. Continuous cultivation and enrichment were carried out immediately in an anaerobic chamber after the samples were transported back to our laboratory. Specifically, 10 mg soil was transferred into a 250 ml serum bottle containing 100 ml sterilized culture media (Figure 5). The media had a composition (mg/l) of KH_2PO_4, 160; K_2HPO_4, 420; Na_2HPO_4, 50; NH_4Cl, 40; $MgSO_4 \cdot 7H_2O$, 50; $CaCl_2$, 50; $FeCl_3 \cdot 6H_2O$, 0.5; $MnSO_4 \cdot 4H_2O$, 0.05; H_3BO_3, 0.1; $ZnSO_4 \cdot 7H_2O$, 0.05; $(NH4)_6Mo_7O_{24}$, 0.03; glucose, 200; and ammonia chloride, 60. The pH of the media was adjusted to 7.4 with 1 M HCl or 1 M NaOH, after which the media

were sterilized by autoclaving (121^{o}C and 1 atm) for 20 min. Glucose was filter-sterilized and aseptically added to the autoclaved media. The serum bottle was equipped with CO_2

Figure 4. Soil Sampling Site at Santa Rosa Central Landfill

entrapping devices. For this research, 1 M KOH was used to entrap CO_2. Resazurin (1 mg/l) was added as a redox indicator to indicate contamination by molecular oxygen and cysteine (3.0 g/l) was added to reduce the trace amount of oxygen remaining in the media after autoclaving. The headspace of the serum bottle was pressurized with ultra-pure nitrogen and the serum bottle was capped with butyl rubber septa and crimped with an aluminum seal. The inoculated serum bottle was put into a rotary-shaker (150 rpm at 35 ℃) in the dark for at least 1 week until the formation of black precipitate at the bottom and on the wall of the serum bottle can be observed. Then 10 ml enriched culture was transferred into 100 ml fresh culture media with approximately 50 mg/l Fe^{3+} for the second phase culture enrichment. 10 ml enriched culture from the second phase was transferred into 100 ml fresh culture media with approximately 50 mg/l Fe^{3+} for the third

phase culture enrichment and 10 ml enriched culture from the third phase was transferred into 100 ml fresh culture media with approximately 50 mg/l Fe^{3+} for the fourth phase culture enrichment. After the fourth phase enrichment was completed, bacterial cells were harvested by centrifugation (6000 *g*, 15 min) and washed twice with fresh, anoxic $NaHCO_3$ buffer (0.05 M) under an extra-pure nitrogen atmosphere. The

Figure 5. *Shewanella putrefaciens* Culturing

concentrated cells were re-suspended in a serum bottle containing fresh, anoxic $NaHCO_3$ buffer (0.05 M) to give a final concentration of approximately 5×10^9 cells/ml. *Shewanella putrefaciens* was identified by polymerase chain reaction (PCR) analysis. This method amplified specific regions of DNA in the microorganism's genome by selectively catalyzing the replication of those regions. The replicated regions were then compared to a database where DNA of *S. putrefaciens* has already characterized. Microbial Genome Database (MBGD) developed by National Institute for Basic Biology and Okazaki National Research Institutes and the database developed by SRI International, Marine Biological Laboratory, Double Twist Inc., Institute for Genomic Research, University of

California at San Diego and Universidad Nacional Autónoma de México were used for *Shewanella putrefaciens* screening. Once *Shewanella putrefaciens* was screened out, it was enriched in 100 ml fresh culture media with approximately 50 mg/l Fe^{3+}.

3.3 Laboratory Scale MFC Experiments

Dual-chamber MFCs were constructed in this research. Sketches of the MFCs are illustrated in Figure 6. As shown in Figure 6, a graphite rod, without a catalyst coating, was installed in the center of the inner chamber as the anode. The anode was inoculated with the cultured *Shewanella putrefaciens*, the dominant organism in the process of iron reduction in the iron rich soil of Northwest Florida. Carbon cloth (effective area of 12.6 cm^2, 30% wet proofing), coated with platinum catalysts (0.15 mg/cm^2, 5% Pt) was placed on the outside layer of the inner chamber, serving as the cathode. Connections between the two electrodes were a copper wire through a rheostat (10 - 1000 Ω). Synthetic polymeric nanoporous membranes were tested in this research and used as the cation-selective membrane or cation-exchange membrane (CEM). During the operation, *Shewanella putrefaciens* was attached to the anode and collected landfill leachate was introduced to the anodic chamber. The operation proceeded in the absence of oxygen. The generated carbon dioxide was trapped in the CO_2 entrapping device. In the cathodic chamber, oxygen was provided.

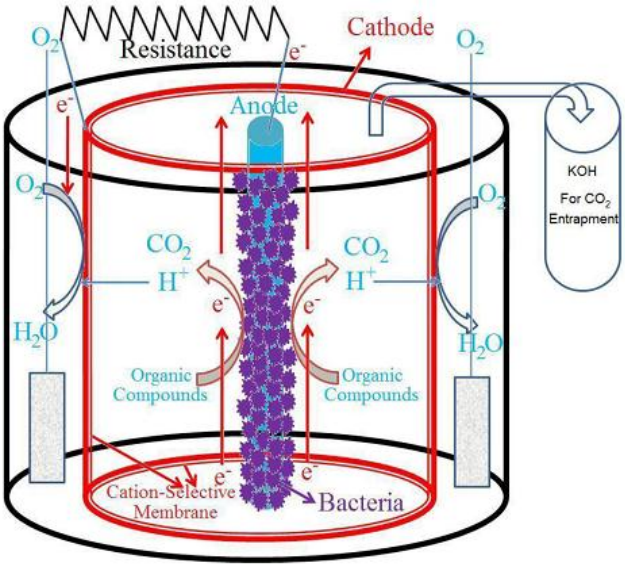

Figure 6. Sketches of the MFC Developed for This Research

Based on above sketches, two MFC setups were developed in this research, i.e., a static MFC (Figure 7) and a continuous MFC (Figure 8). The static MFC was a batch-focused setup and the continuous MFC provided the results for continuous systems.

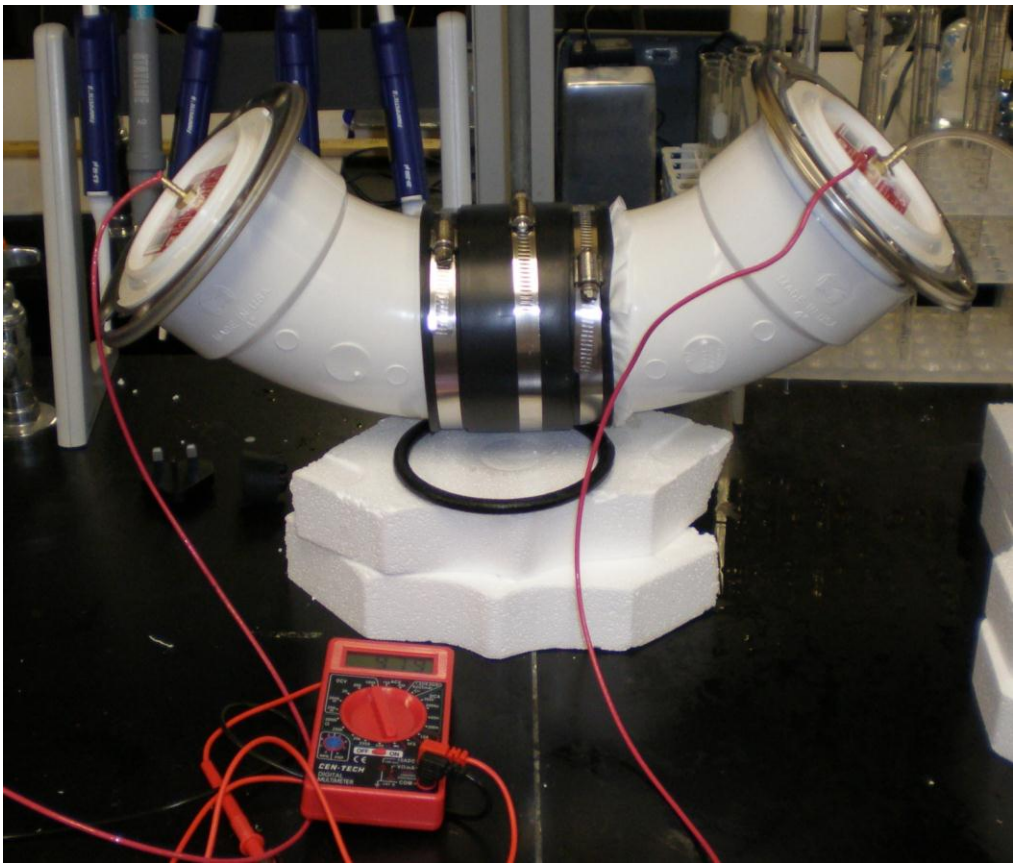

Figure 7. Static MFC Setup

3.3.1 Anode Selection

Anodic materials must be conductive, biocompatible and chemically stable in the reactor solution. Metal anodes consisting of noncorrosive stainless steel mesh can be utilized, but copper is not useful due to the toxicity of even trace copper ions to the bacteria. The most versatile electrode materials are carbon, available as compact graphite plates, rods, or granules, as fibrous material (felt, cloth, paper, fibers, foam), and as glassy carbon. The simplest materials for anode electrodes are graphite plates or rods as they are relatively inexpensive, easy to handle, and have a defined surface area. As the anode receives the electrons, its potential decreases to a lower level than that of the cathode in the cathodic

chamber (Cheng et al. 2008). The performance of the anode thus plays an important role for a maximum power output. For this research, highly porous graphite electrodes were used as the anode (Figure 9). After introduction of landfill leachate, the anodic chamber was sparged with nitrogen to remove oxygen.

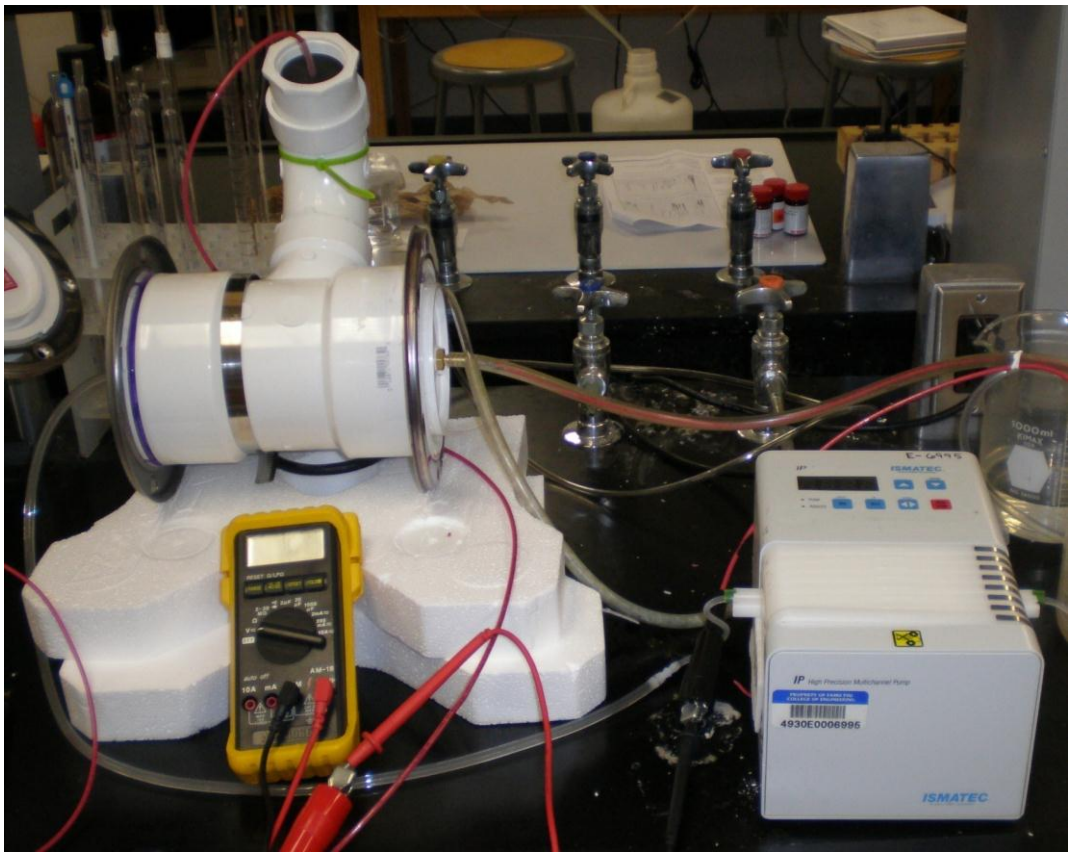

Figure 8. Continuous MFC Setup

3.3.2 Cathode Selection

The choice of the cathode materials also greatly affects the MFC performance. Besides oxygen, various catholytes such as hexacynoferrate or acidic permanganate have been used in MFCs (Rabaey et al. 2005; You et al. 2006). In comparison to these oxidants, oxygen is more suitable as the electron acceptor for the MFCs due to its high oxidation potential, availability, low cost, sustainability, and the lack of a chemical waste product (water is formed as the only end product). Based on prior research, MFCs with O_2 or air as the cathodic electron acceptor often need expensive platinum as the catalyst to

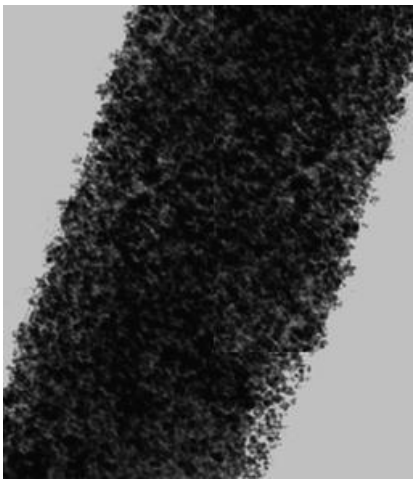

Figure 9. High Porous Graphite Electrode

accelerate the O_2 reduction reaction (Liu and Logan 2004), although novel non-noble metal catalysts such as pyrolyzed iron (II) phthalocyanine (FePc) or cobalt tetramethylphenylporphyrin (CoTMPP) are proposed to replace platinum (Cheng et al. 2006b; Zhao et al. 2006). Recently, potassium ferricyanide ($K_3[Fe(CN)_6]$) is also popularly utilized as the electron acceptor in MFCs owing to its good performance (Park and Zeikus 2003). The greatest advantage of potassium ferricyanide is the low over potential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. A 50 - 80% increase in maximum power using potassium ferricyanide in the cathodic compartment as compared to an oxygen-saturated aqueous cathode or a platinum-coated air-cathode has been reported (Oh et al. 2004). The observed differences can be attributed to high open circuit potential and a greater mass transfer efficiency of potassium ferricyanide solution than that of dissolved oxygen. The greatest disadvantage, however, is that potassium ferricyanide is not a suitable choice for sustainable electricity generation in MFCs. It is potentially toxic, requires regular replenishing due to its low rate of regeneration by oxygen, and diffuses through the membrane over long-term operation which eventually reduces the overall performance of the MFCs (Logan and Regan 2006). For the landfill applications, sustainability is always the priority. Therefore, for this research, O_2 was chosen as the electron acceptor. Since O_2 served as the electron acceptor, phosphate buffer (50 mM K_2HPO_4) was used as the electrolyte.

3.3.3 Membrane

The majority of MFC designs require the separation of the anodic and the cathodic compartments by a CEM. Exceptions are naturally separated systems such as sediment MFCs or specially designed single-compartment MFCs (Cheng et al. 2006a; Reimers et al. 2001). The most commonly used CEM is Nafion (Dupont Co., USA), which is available from numerous suppliers (e.g., Aldrich and Ion Power, Inc.). Alternatives to Nafion, such as Ultrex CMI-7000 (Membranes International Inc., Glen Rock, NJ) also are well suited for MFC applications and are considerably more cost-effective than Nafion (Rabaey et al. 2004). When a CEM is used in an MFC, it is important to recognize that it may be permeable to chemicals such as oxygen, ferricyanide, or organic matter used as the substrate. For this research, Ultrex CMI-7000 was used as the CEM.

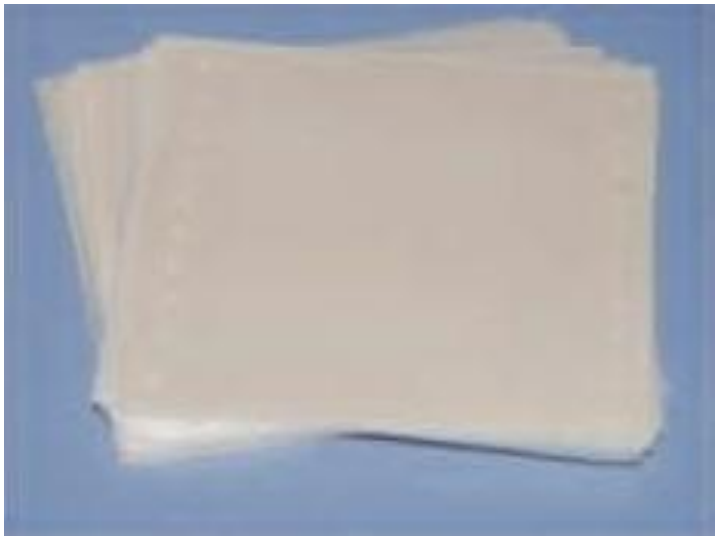

Figure 10. Ultrex CMI-7000

3.4 Iron Release Prevention Experiments

To test that the proposed MFC technology can be practically applied to decompose landfill leachate in the field and prevent iron release, the following experiments were conducted. The design was similar as the laboratory scale MFC setup and the major difference was that iron rich soil collected from the landfills in Northwest Florida was

introduced in the reactions (Figure 11). The optimal conditions from the laboratory scale experiments such as the anode and cathode selections as well as the optimal DO concentration were applied. In addition to the parameters monitored before, ferrous iron

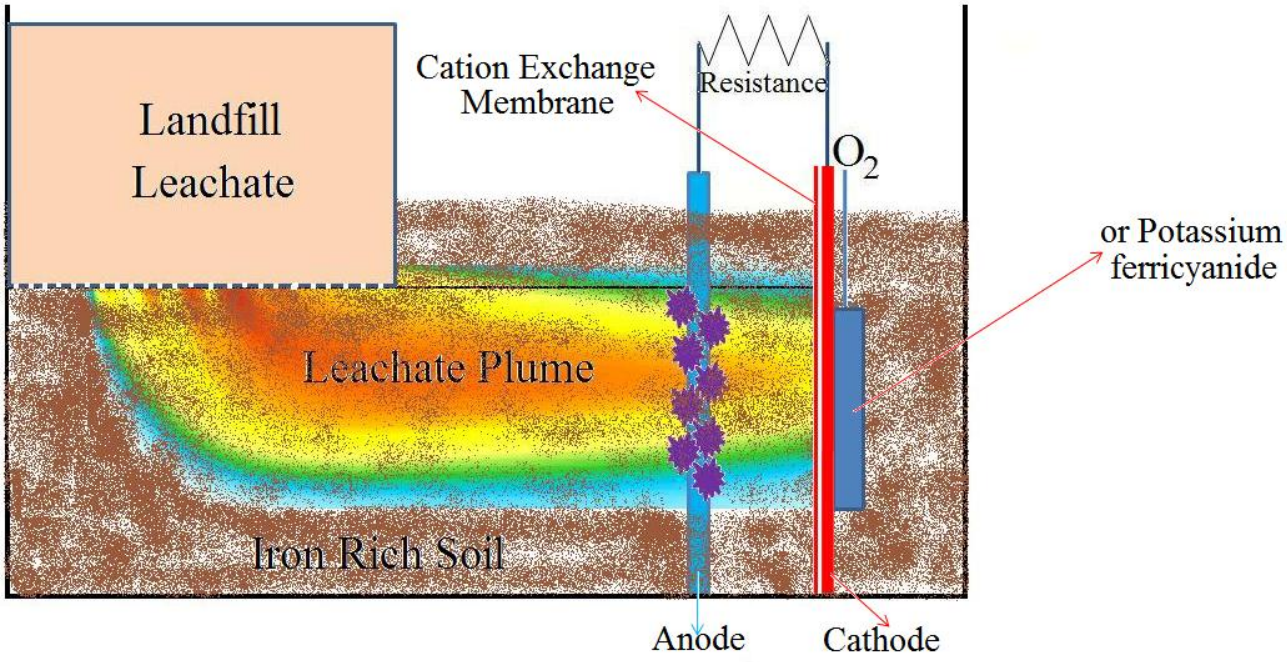

Figure 11. Illustration of Iron Release Prevention Experiments

concentration was monitored in both the anodic chamber and the cathodic chamber. To provide evidence that the MFC technology can indeed prevent iron release, a parallel control experiment was conducted: The cathode and anode were removed and landfill leachate contacted and reacted with iron rich soil directly (Figure 12). For the control experiment, landfill leachate organic component decomposition and ferrous iron release was monitored and compared with the iron release prevention experiments. For the control experiment, it was suspected that electrons were released from organic compound decomposition, which would be consumed by iron oxides surrounding the anode in the anodic chamber. Consequently, iron was supposed to be leased. The effect of oxygen concentration (DO) on iron release prevention was studied by aerating the solution in the cathodic chamber using O_2 in combination with nitrogen. Specifically, DO concentrations varied from 0 to 8 mg/l.

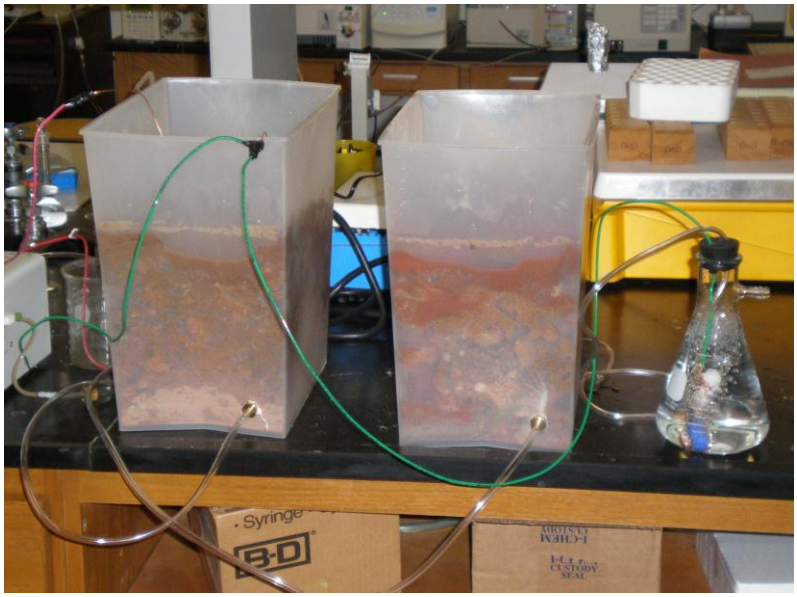

Figure 12. Iron Release Prevention Experiment and Control Experiment Setups

3.5 Impact of Temperature on MFC Performance

Impact of temperature on MFC performance was conducted by putting the static MFC in an incubator (Figure 13). The temperature investigated was in the range from 25^{o}C to 40^{o}C.

Figure 13. Impact of Temperature on MFC Performance Setup

4. Results

4.1 *Shewanella putrefaciens* Culturing

In this research, we investigated iron release prevention and energy generation using mediator-less MFCs. These MFCs depended on the bacteria to transfer electrons to the anode. For this research, we used *Shewanella putrefaciens*, which are commonly found in sediments, especially in the iron rich Northwest Florida subsurface soil, in the MFC studies. We cultured electrochemically active *Shewanella putrefaciens* using collected soil samples from Franklin County Landfill, Quincy-Byrd Landfill (Gadsden County), Baker Landfill (Okaloosa County), and Santa Rosa Central Landfill (Santa Rosa County) as the inocula. Continuous cultivation and enrichment were carried out in an anaerobic chamber after the samples were transported back to the laboratory. Bacterial cells were harvested by centrifugation (6000 *g*, 15 min) and washed twice with fresh, anoxic $NaHCO_3$ buffer (0.05 M) under an extra-pure nitrogen atmosphere. The concentrated cells were re-suspended in a serum bottle containing fresh, anoxic $NaHCO_3$ buffer (0.05 M) to give a final concentration of approximately 5×10^9 cells/ml. *Shewanella putrefaciens* was identified by PCR analysis.

4.2 Laboratory Scale MFC Experiments

4.2.1 Power Generation

Two dual-chamber MFCs, one batch MFC and one continuous MFC were constructed for this research. Graphite rods, without coated catalysts, were installed in the center of the inner chambers as the anodes. The anodes were inoculated with the cultured *Shewanella putrefaciens*. Carbon cloth (effective area of 12.6 cm^2, 30% wet proofing), coated with platinum catalysts (0.15 mg/cm^2, 5% Pt) served as the cathode. In the cathode chamber, O_2 served as the electron acceptor. The anodes and cathodes were connected through digital multimeters. Synthetic polymeric nanoporous membranes were used as the cation-exchange membrane (CEM).

Besides landfill leachate, glucose was also used as a comparison for power generation. Glucose generated higher voltage (up to 0.4 V) as compared to that of landfill leachate

(up to 0.1 V) (Figure 14). In addition, a self-sharpening power generation front was observed for glucose. However, for landfill leachate, there was an obvious lag, indicating that *Shewanella putrefaciens* needed time to adapt to the landfill leachate. Among the landfill leachate collected from the four locations, landfill leachate collected from Okaloosa County generated the most power, followed by Leon County, Gadsden County and Santa Rosa County. By translating the voltage to power, it was discovered that the power generation was as high as 68 mW/m^2 for glucose and 25 mW/m^2 for landfill leachate.

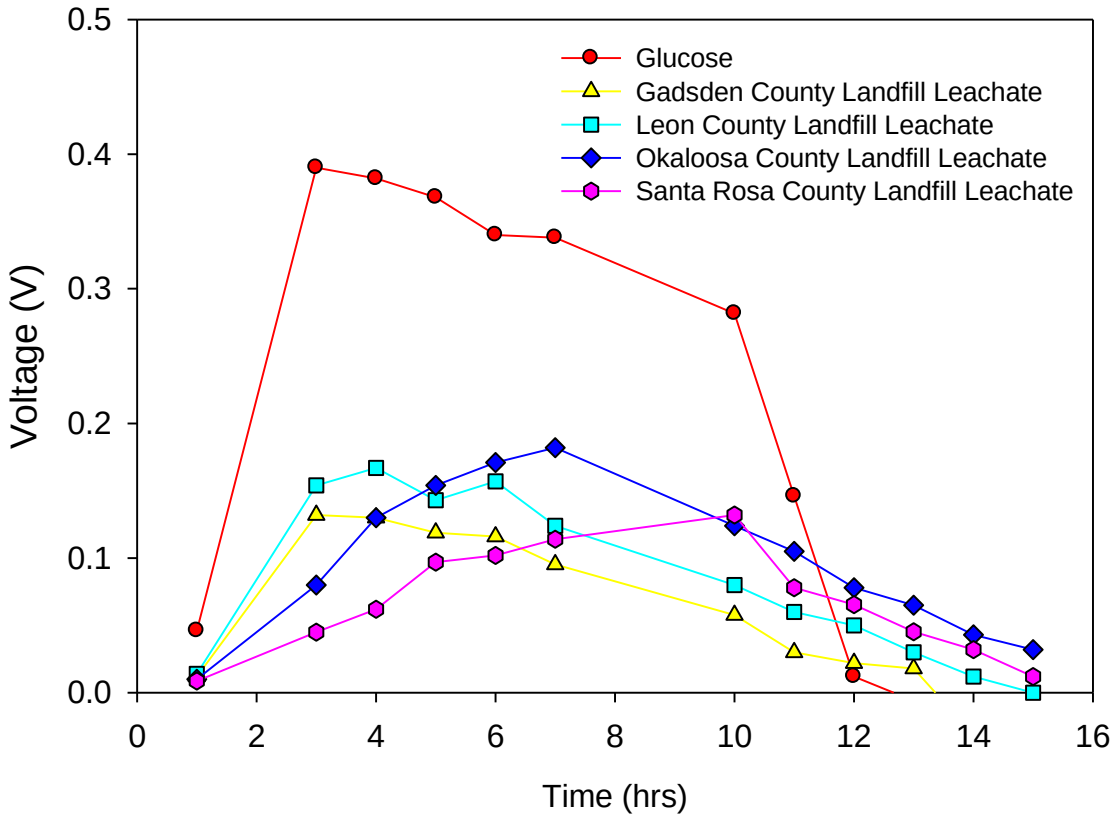

Figure 14. Voltage Generation of the Static MFC

In the continuous MFC, the carbon source was continuously supplied and uninterrupted current was produced (Figure 15). The input landfill leachate was diluted to a BOD_5 value ~ 250 mg/l. After the MFC treatment, the effluent BOD_5 was in the range of 40 ~

120 mg/l, i.e., around 50 ~ 80% of BOD was remediated (Figure 16). Correspondingly, a stable voltage of ~ 0.3 V and ~ 0.1 V was maintained for glucose and landfill leachate.

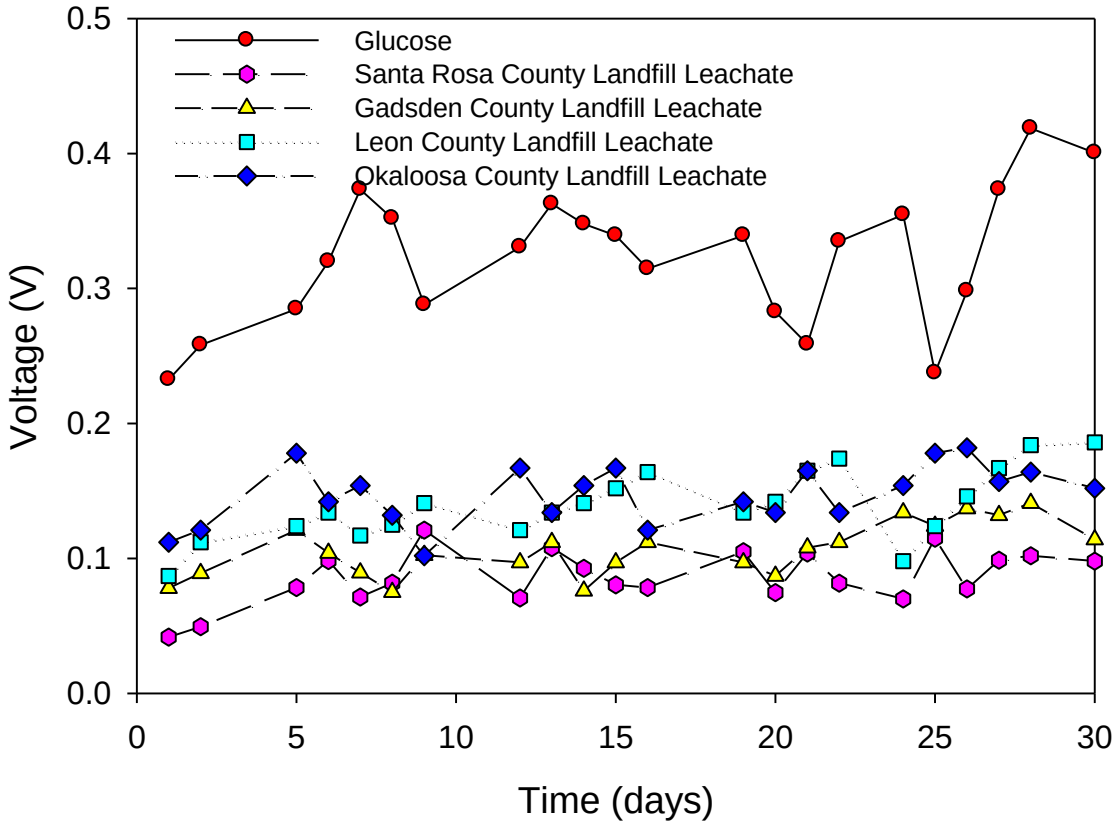

Figure 15. Voltage Generation of the Continuous MFC

Among the landfill leachate collected from the four landfill locations, there was a general trend that landfill leachate collected from Okaloosa County generated the most power, followed by Leon County, Gadsden County and Santa Rosa County. The power generation had no relationship with the effluent BOD_5 values. By comparing the power generation with BOD_5 consumption, it was discovered that power generation corresponded to the BOD_5 consumption (Figure 17).

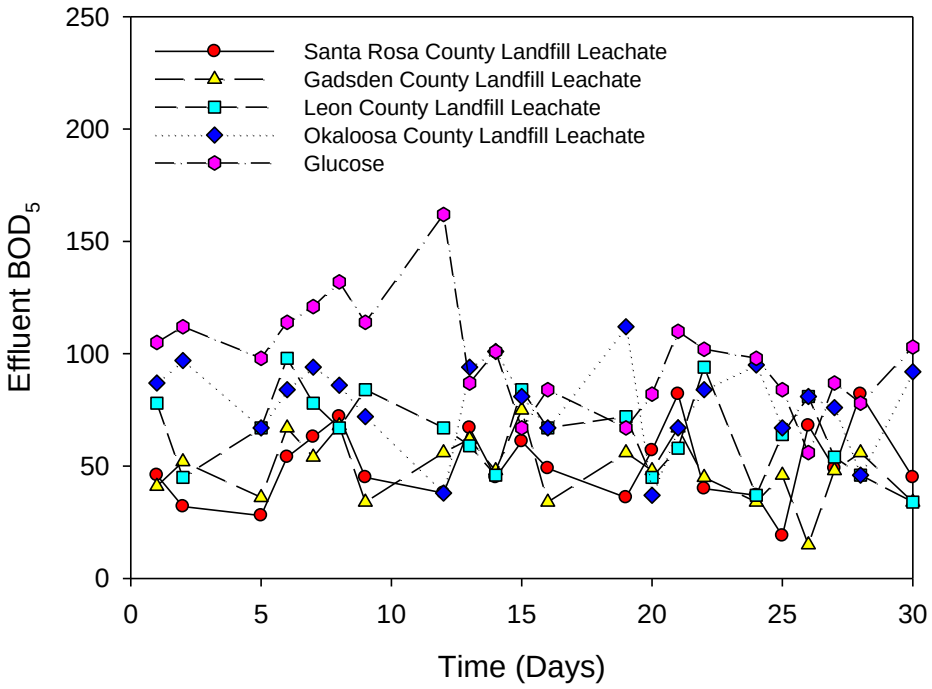

Figure 16. Continuous MFC Effluent BOD_5 Values

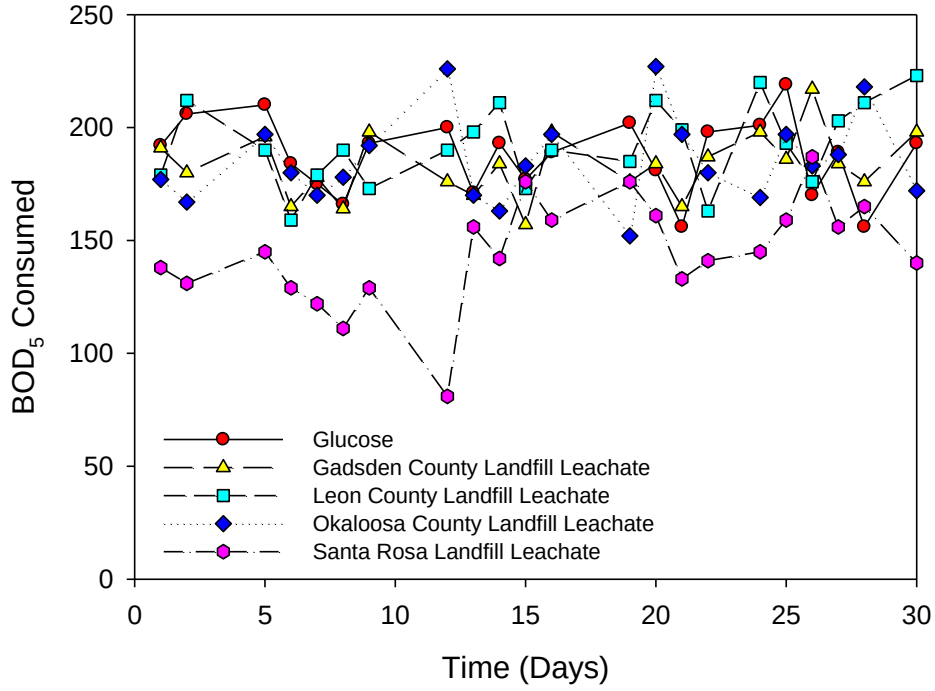

Figure 17. Continuous MFC BOD_5 Consumption

4.2.2 Impact of pH on Power Generation

Using the two dual-chamber MFC setups, the impact of pH on power generation from landfill leachate collected from Leon County was tested. For these processes, O_2 served as the electron acceptor in the cathode chamber, and the anodes and cathodes were connected through a digital multi-meter.

The impact of pH on power generation was illustrated in Figure 18 and Figure 19. High pH (i.e., pH 8) generated more power as compared to low pH (i.e., pH 6) for both the static MFC (Figure 18) and continuous MFC (Figure 19). It should be noted that the pH control was achieved at the anode chamber where organic compounds (glucose or landfill leachate) were decomposed. According to the following equation, raising the pH should favor electron release:

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^- \quad (5)$$

However, when free electrons are picked up by oxygen in the cathode chamber, lowering the pH should favor the reaction:

$$1/4O_2 + H^+ + e^- \rightarrow 1/2H_2O \quad (6)$$

Since the cathode chamber is totally separated from the anode chamber, for above experiments, we only examined pH variations at the anode chamber. In addition, we only focused on typical pH ranges of the soils nearby the landfills, i.e., pH 6 to pH 8.

The pH of landfill leachate ranges from 3 to 10. However, the typical values are usually in the range of 6 to 8. Based on this research, there is a general trend that high pH favors the MFC performances since organic decomposition consumes alkalinity."

4.2.3 Impact of Temperature on Power Generation

Impact of temperature on power generation was examined using Leon County landfill leachate as the substrate for the static MFC. As shown in Figure 20, more power was generated at higher temperature as compared to room temperature. At 35^{o}C, a voltage of 0.4 V can be reached as compared to 0.15 V at room temperature.

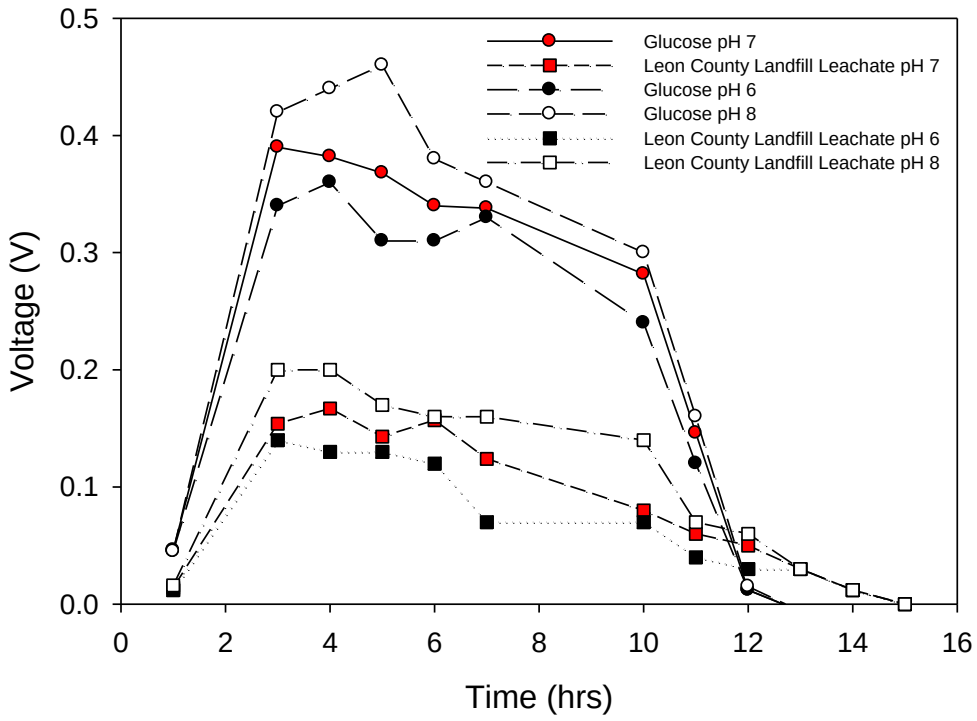

Figure 18. Impact of pH on Power Generation from Static MFC

Temperature affects reaction rates considerably. Since MFCs decompose organic compounds under anaerobic conditions, growth rates in general roughly double for each 10^{o}C rise in temperature within the usual mesophilic operational range from 10^{o}C to 35^{o}C. This is demonstrated in this research. As shown in Figure 20, voltage generation was doubled when the temperature increased from room temperature to 35^{o}C. For landfills located in Florida, owing to the tropical conditions, high temperature favors MFC performances. However, on the other hand, dissolved oxygen decreases with the increase of temperature, which may have adverse effect on MFC efficiency. Therefore,

for field applications, impact of temperature must be carefully evaluated before MFC implementation."

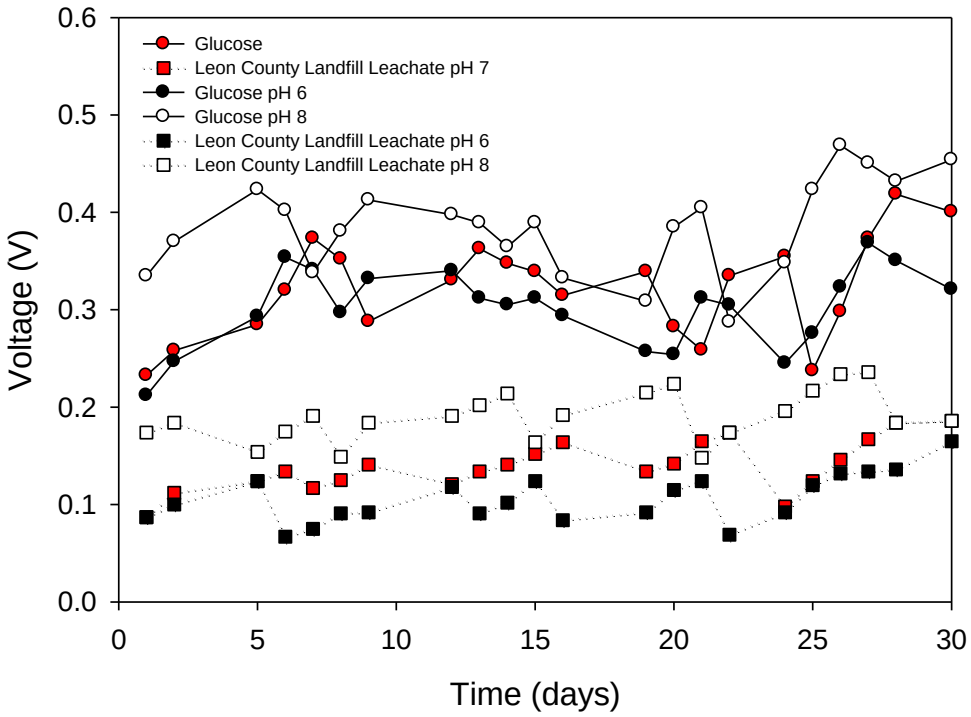

Figure 19. Impact of pH on Power Generation from Continuous MFC

4.3 Applications of MFC Technology in Preventing Iron Release nearby Landfills

As a control, landfill leachate collected from Santa Rosa County Landfill and Leon County Landfill was sprayed to the soil samples collected from Santa Rosa County Landfill (Figure 21). After 5 days, iron started to be released from the soil. Within two weeks, iron can be released as high as 150 mg/l (Figure 21). In a parallel setup, MFC technology was applied. Specifically, a Nafion membrane was used to create an anode region where landfill leachate was applied. Outside this region, a cathode region was created with wires connected to an oxygen source (armed Erlenmeyer flask in Figure 12). Within the anode region, *Shewanella putrefaciens* was inoculated. As evidenced by Figure 21, much less iron was released when MFC technology was applied.

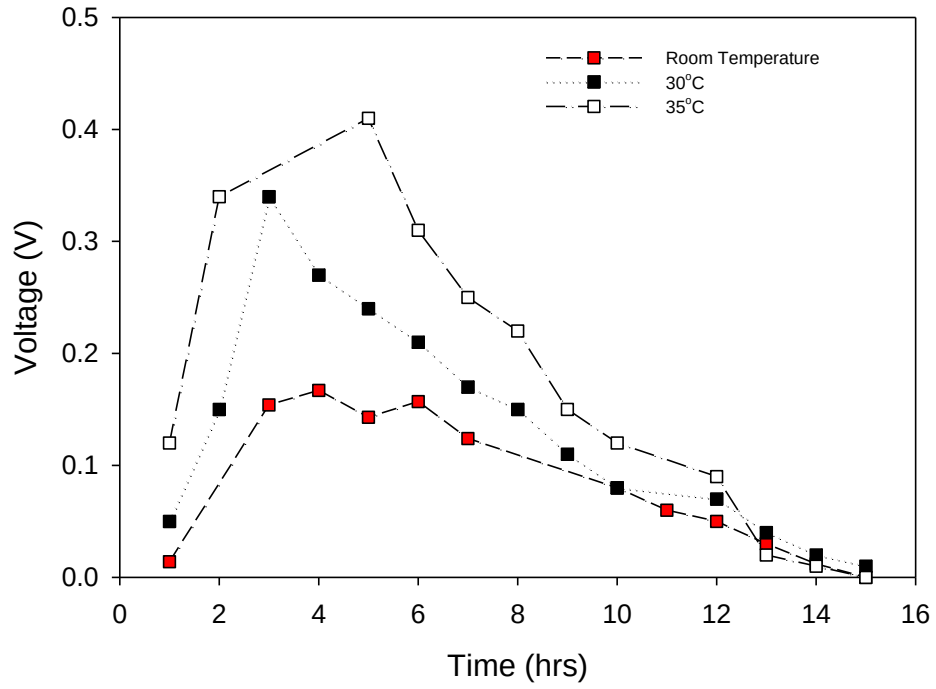

Figure 20. Impact of Temperature on Power Generation from Static MFC

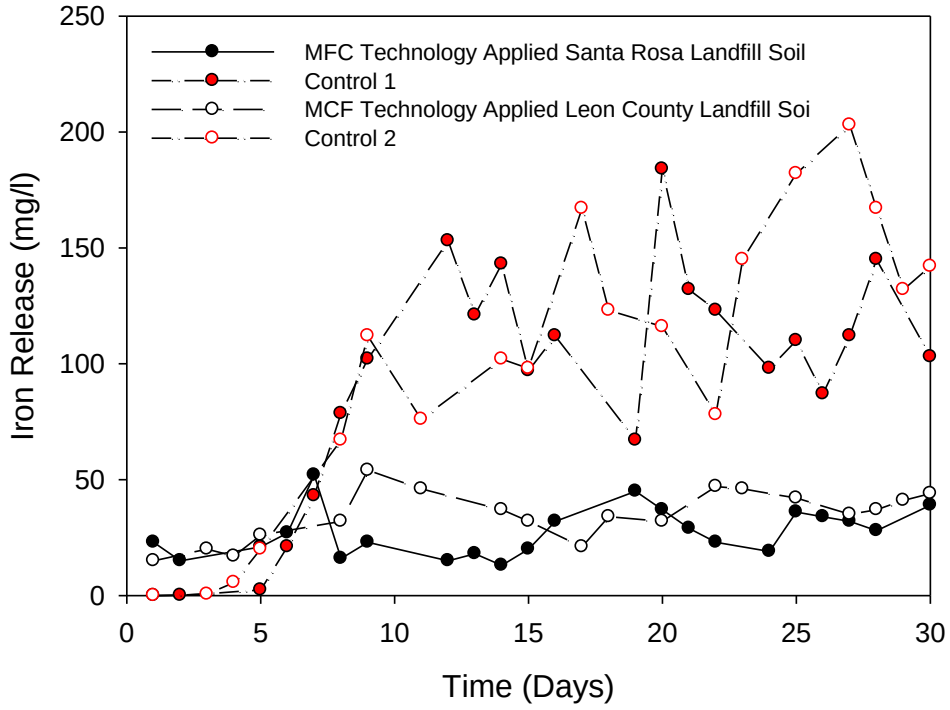

Figure 21. MFC Technology Applications on Soil Samples Collected from Landfills

Even with the application of the MFC technology, there was approximately 30 to 40 mg/l of iron released. This was because the obstacles of the electron transfer within the anode region. Another important factor that impacted the iron release prevention was the potential difference between the cathode and anode. To address this issue, the impact of dissolved oxygen on iron release prevention was further investigated.

4.4 Impact of Dissolved Oxygen on Iron Release Prevention

The difference of the potential between the anode and cathode was the driving force for iron release control, which was controlled by the dissolved oxygen level in the cathode region. The effect of dissolved oxygen on iron release prevention was studied by aerating the solution in the cathodic chamber using O_2 in combination with nitrogen. Specifically, DO concentrations varied from 0 to 8 mg/l.

For soil samples collected from Santa Rosa Landfill, a high dissolved oxygen level (i.e., 7.87 mg/l) resulted in much lower iron release (Figure 22). With the decrease of dissolved oxygen, more iron was released. For dissolved of oxygen at levels of 7.87, 4.23 and 2.41 mg/l, corresponding iron release was around 30, 52 and 92 mg/l. With no MFC technology application, iron release was around 120 mg/l.

For soil samples collected from Leon County Landfill, a high dissolved oxygen level (i.e., 7.81 mg/l) also resulted in much lower iron release (Figure 23). With the decrease of dissolved oxygen, more iron was released. For dissolved of oxygen at levels of 7.81, 4.12 and 2.81 mg/l, corresponding iron release was around 35, 41 and 61 mg/l. With no MFC technology application, iron release was around 132 mg/l.

Oxygen content decreases with the depth of the soil. Especially, the dissolved oxygen decreases with the increase of temperature. Nearby landfills in Florida, owing to the tropical conditions, the dissolved oxygen tends to be low. Therefore, the impact of dissolved oxygen on MFC applications should be taken into consideration.

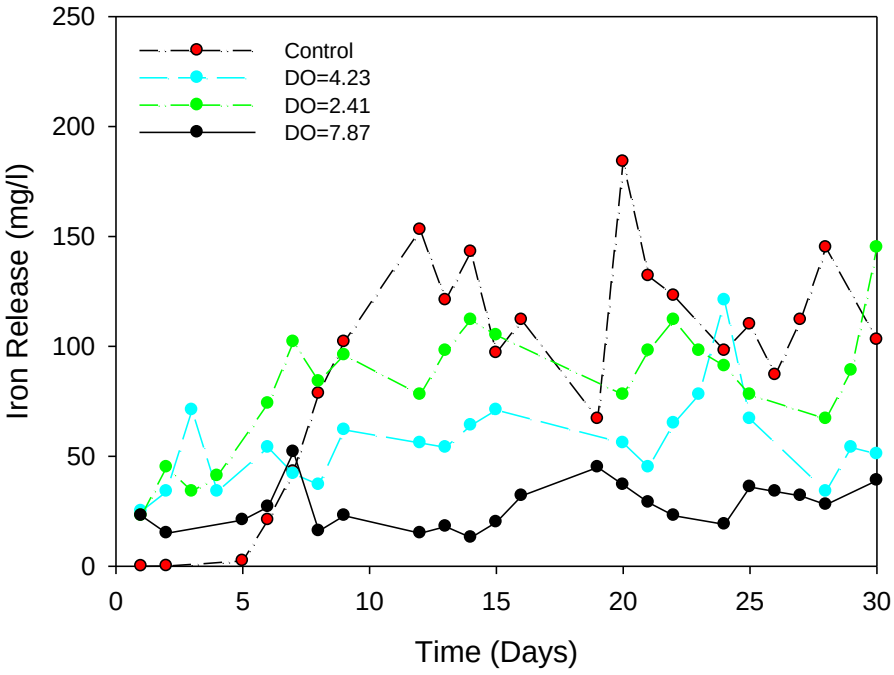

Figure 22. Impact of Dissolved Oxygen on Iron Release for Soil Samples Collected from Santa Rosa Landfill

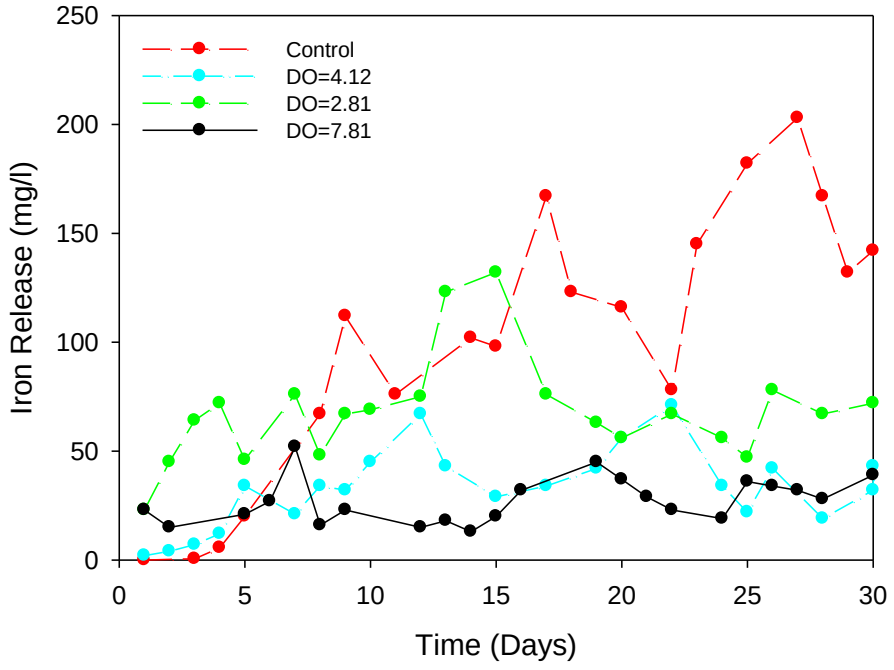

Figure 23. Impact of Dissolved Oxygen on Iron Release for Soil Samples Collected from Leon County Landfill

5. Discussion

5.1 Organic Composition and MFC Performance

The high energy requirement of conventional landfill leachate treatment is warrant for alternative treatment technologies which require less energy for its efficient operation and recover useful energy to make operations sustainable (Depountis et al. 2009; Fang et al. 2010). In the past two decade high rate anaerobic processes are finding increasing applications for the treatment of landfill leachate as well as industrial wastewater. Although energy can be recovered in the form of methane gas during anaerobic treatment, the utilization of methane is not attractive (Iza et al. 1992). When treating small quantities of low strength leachte, the generated methane is usually flared. In addition, due to global environmental concerns and energy insecurity, there is emergent interest in finding sustainable and clean energy sources (Gebert and Groengroeft 2006).

MFCs are capable of providing clean energy, apart from effective treatment of landfill leachate. MFCs utilize bacterial catalysis to directly generate electric power from carbohydrates, which can be found in a diverse range of sources such as crops, industrial and agricultural waste, including landfill leachate. Although MFCs have the advantage of clean power generation and simultaneous waste utilization, their commercialization has been halted due to their low power output. In MFCs, the current is generated by diverting the catabolic electrons to the anode. The low power capability of a MFC is mainly due to the sluggish kinetics of the electron transfer between the bacterial cells and the fuel cell anode. Although redox mediators such as methylene blue can be used to facilitate the electron transfer, these synthetic redox mediators are mostly expensive and toxic to microorganisms, making mediator-type fuel cells difficult for practical applications. Mediator-less MFCs thus have the highest potential for MFC applications. However, the isolation and cultivation of these microorganisms involve rather complicated procedures.

During the operation of mediator-less MFCs, factors that limit electricity generation include organic compound oxidation at the anode, electron transfer from the microorganisms to the anode hence, presence of electrochemically active redox enzymes,

external resistance of the circuit, proton transfer through the membrane to the cathode, and oxygen reduction at the cathode. Among above factors, the most important one is the organic compound oxidation, which is a function of organic composition (Hou et al. 2009; Liu and Zheng 2009; Luo et al. 2010). For organic compounds with different compositions, the energy generation is different (Table 1). As shown in Table 1, glucose can release more energy than other organic compounds such as acetate, etc. Since landfill leachate is a combination of variable compounds, the energy release would be different once they are applied in MFCs.

Table 1. Gibbs Free Energy of Organic Compound Oxidation

Reactions for Organic Compounds	$\Delta G^0(w)$ kJ/e^- eq
Acetate: $1/8\ CH_3COO^- + 3/8\ H_2O = 1/8\ CO_2 + 1/8\ HCO_3^- + H^+ + e^-$	-27.40
Ethanol: $1/12\ CH_3CH_2OH + 1/4\ H_2O = 1/6\ CO_2 + H^+ + e^-$	-31.18
Formate: $1/2\ HCOO^- + 1/2\ H_2O = 1/2\ HCO_3^- + H^+ + e^-$	-39.19
Glucose: $1/24\ C_6H_{12}O_6 + 1/4\ H_2O = 1/4\ CO_2 + H+ e-$	-41.35
Lactate: $1/12\ CH_3CHOHCOO^- + 1/3\ H_2O = 1/6\ CO_2 + 1/12\ HCO_3^- + H^+ + e^-$	-32.29
Propionate: $1/14\ CH_3CH_2COO^- + 5/14\ H_2O = 1/7\ CO_2 + 1/14\ HCO_3^- + H^+ + e^-$	-27.63

Since landfill leachate collected from Okaloosa County generated the most power, it was suspected that landfill leachate collected from this landfill had more energy-rich organic waste.

5.2 Landfill Leachate Decomposition

During MFC applications, organic compounds were decomposed. Samples were periodically withdrawn from the MFC reactors and analyzed for organic concentration in terms of BOD_5. If microbial activities are coupled with organic depletion and Monod-type kinetics are assumed to describe microbial growth, substrate and microbial concentrations over time can be described by the following equations (Monod 1949):

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m SX}{K_s + S} \tag{7}$$

$$\frac{dX}{dt} = \frac{\mu_m SX}{K_s + S} - \frac{bX}{K_s + S} \tag{8}$$

where S is the organic concentration, which is expressed in terms of BOD_5 (mg/L); μ_m is the maximum specific growth rate (hr^{-1}); X is the microbial concentration (g/L); t is the elapsed time (hr); Y is the growth yield coefficient (g biomass per g substrate); K_s is the half-saturation coefficient (g/L); and b is the microbial decay coefficient (hr^{-1}). By ignoring the decay rate coefficient, Y can be used to estimate the microbial production based on organic substrate depletion, such that:

$$Y = -\frac{\Delta X}{\Delta S} \tag{9}$$

$$X = X_0 + Y(S_0 - S) \tag{10}$$

By substituting equation (10) into equation (7), substrate depletion can be expressed as:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m S[X_0 + Y(S_0 - S)]}{K_s + S} \tag{11}$$

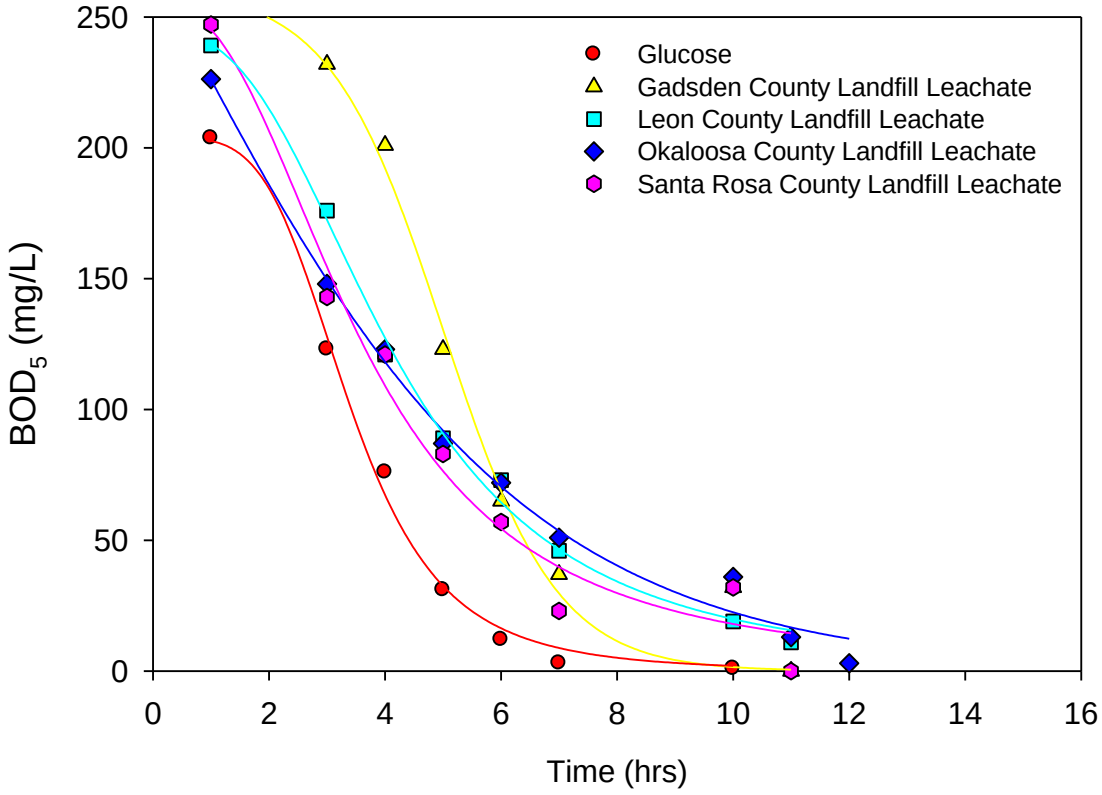

Figure 24. Organic Decomposition in the Static MFC

The simulated half-saturation coefficient K_s (mg/l), growth yield coefficient Y (g biomass per g substrate), and maximum specific growth rate μ_m (day^{-1}) are listed in Table 2. Except landfill leachate from Gadsden County, all the other landfill leachate had similar K_s values, indicating that the culture had similar affinity to the leachate. However, all these K_s values were larger than that of glucose. Gadsden County Landfill leachate also had the least Y value and μ_m value. All the other leachate had similar Y and μ_m values. Based on the above analysis, it might be concluded that landfill leachate from Gadsden County Landfill contained some organic compounds that were more difficult for *Shewanella putrefaciens* to decompose. However, since similar power was generated as compared to other landfill leachate samples, there was not much difference in the energy content of the organic compounds from this landfill as compared to others.

Table 2. BOD_5 Decomposition Parameters in the Static MFC

	K_S (mg/L)	Y (g/g)	μ_{max} (day^{-1})
Glucose	154.3	0.678	0.0124
Gadsden County	271.6	0.323	0.0072
Leon County	172.1	0.412	0.0089
Okaloosa County	163.7	0.486	0.0105
Santa Rosa County	174.5	0.421	0.0093

5.3 Iron Release Prevention

A range of more complex organics, containing a large variety of different readily and non-readily degradable molecules such as domestic wastewater (Liu et al.), brewery wastewater (Feng et al. 2008), paper recycling wastewater (Huang and Logan 2008) or the effluent of anaerobic digesters (Aelterman et al. 2006) have been demonstrated to generate electrical power in MFCs. Nevertheless, the power outputs using wastewater are about a factor of 10 lower compared to pure substrates (Aelterman et al. 2006). Moreover, the composition of the wastewater is strongly affecting the power output of MFCs. This is also the case for landfill leachate. So far, although landfill leachate can be used to generate electricity, the level is still too low to be utilized directly.

However, the MFCs provide excellent technology to be used nearby landfill to prevent iron release. As demonstrated in this research, 75 to 80% of iron release can be prevented if the MFC technology is applied. Theoretically, the electrons released from organic compounds will be transferred to iron oxide. Consequently, iron will be reduced and released:

$$CH_2O + 2Fe_2O_3 + 3H_2O = CO_2 + 4Fe^{2+} + 8OH^- \qquad (12)$$

Based on above equation, 55.8 g ferrous iron can be released per electron transfer. The energy requirement for iron reduction is listed in Table 3.

Table 3. Gibbs Free Energy of Iron Reduction

Reactions for Iron Reduction	$\Delta G^0(w)$ kJ/e^- eq
$Fe^{3+} + e^- = Fe^{2+}$	74.3

The energy that *Shewanella putrefaciens* obtained from the oxidation of the landfill leachate through respiration must balance their need to synthesize the new cells. Consequently,

$$\varepsilon A \Delta G_r + \Delta G_s = 0 \qquad (13)$$

where ε is the efficiency of energy transfer to or from the energy carrier (e.g., ATP) which is assumed to be 0.6; ΔG_r is the free energy released per electron equivalent (eeq) (amount of the substrate that releases 1 mole e^- during a specified oxidation reaction) of electron-donor substrate converted for energy (e.g., respiration); ΔG_s is the carrier (ATP) energy required to synthesize 1 eeq of cells which includes energy loss incurred in using the energy carrier (e.g., ATP); and A is the balance ratio between ΔG_r and ΔG_s. For heterotrophic growth with ammonia as nitrogen source, A can be estimated by equation by:

$$A = \frac{\frac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon \Delta G_r} \qquad (14)$$

where ΔG_p is the free energy required (or evolved) in conversion of the carbon source to pyruvate (kcal per eeq pyruvate); ΔG_c is the ATP energy required to form 1 eeq cells from pyruvate and ammonia which is assumed to be 7.5 kcal; m = +1 when $\Delta G_p > 0$ and m = -1 when $\Delta G_P < 0$. For heterotrophic growth with nitrate as the nitrogen source, as

nitrate needs to be converted to ammonia first before it can be used for synthesis, A is estimated by:

$$A = \frac{\frac{-\Delta G_p}{\varepsilon^m} - \frac{5}{7}\Delta G_c - 0.89}{\varepsilon \Delta G_r} \quad (15)$$

Stoichiometric yield coefficient Y can be estimate as:

$$Y = \frac{\alpha}{\beta(1+A)} \quad (\text{g biomass formed per g substrate used}) \quad (16)$$

where α is the mole weight of 1 eeq biomass which equals to 5.65 g for ammonia served as the nitrogen source and 4.04 g for nitrate as the nitrogen source if *Shewanella putrefaciens* is assumed to have a formula of $C_5H_7O_2N$, and β is the mole weight of 1 eeq substrate which equals to 7.37 g, 3.83 g, 22.5 g, 7.50 g, 7.42 g and 5.21g for acetate, ethanol, formate, glucose, lactate and propionate, respectively.

The maximum specific growth rate can be estimated by:

$$\mu_{max} = Yk \quad (17)$$

where k is the maximum specific utilization rate (g substrate used per g microbe per day) when ignoring decay or maintenance. It is asserted that the rate of electron transfer in energy-yielding reactions (e.g., respiration) is relatively constant (per g biomass per day) varying between 0.5 and 2.0 among many types of microorganisms — including heterotrophs, autotrophs, aerobes, and anaerobes. Based on this assertion, the maximum specific utilization rate is:

$$k = \frac{\beta(0.5 \sim 2.0)(1+A)}{A} \quad (18)$$

Table 4. Theoretical *Shewanella putrefaciens* Activity Parameters

Organic Compounds	A	Y (g/g)	μ_{max} (day^{-1})
Acetate	0.73	0.44	~ 0.011
Ethanol	0.60	0.92	~ 0.013
Formate	0.43	0.17	~ 0.018
Glucose	0.40	0.53	~ 0.019
Lactate	0.56	0.48	~ 0.014
Propionate	0.72	0.63	~ 0.011

The theoretically calculated Y and k values were in a similar range as those measured in the experiments. It should be noted that the theoretically estimated stoichiometric yield coefficient Y and maximum specific growth rate μ_{max} were obtained based on the assumption that the ammonia served as the nitrogen source and iron served as electron acceptor.

6. Conclusions

Electrons produced in the MFCs flow from the anode through an external electrical circuit to the cathode to generate electrical current. While electrons move externally, protons diffuse from the anode to the cathode via the cation exchange membrane to complete the internal circuit. At the cathode, the electrons and protons combine to reduce the terminal electron acceptor, which in many applications is oxygen. Therefore bacteria in the anode are physically separated from their terminal electron acceptor in the cathode compartment.

Using the MFC technology, iron reduction and release can be prevented, which is achieved by transiting the electrons to the designated electron acceptors avoiding the consumption of electrons by the iron rich soil. At the same time, leachate is simultaneously bioremediated. This study investigated the feasibility of the usage of MFC technology for landfill leachate decomposition, iron release prevention and possible power generation. *Shewanella putrefaciens* was identified as the dominating strain that can utilize landfill leachate as the energy source and transport the released electrons. It is also demonstrated that electrons released from landfill leachate decomposition can flow from the anode to the cathode, where they can be accepted by selected electron acceptors instead of iron oxides. For this research, several landfill leachate and soils in Northwest Florida were sampled and tested for power generation and iron release prevention. The impact of other factors such as pH, temperature and dissolved oxygen on power generation and iron release prevention was also investigated. This research will provide guidelines for MFC technology applications in landfills.

7. Future Work

The conversion of organic waste, especially landfill leachate, to energy is considered an essential part of a sustainable energy portfolio. A variety of potentially valuable underutilized energy sources exist in the United States. MFCs can generate electricity from most organic waste. Thus it is feasible for MFCs to decompose waste material while generating electricity. Coupling these technologies to minimize production costs and increase energy recovery could help make "green energy" profitable and sustainable.

Currently, the power generated by MFCs using landfill leachate is too low to be fully utilized. Part of our future work is to improve MFC performance to fully utilize the generated power. Power generation is limited by the leachate composition. There are many factors affecting the composition of leachate, i.e., age, precipitation, seasonal weather variation, and waste type. In particular, the composition of landfill leachate varies greatly depending on the age of the landfill. As landfill age increases, organics concentration in leachate decreases and ammonia nitrogen concentration increases. Landfill leachate from old sites is usually highly contaminated with ammonia resulting from the hydrolysis and fermentation of nitrogen containing fractions of biodegradable refuse substrates. The existing relation between the age of the landfill and the organic matter composition may provide useful criteria for MFC technology applications. In general, leachate may contain large amounts of organic matter (biodegradable, but also refractory to biodegradation), where humic-type constituents consist an important group, as well as ammonia-nitrogen, heavy metals, chlorinated organic and inorganic salts.

Beyond iron release prevention, MFCs also have other applications besides electricity production. MFCs can be used to power cathodic reduction reactions for bioremedial or industrial processes. Since electricity is not being harvested, the biologically generated current can thus be used to stimulate microbial metabolism on a cathode. In addition, MFCs can also be modified to produce hydrogen gas. Microbial electrolysis cell (MECs), based on bacterial oxidation of organic substrates occurring at the anode and electrons flowing to the cathode, can generate renewable hydrogen from waste materials. In MECs

an electrochemical potential achieved in the anode is supplemented with an additional ~250 mV from an exogenous source so that electrolysis of water occurs at the cathode, producing hydrogen. Over the past two years, research in this area has advanced significantly with the amount of hydrogen generated close the U.S. Department of Energy's target for technology viability. Possible hydrogen generation from landfill leachate is also part of our future work.

For MFC technologies to be applied in the field, one of the TAG members recommends a pilot scale experiment be conducted to evaluate the performance of iron release prevention. Upscale from laboratory to pilot and field is always an issue for technology applications. Parameters generated from laboratory experiments usually cannot be directly applied to field systems. We envision that iron release might be higher than the data we obtain from laboratory experiments. This is because more uncertainties may be encountered in field systems including the uneven oxygen diffusion in the cathode region, and the possible leakage of leachate from the anode region to the cathode region. If above issues can be addressed, MFC technology should efficiently prevent iron release nearby landfills. Iron release prevention will not be impacted by the variation of landfill leachate quality since organic compound decomposition is totally separated from iron reduction. We will work with the local community and the Hinkley Center to seek possibility to conduct a pilot experiment in the future."

8. Student Training

One graduate student, Pawan Subramaniam was trained in this project. Pawan was very active and productive in this research. So far, he has published two technical journal papers in leading journals based on the work sponsored by the Hinkley Center for Solid and Hazardous Waste management. In addition, he has presented his research work four times in national conferences his research work. He holds a Master of Science Degree from Florida State University and currently he is a Ph.D. candidate in the Department of Civil and Environmental Engineering at FAMU-FSU College of Engineering. He will graduate in April, 2011.

9. Acknowledgements

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9.1 Peer-reviewed journal paper publication:

1. Subramaniam, P. K., Martin, L., Grasel, P., Tawfiq, K. and Chen, G. (2110) *Iron Reduction and Adsorption on Shewanella putrefaciens nearby Landfills in Northwest Florida*, Journal of Environmental Science and Engineering, 4(9):60-69.
2. Subramaniam, P. K., Martin, L., Grasel, P., Taqfiq, K. and Chen, G. (2010) *Iron Release Prevention using Microbial Fuel Cell Technology in Northwest Florida*, Environmental Technology, to be submitted.

9.2 Conference presentation:

1. Subramaniam, P., and Chen, G. (Presented November, 2009). *Usage of microbial fuel cell technology to prevent iron release nearby landfills in Northwest Florida*. 95th Annual American Society of Microbiology Southeastern Branch Conference, Savannah, GA: American Society of Microbiology Southeastern Branch.
2. Subramaniam, P., and Chen, G. (To be presented November, 2010). *Landfill leachate treatment and electricity generation using microbial fuel cell technology*. 96th Annual American Society of Microbiology Southeastern Branch Conference, Montgomery, AL: American Society of Microbiology Southeastern Branch.

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