Usage of Microbial Fuel Cell Technology in Landfills. Year II. Enhanced Organic Compound Decomposition and Nitrogen Removal

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Gang Chen
Amy Chan Hilton
Kamal Tawfiq
Yongwoo Lee

Department of Civil and Environmental Engineering
FAMU-FSU College of Engineering

State University System of Florida
Hinkley Center for Solid and Hazardous Waste Management
University of Florida
4635 NW 53rd Avenue, Suite 205
Gainesville, FL 32653
www.hinkleycenter.org

Report #

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PRINCIPAL INVESTIGATOR(S): Gang Chen
AFFILIATION: Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering

ASSOCIATE INVESTIGATOR(S): Amy Chan Hilton and Kamal Tawfiq
AFFILIATION: Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering

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PHONE NUMBER: 850-410-6303

TAG MEMBERS: Lee Martin, Peter Grasel, Michael Watts, and Clayton Clark

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ABSTRACT (1 page only)

Low cost, low maintenance and energy-generating onsite systems for the treatment of landfill leachate with high ammonium content are in urgent need, especially for landfills located in low population areas where landfills are smaller and often at a distance from sewage systems and lack trained personnel. The purpose of this study was to design and test two continuous microbial fuel cell (MFC) reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor for the treatment of landfill leachate in terms of power generation, organic compound decomposition and nitrogen removal. For both of the reactors, in addition to energy generation from landfill leachate treatment, combined carbon and nitrogen removal was achieved. Energy generation resulted from the oxidation of organic components in the landfill leachate was achieved by separating electron release in the anodic chamber from its consumption in the cathodic chamber. Nitrate served as the electron acceptor for the ammonium oxidation/MFC reactor and nitrite served as the electron acceptor for the MFC/Anammox reactor. During the energy generation process, nitrogen was removed through nitrate reduction or nitrite reduction in the cathodic chamber. Both of these two reactors were “loop-operated”, during which the treated landfill leachate was looped from the anodic chamber to the cathodic chamber. Consequently, the acidity produced in the anodic chamber could partially offset the alkalinity produced by nitrate or nitrite reduction. This technology has the potential to be applied to small landfills located at a distance from sewage systems.

EXECUTIVE SUMMARY

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AFFILIATION: Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering

PROJECT WEBSITE ADDRESS (URL): http://www.eng.fsu.edu/~gchen

PROJECT TAG MEMBERS: Lee Martin, Peter Grasel, Michael Watts, and Clayton Clark

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Objectives:

Low cost, low maintenance and energy-generating onsite systems for the treatment of landfill leachate with high ammonium content are in urgent need, especially for landfills located in low population areas where landfills are smaller and often at a distance from sewage systems and lack trained personnel. The purpose of this study was to design and test two continuous microbial fuel cell (MFC) reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor for power generation as well as organic compound decomposition and nitrogen removal from landfill leachate. Specific objectives of this research include:

- Landfill leachate collected from landfills located in Northwest Florida was treated in a laboratory scale continuous ammonium oxidation/MFC reactor, which was composed of an in-line nitrification column and a MFC reactor. Ammonium oxidation as well as the impact of pH was investigated.
- Landfill leachate collected from landfills located in Northwest Florida was treated in a laboratory scale continuous MFC/Anammox reactor. The Anammox reaction was explored and the performance of the MFC/Anammox reactor was compared with that of the ammonium oxidation/MFC reactor in terms of power generation and nitrogen removal.

Methodology:

Two custom-made reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor were tested for power generation as well as organic compound decomposition and nitrogen removal from landfill leachate. For both reactors, a graphite rod, without catalysts coated, was used as the anode. The anode was inoculated with the cultured *S. 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 cm2, 30% wet proofing) was used as the cathode. The cathode was inoculated with *G. metallireducens*. Synthetic polymeric nanoporous membrane (Ultrex CMI-7000) was used as the cation-exchange membrane. For the MFC/Anammox reactor, the cathodic chamber was also inoculated with Anammox consortia. During the operation, collected landfill leachate was introduced to the anodic chamber for organic decomposition, after which the treated leachate was looped to the cathodic chamber for nitrogen removal.

Results:

In the ammonium oxidation/MFC reactor, glucose and landfill leachate collected from Leon County Landfill was continuously supplied and uninterrupted current was produced. The input glucose was 250 mg/l (~ 266 mg/l COD). The landfill leachate was diluted to a BOD5 value ~ 250 mg/l and total nitrogen of ~ 120 mg/l. Around 25 mW/m^2 and 10 mW/m^2 were generated by the ammonium oxidation/MFC reactor for glucose and landfill leachate respectively. With the input total N of 120 mg/l, above 92% of nitrogen was removed with an effluent total N concentration below 9 mg/l. Similar as the ammonium oxidation/MFC reactor, uninterrupted current was produced in the MFC/Anammox reactor. Compared to the ammonium oxidation/MFC reactor, the power generation from the MFC/Anammox reactor was higher for both glucose and landfill leachate (around 35 mW/m^2 and 12 mW/m^2). In addition, above 94% of nitrogen was removed with an effluent total N concentration below 7.5 mg/l. For both of the ammonium oxidation/MFC reactor and the MFC/Anammox reactor, pH had impact on the power generation, i.e., high pH favored the MFC operations.

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1. Introduction

There is worldwide consensus that landfilling is the most cost effective, least polluting and safest means of disposing of solid urban waste. However, one of the challenges to be confronted during landfill operations is to handle the landfill leachate with high ammonium content (Iwami et al. 1992; Liang and Liu 2008; Yusof et al. 2010). The main source of ammonium in the landfill leachate is protein, which is hydrolyzed to generate ammonium-nitrogen in the leachate (Burton and Watson-Craik 1998). The release of soluble nitrogen from municipal solid waste into landfill leachate continues over a long period of time when compared with that of soluble carbon compounds since the hydrolysis of the polypeptide chains is energetically disadvantaged (Lokshina et al. 2003; Pichler and Kogel-Knabner 2000; Vieitez et al. 2000). Landfill leachate discharges characterized by high nitrogen concentrations are detrimental to the environment since nitrogen can trigger eutrophication in the receiving watercourses (Jokela et al. 2002). Therefore, nitrogen is usually removed from landfill leachate, e.g., by biological treatment. Traditional biological nitrogen removal is nonreversible and is carried out in two stages: aerobic nitrification of ammonium via hydroxylamine and nitrite to nitrate, and subsequent anoxic denitrification of nitrate via intermediate stages to nitrogen gas (Chiu et al. 2007; Park et al. 2010; Zhou et al. 2008). Practically, suspended processes have been applied in full scale for nitrification and denitrification of wastewater with high nitrogen contents as a means of nitrogen removal (Agdag and Sponza 2008; Huo et al. 2008a). Specifically, for the treatment of landfill leachate with high ammonium contents, a 4-stage Bardenpho process, which consists of a sequence of anoxic and aerobic zones with capacities of nitrification with pre- and post-denitrification biological processes has been proposed (Ilies and Mavinic 2001). However, this process is complicated and very hard to manage and the results vary depending on the system management. New methods such as suspended carrier biofilm processes have also been studied for nitrogen removal from landfill leachate, even at low temperatures (Welander and Henrysson 1998; Welander et al. 1997). These processes are reliable, but they normally require major investments. Currently, there is an evident need for low cost, low maintenance and energy-generating onsite treatment systems to handle landfill leachate with high organic and ammonium contents. This is especially the case in low population areas where landfills are smaller and often are at a distance from sewage systems and lack trained personnel.

Microbial fuel cells (MFCs) have advantages in removing organic components from landfill leachate since MFCs allow microorganisms to break down various substrates while simultaneously generating power (Pant et al. 2010; Rachinski et al. 2010; You et al. 2006a; You et al. 2006b; Zhang et al. 2008). Therefore, microorganisms play the key role in the 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):

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

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, or neutral red (Takagi et al. 1998). MCFs 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 (Davila et al. 2008). If oxygen is present, oxygen would accept the liberated electrons (Clauwaert et al. 2008; Pham et al. 2006; Raghavulu et al. 2009; Rhoads et al. 2005). It should also be noted that most of the available mediators are expensive and toxic. Therefore, mediator-less MCFs have been developed (Kim et al. 2005; Liu et al. 2009). A mediator-less MCF 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 (Chung and Okabe 2009; Kim and Lee 2010; Li et al. 2010; Rachinski et al. 2010). 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. 2005). The electrochemically active bacteria include *Shewanella putrefaciens* (Schaetzle et al. 2008), *Aeromonas hydrophila* (Kim et al. 2006), etc. Some bacteria, which have pili on their external membranes, are also able to transfer their electron production via these pili (Leang et al. 2010). 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 MCF 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 (Lee et al. 2003; Lu et al. 2009; Manohar and Mansfeld 2009; Rabaey and Verstraete 2005). 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 MCFs (Jadhav and Ghangrekar 2008). Oxygen is usually used as the electron accepter in the cathodic chamber. But there are concerns that large volumes of circulating gas are required. Another convenient option is to use a solution of a solid oxidizing agent (Daniel et al. 2009). 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. Nitrogen in the landfill leachate mainly is present in the form of ammonium (NH_4^+). Once NH_4^+ is converted to nitrate (NO_3^-), there is a chance for the electrons generated in the anodic chamber to be consumed by NO_3^- in the cathodic chamber if MFC reactors are perfectly designed. Current research has demonstrated that the energy requirement to provide the necessary reducing power for denitrification can be drastically reduced if bacteria use the cathodic electrode directly as the electron donor (Virdis et al. 2010). *Geobacter* species were first reported to be capable of using a graphite electrode as direct electron donor during nitrate reduction to nitrite (Gregory et al. 2004). The cathodic denitrification without intermediate H_2

production was able to be coupled with anodic oxidation of organic carbon using MFCs (Clauwaert et al. 2007).

Recently, a novel process called anaerobic ammonium oxidation (Anammox) has been introduced to the treatment of municipal landfill leachate with high concentrations of ammonium (Ganigue et al. 2010; Ganigue et al. 2007; Liu et al. 2010). Anammox is a microbiological mediated exergonic process during which ammonium is converted to nitrogen gas under anaerobic conditions with nitrite serving as the electron acceptor (Ganigue et al. 2007). Anammox process is strictly anaerobic and is inhibited by high concentrations of oxygen. Currently, microbial species that are responsible for the Anammox process have been identified, which include *Planctomycetes* genus *Candidatus* "*Brocadia anammoxidans*" and "*Kuenenia stuttgartiensis*" as well as several species of "*Scalindua*" (Strous et al. 1997). Anammox is an autotrophic process and can completely convert ammonium to nitrogen gas without the presence of organic matter. Thus, Anammox not only eliminates the need for complex compromises between organic carbon removal and nitrogen removal, but also saves oxygen supplies and reduces CO_2 emission as compared to the conventional nitrification/denitrification process. For the Anammox process to occur, partial nitrification during which nitrite is accumulated is the prerequisite (Fux et al. 2002; Yan and Hu 2009; Zhang et al. 2010). Also, high organic contents may interfere with partial nitrification and the subsequent Anammox. If these two issues can be addressed, Anammox is a promising means to handle landfill leachate with high organic and ammonium contents. In practice, Anammox has been achieved with two reactors in series, with a partial nitrification reactor as a first step, and a separate unit for Anammox as a second step (Hellinga et al. 1998). With this configuration, the two biological processes can be controlled separately (van Dongen et al. 2001). The key step for Anammox is to achieve stable nitrite accumulation through partial nitrification (Qiao et al. 2010; Zhang et al. 2010). Different strategies and approaches such as control of temperature, hydraulic retention time, pH, dissolved oxygen as well as the presence of free ammonia has been practiced. Specifically, it has been concluded that temperature above 25 ℃, low hydraulic retention time, and high pH favor ammonium oxidizers rather than the nitrite oxidizers (Guo et al. 2009; Jetten et al. 1998; Shinohara et al. 2009; Yan and Hu 2009). Alkalinity is also an important factor for nitrification. Depending on the alkalinity of the wastewater, it is possible to convert a fraction or even the whole load of ammonium into nitrite (Tian et al. 2009). Varying the dissolved oxygen concentration in the reactor is also a possible way for enhancing nitrite accumulation.

In our prior research, we have constructed one batch MFC and one continuous MFC for the treatment of landfill leachate. The illustration of these MFCs is shown in Figure 1. Graphite rods, without catalysts coated, were installed in the center of the anodic chambers as the anodes. The anodes were inoculated with the cultured *S. putrefaciens*. 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 in the center of the cathodic chambers, serving as the cathodes. In the cathodic chambers, O_2 served as the electron acceptor. The anodes and cathodes were connected through a digital multimeter. Synthetic polymeric nanoporous membranes (Ultrex CMI-7000, Membranes International Inc., Glen Rock, NJ) were used as the cation-exchange membrane (CEM). As shown in Figure 2, at pH 7, more power was generated for glucose (up to 68 mV/m^2) than that of landfill leachate (up to 30 mV/m^2). In addition, a self-sharpening power generation front was observed for glucose. However, for landfill leachate, there was an obvious lag, indicating that *S. putrefaciens* needed time to adapt to the landfill leachate. Impact of pH on power generation was also investigated. The higher the pH (i.e., pH 8), the more power was generated. It should be noted that the pH control was achieved in the anodic chamber where organic compounds (glucose or landfill leachate) were decomposed. According to equation (1), raising the pH facilitated electron release. However, when the free electrons are consumed by oxygen in the cathodic chamber, lowering the pH should favor the reaction, i.e., $1/4O_2 + H^+ + e^- \rightarrow 1/2H_2O$. In the continuous MFC, the carbon source was continuously supplied and uninterrupted current was produced (Figure 3). The input landfill leachate was diluted to a BOD_5 value of ~ 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. 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.

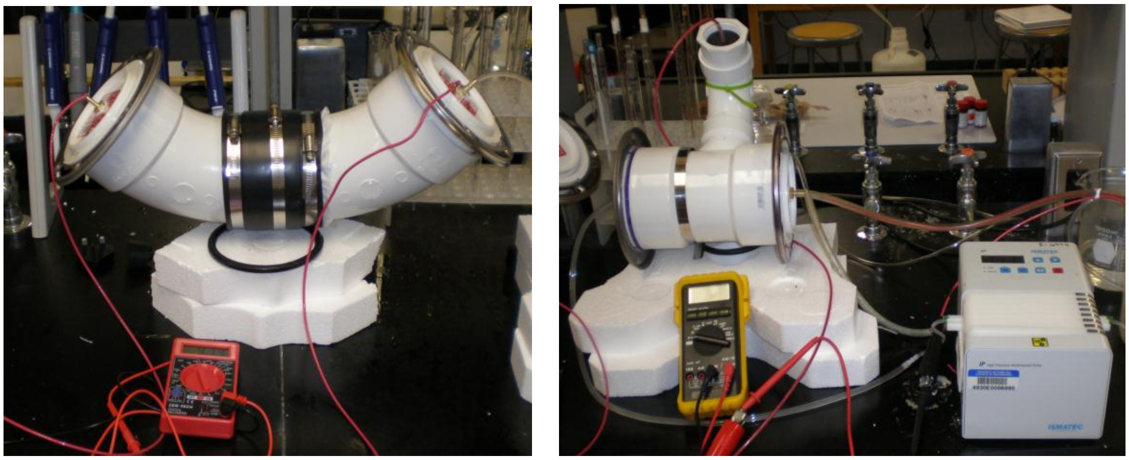

Figure 1. Batch and Continuous MFC Reactor Setups

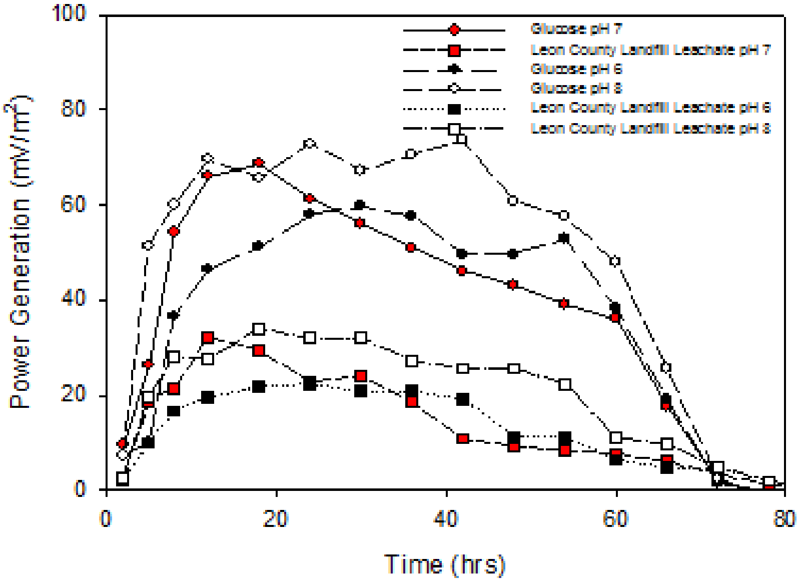

Figure 2. Power Generation from Batch MFC

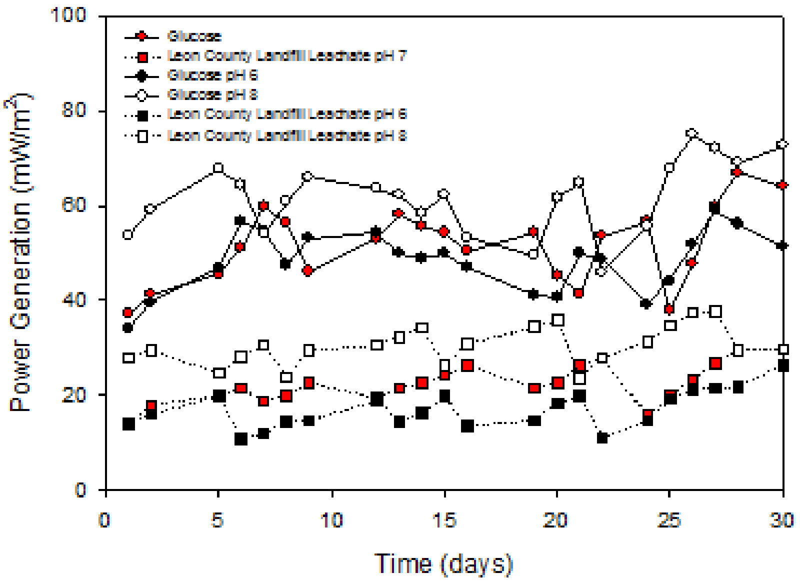

Figure 3. Power Generation from Continuous MFC

During batch MFC applications, organic compounds were dynamically decomposed. Samples were periodically withdrawn from the MFC 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, organic compound and microbial concentrations over time can be described by following equations (Monod 1949):

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m SX}{K_s + S} \qquad (2)$$

$$\frac{dX}{dt} = \frac{\mu_m SX}{K_s + S} - \frac{bX}{K_s + S} \qquad (3)$$

where S is the organic concentration, which is expressed in terms of BOD_5 (mg/l); μ_m is the microbial 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); Ks 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 compound depletion, such that:

$$Y = -\frac{\Delta X}{\Delta S} \tag{4}$$

$$X = X_0 + Y(S_0 - S) \tag{5}$$

By substituting equations (4) and (5) into equation (2), substrate depletion can be expressed as:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_m S[X_0 + Y(S_0 - S)]}{K_s + S} \tag{6}$$

Landfill leachate decomposition was simulated against Equation (6) (Figure 4). 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}) for landfill leachate collected from four landfills in Northwest Florida together with glucose are summarized in Table 1. Except for the landfill leachate collected from Gadsden County, all the other landfill leachate had similar K_s values, indicating that the microbial 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 above analysis, it might be concluded that landfill leachate from Gadsden County Landfill contained some organic compounds that were a little harder for *S. putrefaciens* to decompose. However, since similar power was generated as compared to other landfill leachate samples, there was no much difference of energy content of the organic compounds from this landfill as compared to others.

Table 1. Glucose and Landfill Leachate Decomposition Parameters

	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

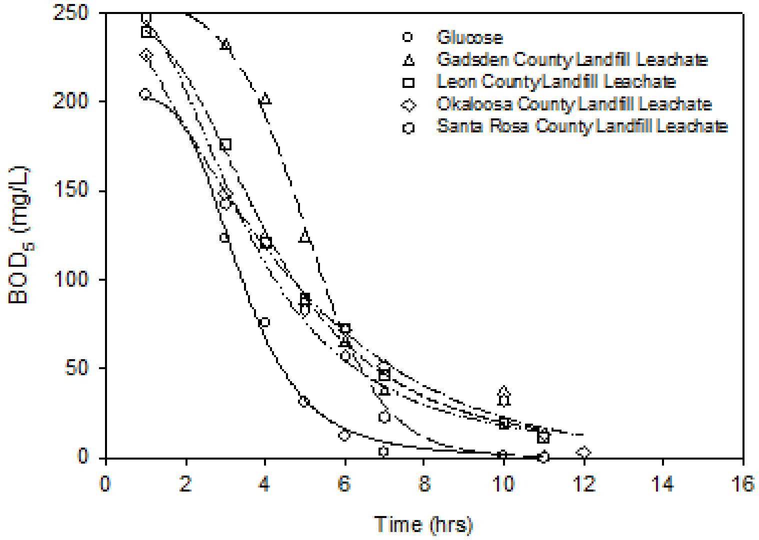

Figure 4. Landfill Leachate Decomposition Profile

In prior research, we have also investigated the usage of Anammox processes to treat landfill leachate. The laboratory scale anaerobic-partial nitrification-Anammox reactor consisted of three sequencing CSTR batch reactors with a working volume of 500 ml each (Figure 5). The first reactor was an anaerobic reactor equipped with a gas capturing device for the removal of produced CO_2 and CH_4. The second reactor was a partial nitrification reactor with a controlled oxygen supply device. These two reactors were continuously stirred by a single mechanical blade. The last reactor was an Anammox reactor, which was operated at 37°C using a temperature-controlled water bath. The key step for this experiment was to achieve partial nitrification and obtain stable nitrite accumulation. Dissolved oxygen and alkalinity were important factors for partial nitrification. Depending on the dissolved oxygen and alkalinity of the leachate, it was possible to convert a fraction or even the whole load of ammonium into nitrite. As shown in Figure 6, N_2 production was a function of both alkalinity and dissolved oxygen. Corresponding to the input ammonium of 90.5 mg/l, 75.4 mg/l and 54.1 mg/l, the optimal alkalinity was 278 mg/l, 380 mg/l and 450 mg/l as $CaCO_3$. For the ammonium input of 90.5 mg/l, the optimal dissolved oxygen was 0.15 mg/l (Figure 7).

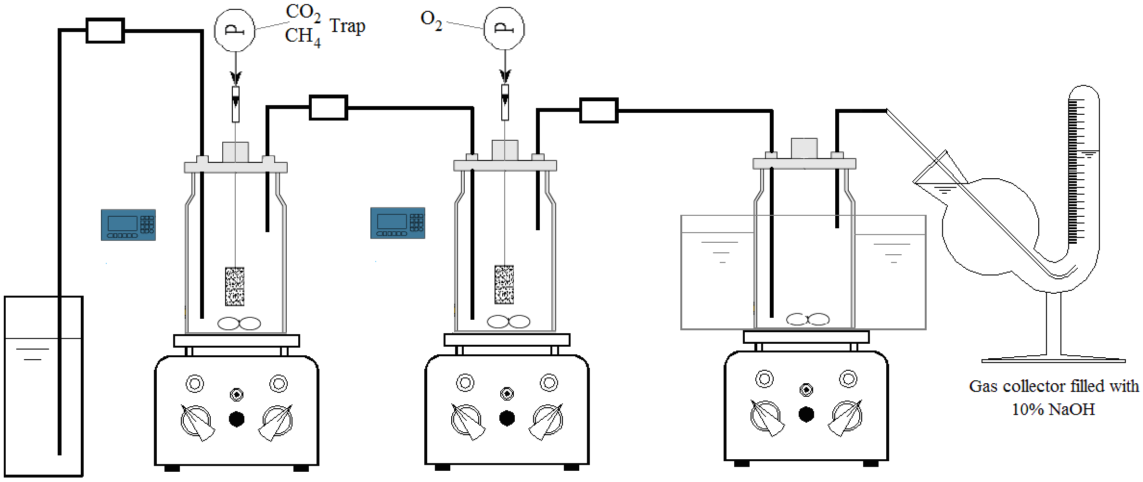

Figure 5. Anaerobic-Partial Nitrification-Anammox Reactor Setup

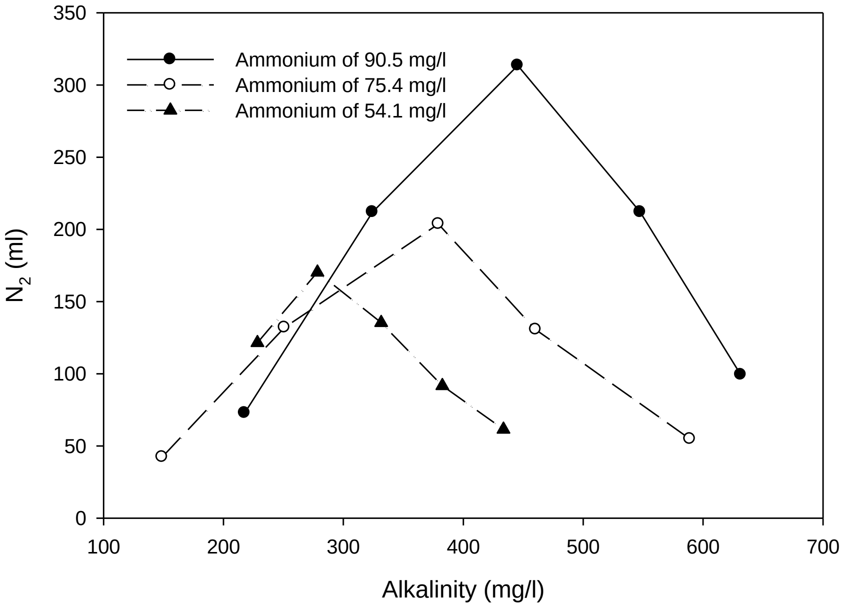

Figure 6. Nitrogen Production as a Function of Alkalinity

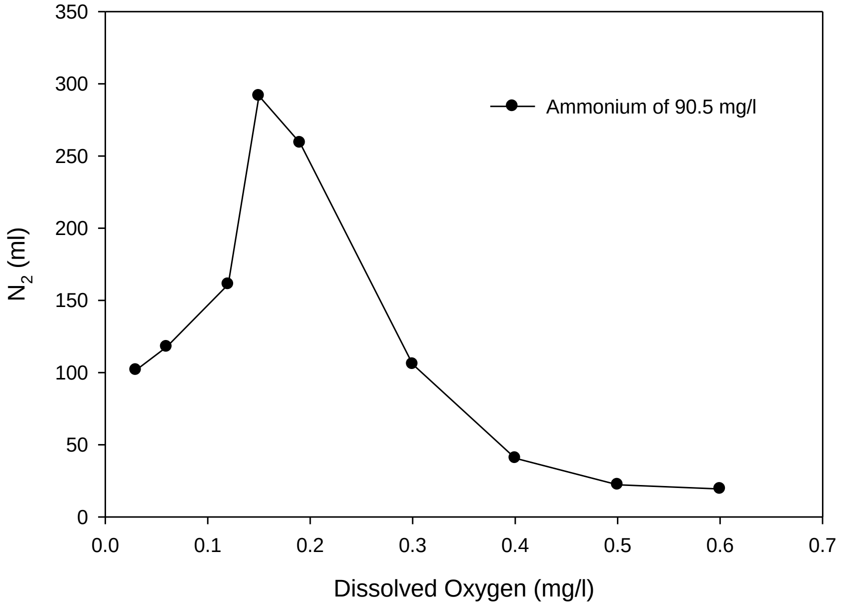

Figure 7. Nitrogen Production as a Function of Dissolved Oxygen

Objectives

Currently, there is interest to incorporate Anammox into MFCs to handle municipal landfill leachate with high organic and nitrogen contents, which should be able to achieve power generation, organic compound decomposition and nitrogen removal in a single unit (Jetten et al. 2001; Strous et al. 1997). Since Anammox is an autotrophic process and can completely convert ammonium to nitrogen gas without the presence of organic matter, the organic components in the landfill leachate can be fully utilized for energy generation in the MFCs. In addition, the organic removal by MFCs prior to Anammox also eliminates the possible interference of high organic contents on partial nitrification, a prerequisite for Anammox during which nitrite is accumulated. Thus, the benefit of the combined system is obvious.

The purpose of this study was to design and test two continuous MFC reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor, for power generation as well as removal of organic compounds and nitrogen from landfill leachate. For the ammonium oxidation/MFC reactor, we hypothesized that the electrons released from organic compound decomposition flow from the anode to the cathode, where they were utilized by nitrate. For the MFC/Anammox reactor, we hypothesized that the organic compounds in the landfill leachate were decomposed in the anodic chamber and the released electrons were transported to the cathodic chamber and consumed by nitrite. Specific objectives of this research include:

1. Landfill Leachate Treatment in Ammonium Oxidation/MFC Reactor: Landfill leachate collected from landfills located in Northwest Florida was treated in a laboratory scale continuous ammonium oxidation/MFC reactor, which was composed of an in-line nitrification column and a MFC reactor. The success of this process depended on two main factors: the capability of *Shewanella* species to transport electrons to the anode and *Geobacter* species to use the electrode as the direct electron donor for nitrate reduction. Ammonium oxidation and the impact of pH on the reactor operations were investigated.

2. Landfill Leachate Treatment in MFC/Anammox Reactor: Landfill leachate collected from landfills located in Northwest Florida was treated in a laboratory scale MFC/Anammox reactor. For this treatment process, more power was expected to be generated since the organic carbon is almost fully utilized for energy generation. However, air supply was required at the bottom of the cathodic chamber for partial nitrification. Anammox reaction was explored and the factors that impacted the performance of the MFC/Anammox reactor were studied.

3. System Comparison: The two onsite treatment systems were compared in terms of power generation as well as organic compound decomposition and nitrogen removal. In addition, treatment cost and energy production profit were discussed.

2. Background

2.1 Landfill Leachate Treatment

Landfilling is widely adopted as one of the most economical processes of solid waste disposal. At the same time, landfill leachate is also a great environmental concern because of its complex composition and high concentration (ElFadel et al. 1997; Kirkeby et al. 2007; Manfredi et al. 2010). Landfill leachate is the liquid generated from the moisture associated with the solid waste deposited in the landfill cell, which mainly is created when infiltrating rainwater dissolves contaminants within the landfill waste and seeps out the bottom of the landfill cell into the leachate collection system. Landfill leachate characteristics present high variation due to several factors such as the composition of the solid waste, site hydrology, compaction, cover design, waste age, sampling procedures, interactions of leachate with the environment, and landfill designs and operations (Comstock et al. 2010; Huo et al. 2008b). Among these factors, the landfill operations, waste age and climatic conditions play the key role. The actual composition of landfill leachate varies dramatically from landfills to landfills. Even within the same landfill, the composition varies over time (Huo et al. 2008b). Typical chemicals contained in the landfill leachate include volatile organic compounds, metals/metal ions, and synthetic organic compounds, etc. In the subsurface soil, landfill leachate becomes anaerobic once all oxygen is consumed in the breakdown of organic matter.

Due to its reliability, simplicity and high cost-effectiveness, biological treatment (aerobic and anaerobic processes) is commonly adopted for the treatment of landfill leachate containing high concentrations of organic compounds. The biodegradation of organic matter in landfill leachate is carried out by microorganisms, which convert organic components to carbon dioxide under aerobic conditions and biogas (a mixture comprising chiefly CO_2 and CH_4) under anaerobic conditions (Nyer 1988; Palit and Qasim 1977). Biological processes have been shown to be very effective in removing organic and nitrogenous matter from immature leachates when the BOD/COD ratio has a high value (> 0.5) (Kurniawan et al. 2010; Xu et al. 2010). For aerobic treatment, besides abatement of biodegradable organic pollutants, ammonium nitrogen nitrification can also be achieved. Aerobic biological processes such as suspended-growth biomass, aerated lagoons, activated sludge processes and sequencing batch reactors (SBR) have been widely utilized (Nyer 1988; Palit and Qasim 1977). For anaerobic treatment, energy can be generated and very few solids remain. However, these processes suffer from low reaction rates (Frigon et al. 1997; Kheradmand et al. 2010). Besides biological treatment, physicochemical methods are also being considered for the treatment of landfill leachate (Novelo et al. 2010; Weng et al. 2011). When treating the young leachate, biological techniques can yield a reasonable treatment performance with respect to COD, NH_3-N and heavy metals. When treating stabilized (less biodegradable) leachate, physicochemical treatments have been found to be suitable as a refining step for biologically treated leachate, in order to remove organic refractory substances (Novelo et al. 2009).

Landfill leachate treatment represents a portion of the broader nexus between energy and water. Collecting, treating, and discharging landfill leachate to acceptable permit standards requires energy, mostly as electricity (Ro et al. 1997). Many methods that are currently used to treat leachate may have several drawbacks. For example, physical methods such as sedimentation, adsorption and membrane filtration, or chemical processes such as coagulation and chemical precipitation are commonly regarded as higher cost or lower effectiveness. Aerobic biological processes are effective for removing organic compounds but do remain inefficient due to large amounts of excessive sludge production, odour generation and high energy consumption (Renou et al. 2008). In addition, with the continuous hardening of the discharge standards in most countries and the ageing of landfill sites with more and more stabilized leachate, conventional treatment technologies (biological or physicochemical) are not sufficient anymore to reach the level of purification needed to fully reduce the negative impact of landfill leachate on the environment. More effective treatments such as membrane technology has emerged as a viable treatment alternative to comply and pending water quality regulations (Ahmed and Lan 2012; Ahn et al. 2002; Mahmoudkhani et al. 2012). Due to the increased enforcement of discharge regulation and escalating surcharges by public owned treatment works, many landfill leachate facilities are taking steps to reduce the discharge and recover energy. Consequently, these treatment facilities may be able to significantly reduce their energy costs through energy efficiency measures and treatment process modifications. Currently, the science of bio-energy, featuring wide-ranging fermentation of materials, clear mechanisms, simple processes and suitable extension of the resources has established its active role in landfill leachate treatment (Pant et al. 2010; Vlaeminck et al. 2012).

2.2 MCF and Energy Generation

The high energy requirements of conventional landfill leachate treatment demand for alternative cost-effective treatment technologies. In addition, due to global environmental concerns and energy insecurity, there is emergent interest to find out sustainable and clean energy sources. Accordingly, MFC reactors have drawn increasing attention in the treatment of landfill leachate (Pant et al. 2010; Puig et al. 2011). MFCs, in which microorganisms oxidize the organic compounds and transfer the released electrons onto an electrode, are a promising biotechnological approach for harvesting energy from the carbohydrates in the landfill leachate. MFCs typically consist of two separate chambers, an anodic chamber and a cathodic chamber (Figure 8). In the anodic chamber, microorganisms oxidize biodegradable organic matter and transfer the released electrons to the anode electrode. The released electrons move along a circuit to the cathodic chamber, where oxygen or other chemicals such as ferricyanide accept the electrons to forming water (from oxygen) or ferrocyanide (from ferricyanide) (Jadhav and Ghangrekar 2008; Strik et al. 2008). There are three ways by which microorganisms can transfer electrons to the anode electrode: using exogenous mediators such as potassium ferric cyanide, thionine, methyl viologen, and humic acid, etc., using mediators produced by the bacteria, or by direct transfer of electrons from respiratory enzymes (i.e., cytochromes) to the electrode (Pant et al. 2010). Mediators provide a method of shuttling electrons from inside of the bacterial cells to the electrode. There are several drawbacks using exogenous mediators, such as short lifetime, high costs, and toxicity to the microorganisms. However, when the bacteria produce their own mediators, or they transfer electrons directly to the electrode, the system can operate at a high, sustained level of activity. These systems are defined as a mediator-less MFCs. Mediator-less MFCs are considered to have more commercial application potentials (Aldrovandi et al. 2009; Du et al. 2008; Roh and Kim 2012). In the mediator-less MFCs, a membrane separates the anode from the cathode, which functions as an electrolyte that plays the role of an electric insulator, allowing protons to move through. Several isolates, including *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens*, and *Rhodoferax ferrireducens*, have been shown to generate electricity in mediator-less MFC systems (Il Park et al. 2008). In addition to microorganisms that can transfer electrons to the anode, the presence of other organisms appears to benefit MFC performances. It is reported that, a mixed culture generated a current that was six fold higher than that of a pure culture (Mohan et al. 2008). Hence, the microbial communities that develop in the anodic chamber may have a similar function as those found in methanogenic anaerobic digesters. Besides, these microorganisms can also transfer electrons to the electrode surface. Such microbial communities have been referred as adapted anodophilic consortia, which include *Geobacteraceae*, *Desulfuromonaceae*, *Alteromonadaceae*, *Enterobacteriaceae*, *Pasteurellaceae*, *Clostridiaceae*, *Aeromonadaceae*, and *Comamonadaceae* (Pham et al. 2008).

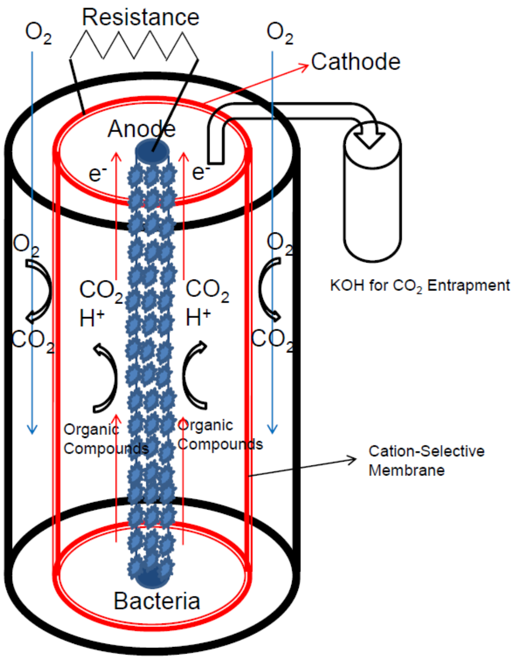

Figure 8. Sketch of Dual Chamber MFC

2.3 MFC Operation Optimization

The major implementation limitations of MFCs for the treatment of landfill leachate are the low power density production. The maximum power density reported in the literature was 3600 mW/m^2, which was observed in a dual-chamber fuel cell treating glucose with an adapted anaerobic consortium in the anodic chamber and a continuously aerated cathodic chamber containing an electrolyte solution that was formulated to improve oxygen transfer to cathode (Rabaey et al. 2003). The system performances varied considerably in different applications (Table 2) (Liu and Logan 2004b).

Table 2. Power Generation Rates Reported in the Literature

Description	**Power (mW/m^2)**
Anaerobic sediments	16
Starch wastewater	19
Starch wastewater	20
Domestic wastewater	24
Anaerobic sediments	28
Domestic wastewater, CE-PEM	28
Domestic wastewater, CE no PEM	146
Lactate	0.6-15
Lactate, Peptone and yeast extract	788
Acetate (salt bridge)	0.3
Acetate	14-49
Glucose	33-3600
Glucose – CE-PEM	262
Glucose – CE (no PEM)	494

In the past few years, there have been substantial increases in power generation. Besides, costs of MFCs can also be minimized by using plain graphite electrodes and commercially available membranes. To further improve the power density, besides anodophilic consortium selection for efficient electron transfer to electrode, MFC configuration also plays an important role.

2.3.1 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, i.e., even trace copper ions are toxic 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, and foam), and as glassy carbon (Mohan et al. 2008). The most commonly used materials for anode electrodes are graphite plates or rods as they are relatively inexpensive, easy to handle, and have a defined surface area (Figure 9). After receiving electrons, the anode potential decreases to a level lower than that of the cathode in the cathodic chamber (Cheng et al. 2008; Cheng et al. 2006b; Logan et al. 2005). The maximum power generation is closely related to the level of potential decrease in the anode.

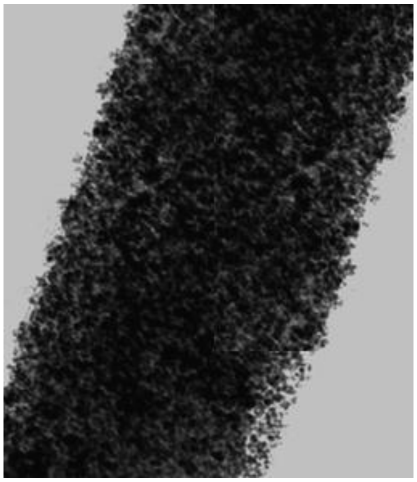

Figure 9. High Porous Graphite Electrode

2.3.2 Cathode Selection

The choice of the cathode materials also greatly affects the MCF performance. Besides oxygen, various catholytes such as hexacynoferrate or acidic permanganate have been used in MFCs (Rabaey et al. 2005; You et al. 2006b). 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 electron acceptor often need expensive platinum as the catalyst to accelerate the O_2 reduction reaction (Liu and Logan 2004a), 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. 2006c; Zhao et al. 2006). Recently, potassium ferricyanide ($K_3[Fe(CN)_6]$) has been tested to be utilized as the electron acceptor in MCFs owing to its good performance (Park and Zeikus 2003). The greatest advantage of potassium ferricyanide is to maintain a low potential for the plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. A 50% to 80% increase in maximum power has been reported using potassium ferricyanide in the cathodic compartment as compared to that of oxygen-saturated aqueous cathode or platinum-coated air-cathode (Oh et al. 2004). The observed differences can be attributed to the 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 the 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 operations which eventually reduces the overall performance of the MFCs (Logan and Regan 2006). For the landfill applications, sustainability is always the priority. Thus, before above problems are solved, O_2 or air is more commonly used as the cathodic electron acceptor.

2.3.3 Membrane

The majority of MFC designs require the separation of the anodic and the cathodic compartments by a CEM. For smooth MFC operations, both protons and electrons need to migrate from the anode to the cathode, be it through the CEM, at the highest possible rate. Membrane resistance, selectivity and permeability thus play an important role in MFC performances. The selection of a membrane represents a choice between two opposing interests: high selectivity and high stability. The higher the selectivity for protons, the better the MFC will operate and the lower the resistance of the membrane. 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 in MFCs 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). Nafion has high selectivity for protons. However, this membrane contains sulfonic acid groups, which are binding with ammonia. Hence, at this moment, this membrane type scores high for selectivity but low for stability. Ultrex has a larger resistance and is less selective but generally shows larger stability (Rabaey et al. 2003). It should be noted that ammonia interferes with Nafion CEM performances. When a CEM is used in an MFC, it is also important to recognize that it may be permeable to chemicals such as oxygen, ferricyanide, or organic matter used as the substrate.

2.4 Landfill Leachate Nitrogen Removal by Anammox

The treatment of municipal landfill leachate has been considered to be very problematic because of its complex chemical composition. It is highly variable, heterogeneous, and depends on the type of waste deposited and the age of the landfill (Renou et al. 2008). Moreover, the leachate quality and quantity changes with time within the same landfill. Apart from the organic and hazardous compounds, leachate contains a high ammonium concentration that could reach a few thousand milligrams per liter (Leite et al. 2011; Wang et al. 2011). At landfills where landfill leachate recirculation is practiced, landfill leachate ammonium concentrations may accumulate at much higher levels than that of conventional single pass leaching. Therefore, it is more difficult to handle (Ellouze et al. 2008). The main nitrogen source in the landfill leachate is protein, which is hydrolyzed to generate ammonium-nitrogen, which can trigger eutrophication in the receiving watercourses if it is not properly removed. Other damaging impacts resulting from nitrogenous discharges include reduction of chlorine disinfection efficiency, an increase in the dissolved oxygen depletion in receiving watercourses, adverse public health effects, and a reduction in suitability for reuse (Gordon et al. 1989; Menser and Winant

1980). Due to the toxic effects that nitrogen produces, the nitrogen content must be treated to an acceptable level, i.e., < 10 mg/l, before it is discharged (Li and Zhao 2003). Traditionally, biological treatment is commonly used as a simple, reliable and highly cost-effective method by removing organic matter as well as nitrogen from the landfill leachate. Specifically, nitrogen removal involves two separate steps, aerobic nitrification of ammonium to nitrate, and anoxic denitrification of nitrate to nitrogen (Nyer 1988; Palit and Qasim 1977). However, landfill leachate usually exhibits a low carbon–nitrogen ratio and a low level of alkalinity, which make the conventional processes of nitrification and denitrification more difficult (Palit and Qasim 1977). To address this issue, it would be necessary to increase the level of alkalinity and/or the organic matter content, thus considerably increasing the cost of treatment. To save energy and obtain stable operations, various novel processes such as shortcut nitrification and denitrification, anaerobic ammonium oxidation (Anammox), aerobic denitrification, and aerobic deammonification have recently been developed (Ganigue et al. 2007; Liang and Liu 2008). Among these novel technologies, Anammox provides a more sustainable alternative, due to the reduced level of aeration and the lower dosage of external organic carbon requirements. In theory, Anammox saves 25% in oxygen consumption and 40% in the carbon resources in comparison to the traditional denitrification process (Liang and Liu 2007; Zhang and Zhou 2006).

3. Materials and Methods

3.1 Landfill Leachate and Soil Sample Collection

Landfill leachate was collected from leachate sumps from Leon County Landfill, located in Tallahassee, FL. Leon County landfill accepts class III commercial and residential wastes through Marpan Recycling, which include yard trash, C&D debris, processed tires, asbestos, carpet, cardboard, paper, glass, plastic, furniture other than appliances, and other materials approved by DEP. Yard debris (leaves and limbs) and waste tires are accepted through the Solid Waste Management Facility. Besides, Leon County landfill also receives electronics, computers and peripherals, televisions, video game systems, handheld electronics, cell phones, household hazardous waste, and stryofoam TM (packaging foam only), etc. 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 this research, the landfill leachate had a composition of COD up to 20,000 mg/l, NH_4^+-N up to 500 mg/l, and phosphorus up to 200 mg/l.

Soil samples that were used for this research were also collected from this landfill site. Specifically, soil samples were collected 1 to 3 feet below the surface, 100 to 300 feet away from the landfills. 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 *S. putrefaciens* and *G. metallireducens* Culturing

In this research, MCFs were used to investigate power generation and nitrogen removal from landfill leachate. Mediator-less MCFs were used which depended on the electrochemically active bacteria to transfer electrons to the anode in this research. Electrochemically active bacteria use the anode in their metabolism, and subsequently 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. Some metal reducing bacteria have been reported to be able 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 *S. putrefaciens* make these strains electrochemically active in case they are grown under anaerobic conditions. In addition, *G. metallireducens* has also been used to transfer electrons from the cathode for nitrate reduction. In this research, *S. putrefaciens* and *G. metallireducens* were cultured using collected landfill soil samples as the inocula.

Continuous cultivation and enrichment of *S. putrefaciens* and *G. metallireducens* 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. The media had a composition of KH_2PO_4, 160 mg/l; K_2HPO_4, 420 mg/l; Na_2HPO_4, 50 mg/l; NH_4Cl, 40 mg/l; $MgSO_4 \cdot 7H_2O$, 50 mg/l; $CaCl_2$, 50 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot 4H_2O$, 0.05 mg/l; H_3BO_3, 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l; $(NH4)_6Mo_7O_{24}$, 0.03 mg/l; glucose, 200 mg/l; and ammonia chloride, 60 mg/l. 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^oC 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 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. 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 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.

3.3 *Anammox Consortia* Culturing

Anammox consortia were cultured from the inocula taken from the nitrifying sludge in the sedimentation tank of the biological nitrogen removal system in the laboratory. Wastewater collected from Smith Wastewater Treatment Facility was modified to have a composition of COD of ~ 60 mg/l, NH_4^+–N of ~ 100 mg/l, NO_2–N < 1 mg/l, NO_3^-–N < 1 mg/l and total phosphorus of 0.18 ~ 0.74 mg/l and used as the substrate. The temperature and pH of the wastewater were in the range of 14.1~24.2 ℃ and 7.65 ~ 7.79 respectively. The alkalinity was adjusted by the addition of $KHCO_3$. During the inoculation, 1 litter of regurgitant sludge with a suspended solid concentration of 4.85 g/l from the biological nitrogen removal system was inoculated into the reactor to initiate the short-cut nitrification SBR system. The dissolve oxygen (DO) concentration of the bulk liquor in the reactor was maintained at 0.15 mg/l and the ammonium concentration in the reactor was maintained at ~ 100 mg/l NH_4^+– N. After around one month's adaptation, the inocula began working functionally with a bulk liquor SS maintained at ~ 1000 mg/l and volatile suspended solid (VSS) at ~ 820 mg/l. The sludge volume and sludge volumetric index of the bulk liquor were kept at 5% and 50 ml/g respectively (Ovreas and Torsvik 1998).

3.4 Landfill Leachate Treatment in Ammonium Oxidation/MFC Reactor

Two laboratory reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/anammox reactor were set up and examined for the treatment of landfill leachate with high organic and nitrogen contents in this research. The laboratory scale continuous ammonium oxidation/MFC reactor included an in-line ammonium oxidation column, followed by a conventional MFC reactor (Figure 10).

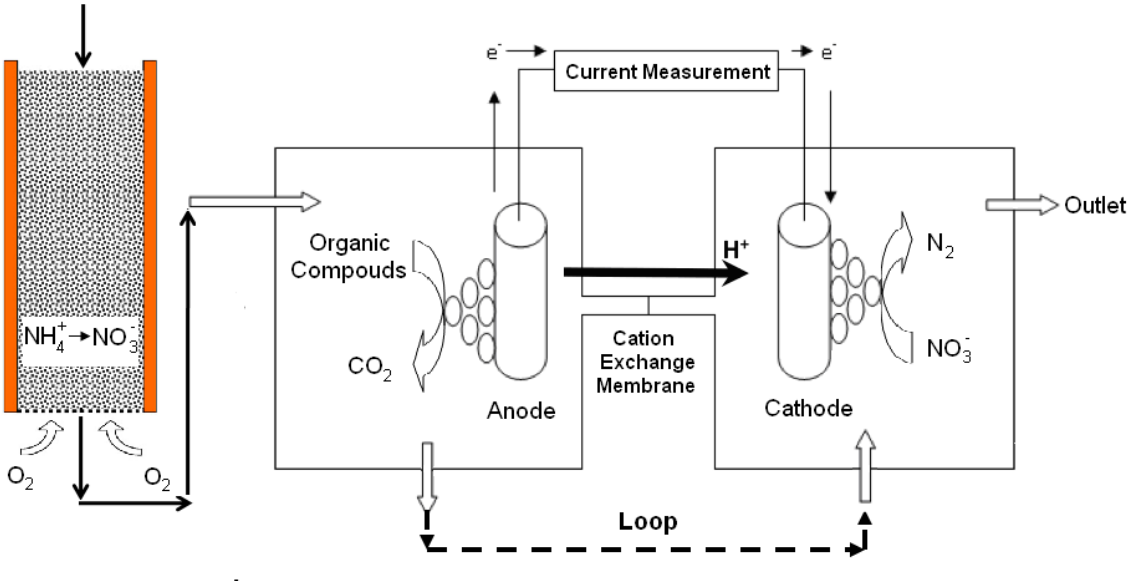

Figure 10. Ammonium Oxidation/MFC reactor Setup

In the ammonia oxidation column, an air flow up to 10 ft^3/hr (4.72 l/min) was supplied. The MFC reactor was a custom-made dual-chamber MFC. A graphite rod, without catalysts coated, was installed in the center of the inner chamber as the anode. The anode was inoculated with the cultured *S. putrefaciens*, the dominant organism in the process of iron reduction in the iron rich soil of Northwest Florida. The anodic chamber was sparged with nitrogen to remove oxygen. Carbon cloth (effective area of 12.6 cm^2, 30% wet proofing) was used as the cathode. The cathode was inoculated with *G. metallireducens*. Measurements of voltage produced during experiments were recorded directly from the potentiostat output every 60 seconds using a dual-channel voltage collection instrument (12 bit A/D conversion chips) connected with a personal computer via universal serial bus interface and calibrated with a digital multimeter (Agilent HP 34970). The measured voltage difference was converted to a current according to Ohm's law (Fu et al. 2010). Synthetic polymeric nanoporous membrane (Ultrex CMI-7000, Membranes International Inc., Glen Rock, NJ) was used as the CEM. Since ammonium was oxidized to nitrate, there was minimal chance for ammonium to pass through the CEM from the anodic chamber to the cathodic chamber. During the operation, collected landfill leachate was introduced to ammonium oxidation column for ammonium to be oxidized to nitrate, after which, the leachate was introduced to the anodic chamber for organic decomposition. The operation in the anodic chamber proceeded in the absence of oxygen and the generated carbon dioxide was trapped in the CO_2 entrapping device. Freed electrons from organic decomposition were transported to the cathodic chamber, where the treated leachate was looped in. Consequently, nitrate was reduced to nitrogen gas. Two main factors, the capability of *S. putrefaciens* to transport electrons to the anode and *G. metallireducens* to use the electrode as the direct electron donor for nitrate reduction were the key for the success of above operation.

3.5 Landfill Leachate Treatment in MFC/Anammox Reactor

A laboratory scale MFC/Anammox reactor, in which Anammox was incorporated into the cathodic chamber, was also be set up for this research (Figure 11).

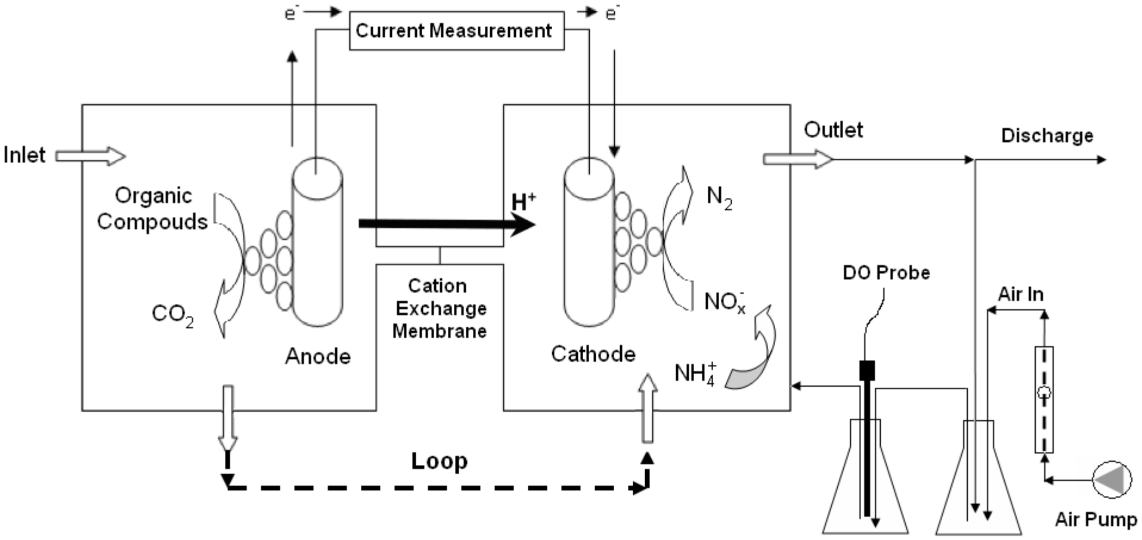

Figure 11. MFC/Anammox Reactor Setup

The design of the anodic chamber of the MFC reactor was similar to that of the ammonium oxidation/MFC reactor. The same strain of *S. putrefaciens* was used to coat the anode. For the cathodic chamber, carbon cloth (effective area of 12.6 cm^2, 30% wet proofing) was used as the cathode. The cathode was inoculated with *G. metallireducens*. The cathodic chamber was also inoculated with Anammox consortia. Again, measurements of voltage produced during experiments were recorded directly from the potentiostat output every 60 seconds using a dual-channel voltage collection instrument

(12 bit A/D conversion chips) connected with a personal computer via universal serial bus interface and calibrated with a digital multimeter (Agilent HP 34970). The measured voltage difference was converted to a current according to Ohm's law (Fu et al. 2010). Similarly, synthetic polymeric nanoporous membrane (Ultrex CMI-7000, Membranes International Inc., Glen Rock, NJ) was used as the CEM. During the operation, collected landfill leachate was introduced to the anodic chamber for organic decomposition. The operation proceeded in the absence of oxygen. The treated leachate was then introduced to the cathodic chamber, where low level oxygen was supplied. Depending on the dissolved oxygen level and pH and alkalinity conditions, ammonium may be partially oxidized to nitrite. Consequently, nitrogen was removed from the system by means of Anammox. Freed electrons from organic decomposition were transported to the cathodic chamber to further enhance the denitrification process. Since ammonium was partially oxidized in the cathodic chamber, ammonium's passing through the CEM from the anodic chamber to the cathodic chamber had minimal adversely impact on the reactor performance. In addition, the freed electrons form organic decomposition ensured that nitrate produced through over oxidation was reduced to nitrogen gas and removed. It was expected that more energy would be generated from this setup as compared with that of the ammonium oxidation/MFC reactor. The laboratory setup of the MFC/Anammox reactor is illustrated in Figure 12.

3.6 *S. putrefaciens* and *G. metallireducens* Attachment to CEM

The performance of the MFCs depended on the suitable function of the CEM. However, during the operation, *S. putrefaciens* in the anodic chamber and *G. metallireducens* in the cathodic chamber tended to aggregate on the surfaces of the CEM and interfered with the MFC performance. The following experiment was conducted to examine the attachment of *S. putrefaciens* and *G. metallireducens* on CEM. *S. putrefaciens* and *G. metallireducens* cells collected at late logarithmic physiological state (concentration predetermined by ATP assay) were centrifuged at 2500 rpm (Damon/IEC Divison, Needham Heights, MA) and washed twice with sterilized buffer solution before re-suspending in the sterilized nano-pure deionized water (NPDI) to make a bacterial suspension (~ 5×10^9 cells/ml). A sterilized 2 cm × 2 cm CEM was gently emerged into

100 ml bacterial suspension diluted to different concentrations. After agitating on a Wrist Action Shaker (Model 75, Burrell Scientific, Pittsburgh, PA) for 1 hr, the CEM was removed and the solution was measured for *S. putrefaciens* and *G. metallireducens* concentration through ATP measurements.

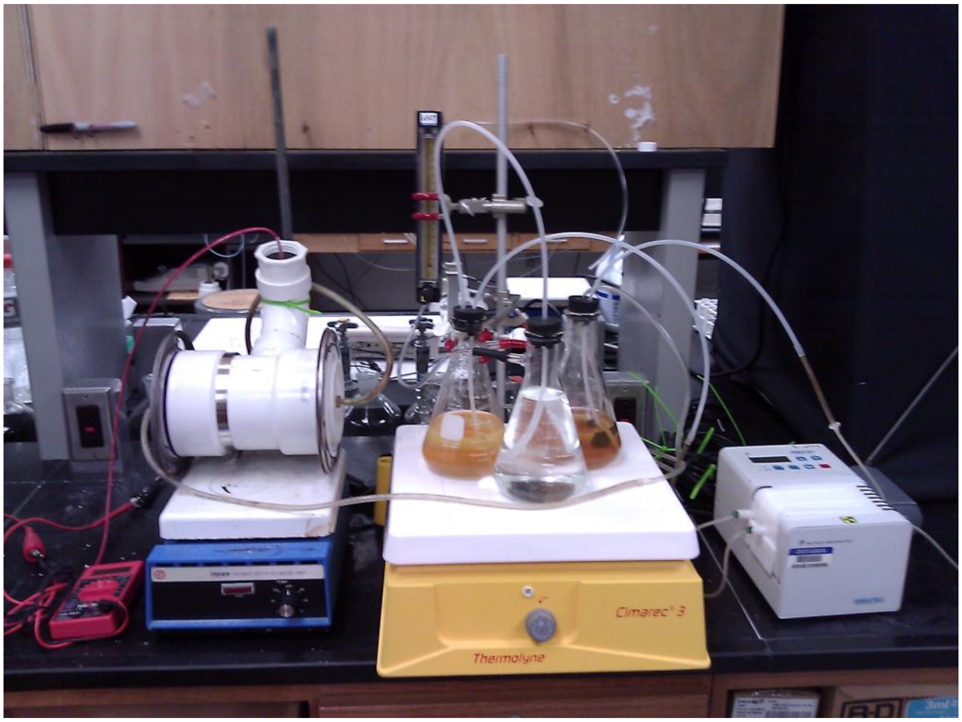

Figure 12. Laboratory MFC/Anammox Reactor Setup

3.7 *S. putrefaciens, G. metallireducens* and CEM Surface Thermodynamic Characterization

Solid surface thermodynamics can be well described by the surface tension that is defined as half of the free energy change due to cohesion of the material in *vacuo* (van Oss 1994). The surface tension of a material is contributed by a number of relatively independent forces such as dispersion, dipolar, induction, hydrogen-bonding, and metallic interactions (Fowkes 1963). According to the traditional and extended Derjaguin, Landau, Verwey, & Overbeek (DLVO) theory, the solid surface tension is mainly composed of apolar, or Lifshitz-van der Waals (LW) component; polar, or Lewis acid-base (AB) component; and electrostatic (EL) component (Besseling 1997; Marcelja and Radic 1976; Vanoss 1993).

The contact angle on a given solid surface is the most practical way to obtain the surface thermodynamic properties of a solid surface (Kwok et al. 1994). According to Kwok *et al*. (Kwok et al. 1994), van der Waals components of solid surface tensions were relatively stable when measured using dispersive liquids, which was also favored by van Oss *et al*. (van Oss 1994). After examining the experimental results of Kwok *et al*. (1994) using different polar liquids for the contact angle measurement, it seemed that the combination of glycerol and water could offer stable and reliable acid/base component estimations for solid surfaces.

S. putrefaciens and *G. metallireducens* were collected and centrifuged at 2500 RPM (Damon/IEC Divison, Needham Heights, MA) for 20 min. After washed twice with a sterilized buffer solution (potassium phosphate monobasic-sodium hydroxide buffer, Fisher Scitific, Pittsburgh, PA), they were re-suspended in sterilized nano-pure de-ionized water (NPDI, Barnstead, Dubuque, IA) at a concentration of 10^8 cells/ml to make a bacterial suspension. The *S. putrefaciens* and *G. metallireducens* suspensions were then vacuum-filtered on silver metal membrane filters (0.45 μm, Osmonic, Inc., Livermore, CA) to make bacterial lawns. *S. putrefaciens* and *G. metallireducens* surface thermodynamic properties were estimated by the contact angle measurement (Contact Angle Meter, Tantec, Schaumburg, IL) following the method described by Grasso *et al*. (Grasso et al. 1996). Bacterial lawns were air-dried for about 30 min before the contact angle measurement. The amount of cells on the silver filter was approximately 13 mg to ensure a multi-layer covering of the membrane, and moisture content of the lawn was kept in the range of 25% to 30%. An apolar liquid, diiodomethane and two polar liquids, glycerol and water were used for the contact angle measurement (Figure 13).

Each measurement was repeated 30 times and *S. putrefaciens* and *G. metallireducens* surface thermodynamic parameters were estimated by Young-Dupré equation (7) using the average results.

$$(1+\cos\beta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right) \qquad (7)$$

where γ_L, surface tension of the liquid that is used for the measurements (mJ/m^2) can be expressed as:

$$\gamma_L = \gamma_L^{LW} + 2\sqrt{\gamma_L^- \gamma_L^+} \qquad (8)$$

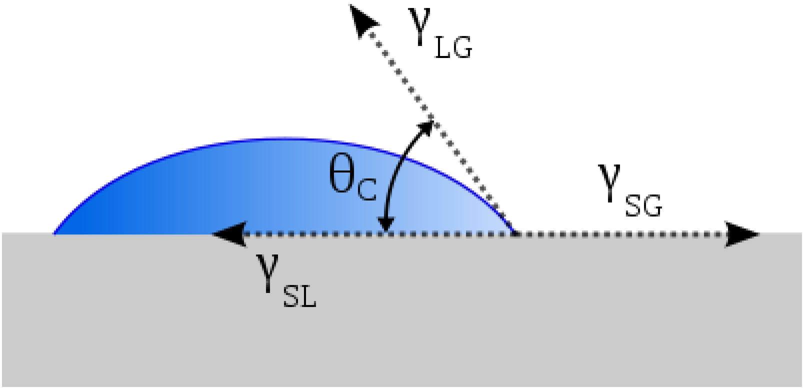

Figure 13. Measuring Liquid on Solid Surface

In above equations, γ^{LW} is the Liftshitz-van der Waals component of surface tension (subscript S for solid and L for liquid) (mJ/m^2); γ^+ is the electron-acceptor parameter and γ^- is the electron-donor parameter of Lewis acid/base component of surface tension (subscript S for solid and L for liquid) (mJ/m^2). ζ-potentials of *S. putrefaciens* and *G. metallireducens* were measured using Lazer Zee Meter (Model 501, Pen Kem, Inc.) by suspending the bacterial pellets in the electrolyte solution (10^{-5} M NaCl) after being centrifuged and washed twice with the sterilized buffer solution.

Surface thermodynamics of the CEM was directly measured using the contact angle measurement (Contact Angle Meter, Tantec, Schaumburg, IL). Similarly to *S. putrefaciens* and *G. metallireducens* measurements, an apolar liquid, diiodomethane and two polar liquids, glycerol and water were used and average readings from 30 measurements were fitted to the Young-Dupré equation to estimate its thermodynamic properties.

4. Results

4.1 *S. putrefaciens* and *G. metallireducens* Culturing

The MFCs in this research relied on *S. putrefaciens* to transfer electrons to the anode and *G. metallireducens* to release electrons from the cathode, which are commonly found in the sediments, especially in the iron rich Northwest Florida subsurface soil. The electrochemically active *S. putrefaciens* and *G. metallireducens* were cultured in this research using the collected soil samples from Leon County Landfill as the inocula. After continuous cultivation and enrichment, *S. putrefaciens* and *G. metallireducens* cells were harvested by centrifugation at 6000 $\times g$ for 15 min. They were then 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. *S. putrefaciens* and *G. metallireducens* were identified by polymerase chain reaction (PCR) analysis (Figure 14 and Figure 15).

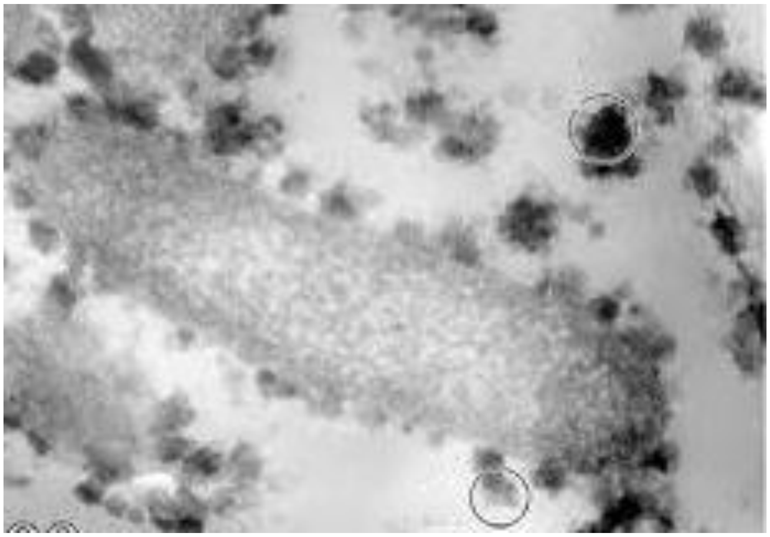

Figure 14. Image of *S. oneidensis*

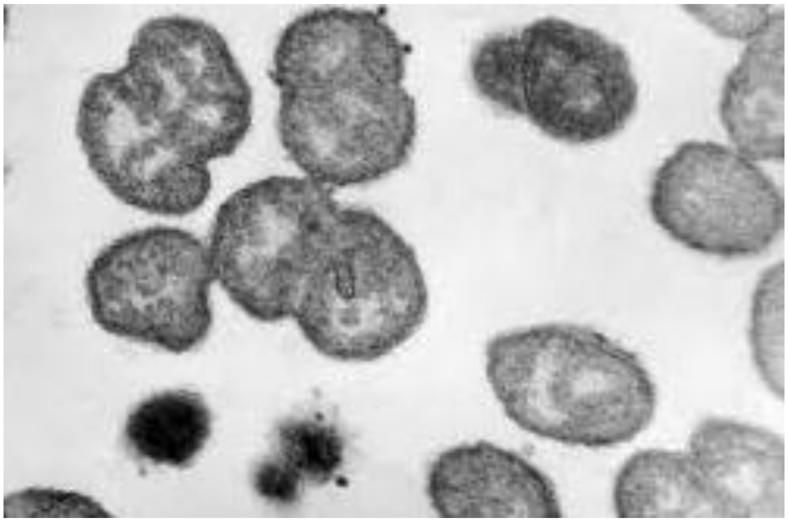

Figure 15. Image of *G. metallireducens*

PCR amplified specific regions of DNA in the microorganism's genome by selectively catalyzing the replication of those regions. Upon verification of the PCR reaction by viewing the gel bands, the PCR samples were purified using a QIAGEN QIAquick-spin PCR purification kit. After the purification, the samples were amplified and the resulted sequences were compared with the database of the National Center for Biotechnology Information (NCBI) based on the strands that have been previously identified, which were done at Florida State University DNA Sequencing Laboratory. The top strains whose DNA codes matched the codes of *S. putrefaciens* and *G. metallireducens* with the highest certainty were selected. Once *S. putrefaciens* and *G. metallireducens* were screened out, they were enriched in 100 ml fresh culturing media.

4.2 Anammox Consortia

For Anammox to occur, ammonium needs to be partially oxidized to nitrite, which requires reducing the activity of the nitrite oxidizing bacteria (NOB), without affecting the ammonia oxidizing microorganisms (AOB). This was achieved in several ways. First, owing to the difference of the activation energies between ammonium oxidation (68 kJ/mol) and nitrite oxidation (44 kJ/mol), increasing the temperature would favor ammonium oxidation. Secondly, at low DO concentrations, ammonium oxidizing bacteria had a higher affinity for oxygen than nitrite oxidizing bacteria. Accordingly, partial nitrification could also be achieved by maintaining low DO levels. In addition, high pH favored ammonium oxidizers rather than the nitrite oxidizers (Jetten et al. 1998). For this research, throughout the course of consortia culturing, the sludge remained in stable conditions. At the end of the culturing, the amounts of the AOB and NOB in the mixed liquid suspended sludge were assayed using the MPN method, which were 0.95×10^6 cell per ml and 2.5×10^4 cell per ml respectively with an AOB/NOB ratio of 38 (Table 3). Therefore, ammonium oxidation dominated over nitrite oxidation during the partial nitrification process.

Table 3. The Amount of AOB and NOB Resulted from MPN Method

Microbial Species	Items	Results								
AOB	Dilution levels	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	Tube amount	3	3	3	3	3	3	3	3	3
	Positive tubes	3	3	3	3	3	2	0	0	0
	Biomass	9.5×10^5 cell per ml								
NOB	Dilution levels	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	Tube amount	3	3	3	3	3	3	3	3	3
	Positive tubes	3	3	3	0	0	0	0	0	0
	Biomass	2.5×10^4 cell per ml								

Typically, aerobic AOB, such as *Nitrosospira* and *Nitrosomonas* are able to oxidize ammonium to nitrite, and aerobic NOB, such as *Nitrospira* and *Nitrobacter* are able to oxidize the nitrite further to nitrate (Mota et al. 2005). NOB are more sensitive to the detrimental environmental conditions than AOB. The most important environmental parameters to obtain partial nitrification are ammonium and nitrous acid concentrations, temperature, pH and dissolved oxygen concentration (Xia et al. 2010). A temperature rise would cause two problems: increased ammonium inhibition and increased activity of the organisms. Temperature above 25℃ leads to an increase of the specific growth rate of

AOB and NOB. In this research, it was discovered that the optimal temperature was 35℃ for AOB and 38℃ for NOB.

Anammox refers to the process during which ammonium is converted to dinitrogen under anaerobic conditions with nitrite serving as the electron acceptor (Caffaz et al. 2006; vandeGraaf et al. 1997). Being strictly anaerobic, Anammox can only proceed in the absence of oxygen and phosphates (Strous et al. 1999a). Anammox presents significant potential for efficient nitrogen cycling of ammonium-rich wastewater since Anammox is a microbiologically mediated exergonic process. The dominating microbial species that are responsible for the Anammox process include *Brocadia anammoxidans* and *Kuenenia stuttgartiensis* as well as several species of *Scalindua* (Strous et al. 1999a; Vandegraaf et al. 1995). The most important environmental parameters that impact Anammox processes include substrate and product inhibition, phosphate and sulfide concentrations, DO, temperature, pH, biomass concentration and suspended solid concentration (Kartal et al. 2010). Specifically, nitrite concentration is an important parameter since it inhibits the Anammox activity. This inhibition can be restored by adding trace amounts of the Anammox intermediates such as hydroxylamine (NH_2OH) and hydrazine (N_2H_4), even after long-term exposure to high concentrations of nitrite. However, different Anammox genera show different tolerance for nitrite. Furthermore, increasing the nitrite concentration would change the stoichiometry of ammonium and nitrite consumption from 1.3 g nitrite/g ammonium at 0.14 g-N/l to almost 4 g nitrite/g ammonium at 0.7 g-N/l (Strous et al. 1999b). So at high nitrite concentrations, the microorganisms did not only use ammonium as the electron donor but also must have generated an internal electron donor to reduce the nitrite. The Anammox process is not inhibited by ammonium or nitrate up to concentrations of at least 1 g-N/l (Strous et al. 1999b). In addition, the bicarbonate concentration is also an important factor to affect the Anammox enrichment. Low bicarbonate/ammonium ratio might lead to low Anammox activity owing to the lack of CO_2. But a too high bicarbonate/ammonium ratio might also lead to inhibition of Anammox due to the formation of a high amount of free ammonia. The image of the cultured Anammox consortia is illustrated in Figure 16.

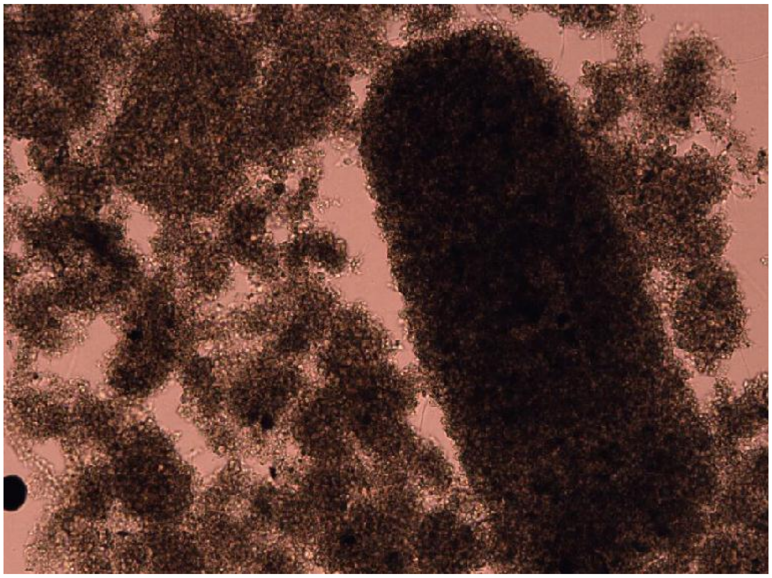

Figure 16. Image of Anammox Consortia

4.3 Landfill Leachate Treatment in Ammonium Oxidation/MFC Reactor

4.3.1 Ammonium Oxidation

Ammonium oxidation was a function of dissolved oxygen (DO). In this research, variable DO concentrations from 2.0 mg/l to 8.5 mg/l were achieved in the ammonium oxidation reactor. As shown in Figure 17, at DO above 4.0 mg/l, most of the ammonium can be oxidized within 10 hours. To ensure the ammonium in the landfill leachate was completely oxidized to nitrate before the landfill leachate was introduced to the MFC, a DO level of 7.0 mg/l was selected for this research. At this DO level, nearly all of the ammonium in the landfill leachate was oxidized to nitrate. By monitoring the nitrate concentration during ammonium oxidation, it was observed that nitrate production increased accordingly with the depletion of ammonium (Figure 18). Minimal nitrite was observed in the reactor, which was confirmed by the mass balance calculation of ammonium depletion and nitrate production. Sufficient ammonium oxidation also ensured that nitrogen could be effectively removed in the cathodic chamber.

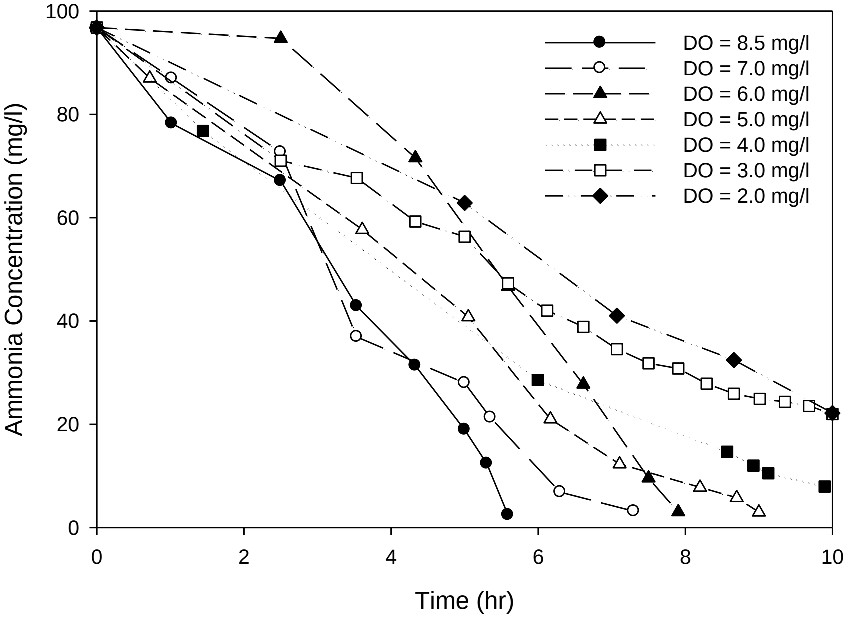

Figure 17. Ammonia Depletion as a Function of Time

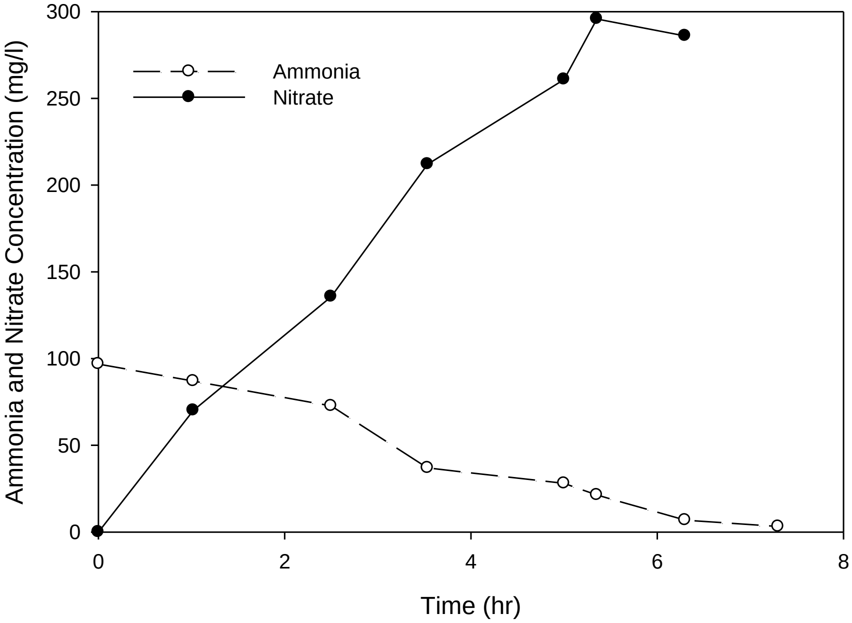

Figure 18. Ammonia Depletion and Nitrate Production as a Function of Time

In the anodic chamber, organic substrates were oxidized by *S. putrefaciens* to produce carbon dioxide, protons and electrons in the absence of oxygen. Aeration in the ammonium oxidation column thus might interfere with the organic decomposition and electron release in the anodic chamber. For this research, in order to achieve a complete ammonium oxidation and reduce the duration of the ammonium oxidation process, DO level of 7.0 mg/l was selected, which might lead to remains of DO in the landfill leachate. This DO level ensured that nearly all ammonium was oxidized to nitrate with minimal nitrite produced, which would compete with nitrate for electrons in the cathodic chamber during MFC operations. To remove the remaining DO and eliminate its impact on the MFC performance, landfill leachate was degased with helium after ammonium oxidation to remove the excess of DO as well as carbon dioxide in a capped container before it was introduced to the MFC reactor. Resazurin (1 mg/l) was used as a redox indicator and no observable oxygen was detected after the degasing process.

4.3.2 Power Generation and Nitrogen Removal

In the ammonium oxidation/MFC reactor, glucose and landfill leachate collected from Leon County Landfill was continuously supplied and uninterrupted current was produced (Figure 19). The input glucose was 250 mg/l (~ 266 mg/l COD). The landfill leachate was diluted to a BOD_5 value ~ 250 mg/l and total nitrogen of ~ 120 mg/l. The power generation was lower as compared with that of oxygen serving as the electron acceptor (Subramaniam et al. 2012). When glucose was used as the carbon source, the ammonium oxidation/MFC reactor generated half of the power produced by that of oxygen in our previous systems which was designed for organic decomposition only (~ 25 mW/m^2 versus ~50 mW/m^2). Less than 10 mW/m^2 of power was generated when landfill leachate collected from Leon County Landfill was used. Ammonia removal was obvious for the ammonium oxidation/MFC reactor. With an input total N of ~ 120 mg/l, above 92% of nitrogen was removed with an effluent N concentration below 9 mg/l (Figure 20).

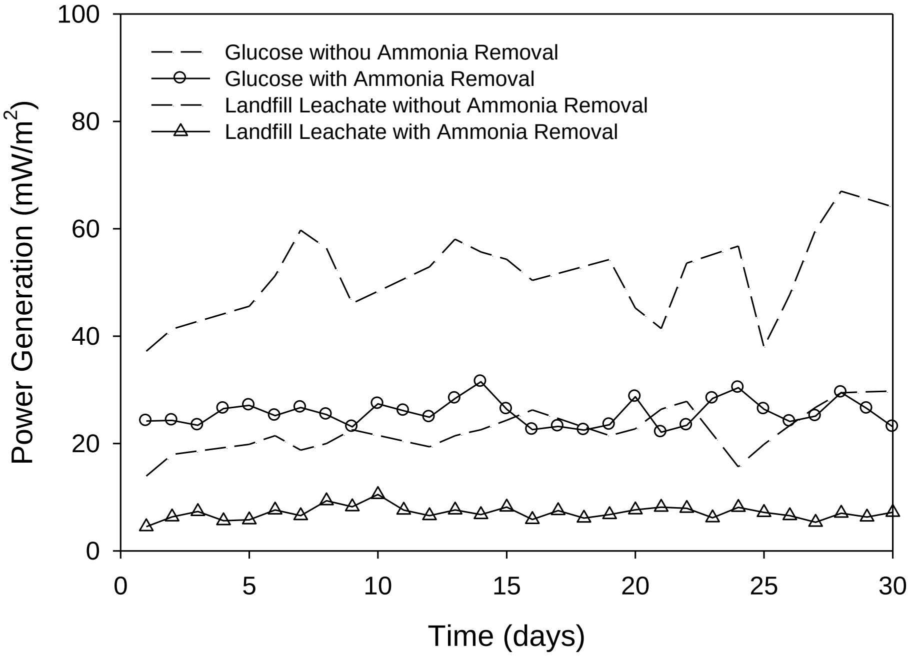

Figure 19. Power Generation of the Ammonium Oxidation/MFC Reactor

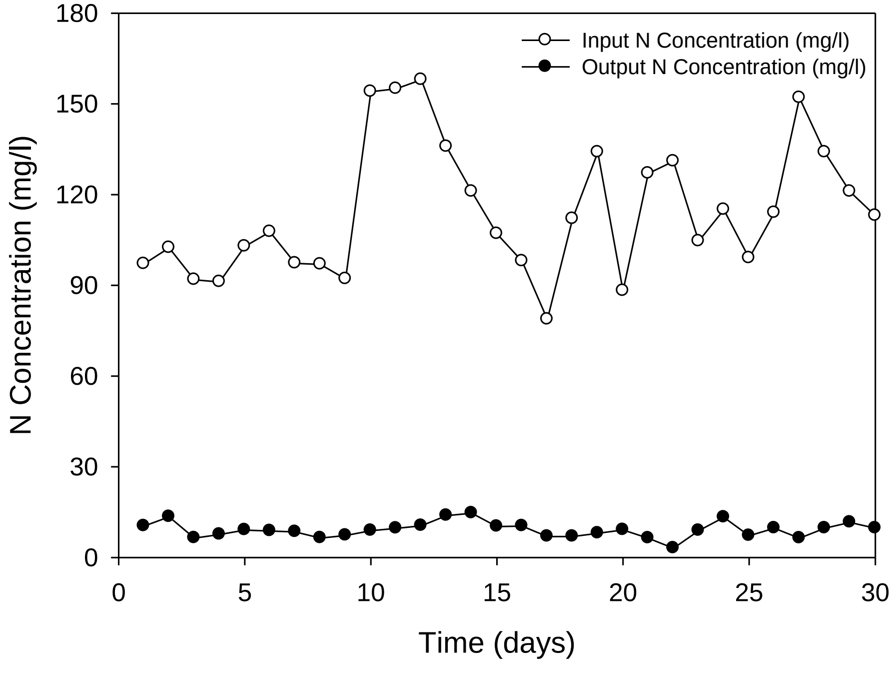

Figure 20. Nitrogen Removal of the Ammonium Oxidation/MFC Reactor

It would be beneficial if energy can also be generated during ammonium oxidation in the same way that such systems have been employed to produce electric power from the oxidation of various organic. However, to date, the production of electricity from ammonium oxidation in MFCs has not been reported. In one study, high levels of ammonium were removed in a MFC used to treat swine wastewater, but further investigation concluded that ammonium was not a substrate for electricity generation, and its removal was largely due to either ammonium volatilization in an air-cathode MFC or ammonium ion diffusion from the anode to the cathode in a two-chambered MFC (Kim et al. 2008; Min et al. 2005). Theoretically, ammonium may contribute to electricity generation in MFCs by two ways. First, ammonium may function as an anodic fuel as ammonium-N is at its lowest oxidation state and electron can be released through ammonium oxidation. Under aerobic conditions, ammonium oxidation has a Gibbs free energy of -275 kJ/mol; under anaerobic conditions, ammonium oxidation has a Gibbs free energy of -357 kJ/mol (Jetten et al. 2001). Consequently, it is theoretically possible for electric energy to be generated in MFCs with ammonium serving as an electron donor and nitrite/nitrate or oxygen as an electron acceptor. Secondly, ammonium may be utilized by nitrifying bacteria to produce organic compounds that are used by heterotrophs to generate electricity. It is known that autotrophic nitrifying bacteria can support heterotrophic growth by producing soluble microbial products (Kindaichi et al. 2004). Although theoretically electricity generation through ammonium oxidation is possible, the power generation should be much smaller compared with that of organic substrate. Therefore, it is not practical for MFC operations with ammonium oxidation serving as the energy source due to the high organic contents of landfill leahcate.

4.4 Landfill Leachate Treatment in MFC/Anammox Reactor

Similar to the ammonium oxidation/MFC reactor, uninterrupted current was produced in the MFC/Anammox reactor with continuous carbon source supply (Figure 21). For comparison purposes, in the MFC/Anammox reactor, glucose (250 mg/l or ~ 266 mg/l COD) and landfill leachate collected from Leon County Landfill (diluted to a BOD_5 value ~ 250 mg/l and total nitrogen of ~ 120 mg/l) were used as the carbon sources.

Compared to the ammonium oxidation/MFC reactor, the power generation from the MFC/Anammox reactor was a little bit higher for both glucose and landfill leachate.

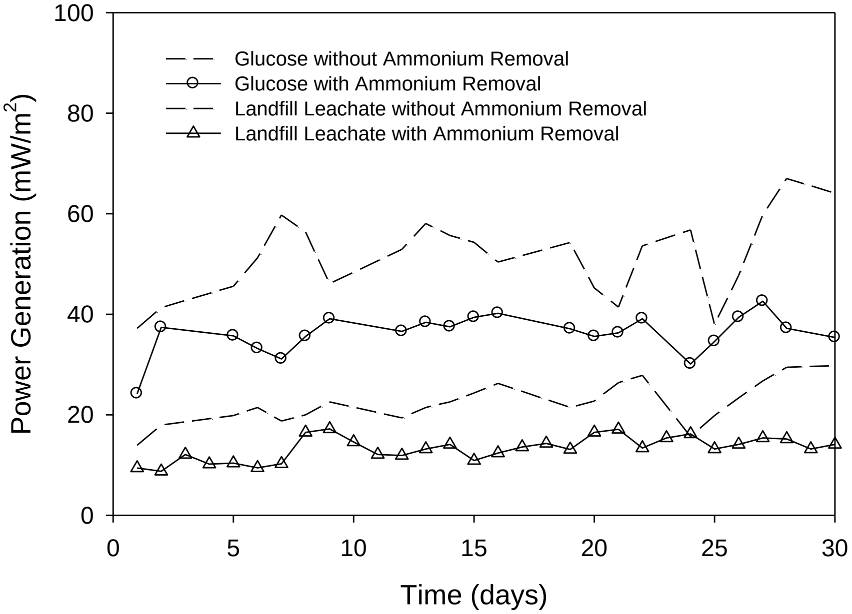

Figure 21. Power Generation of the MFC/Anammox Reactor

For the ANAMMOX process, only 50% of the ammonium needs to be converted to nitrite:

$$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O \qquad (9)$$

This reaction stoichiometry implies that no extra addition of base is required, since the landfill leachate generally contains enough alkalinity (in the form of bicarbonate) to compensate for the acid production if only 50% of the ammonium is oxidized (Zhang et al. 2007). The possibility to produce a 50:50 mixture of ammonium and nitrite has been evaluated extensively. The next step of ANAMMOX is the conversion of nitrite to dinitrogen gas with ammonium serving as the electron donor under anoxic conditions:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O \qquad (10)$$

The Anammox consortia that catalyze the ANAMMOX reaction are autotrophic, meaning that nitrite can be converted to dinitrogen gas without the use of organic compounds or the addition of external methanol. Partial nitrification in the cathodic chamber was to produce nitrite, which served as electron acceptor during the

MFC/Anammox reactor operation. If excess of oxygen was supplied, ammonium would be oxidized to nitrate instead of nitrite. Since nitrate could retrieve less energy than that of nitrite, less power could be generated. This will be discussed in detail in the Discussion section. Certainly, excess of oxygen supply would cost more energy.

Ammonia removal was obvious for MFC/Anammox reactor. Similar to the ammonium oxidation/MFC reactor, around 94% of nitrogen was removed with an effluent N concentration around 7.5 mg/l (Figure 22).

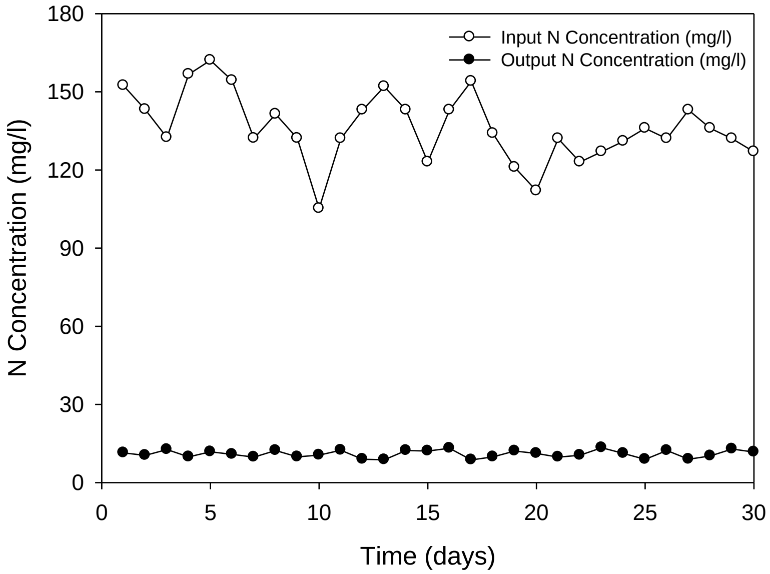

Figure 22. Nitrogen Removal of the MFC/Anammox Reactor

4.5 Impact of pH on Power Generation

For both the ammonium oxidation/MFC reactor and the MFC/Anammox reactor, pH had impact on the power generation. For both reactors, treatment of landfill leachate collected from Leon County was conducted at pH 6, 7 and 8 respectively. The impact of pH on power generation was illustrated in Figure 23 and Figure 24. High pH (i.e., pH 8) generated more power as compared to low pH (i.e., pH 6) for both the ammonium oxidation/MFC reactor (Figure 23) and the MFC/Anammox reactor (Figure 24). It should be noted that the pH control was achieved at the anodic chamber where organic compounds (glucose or landfill leachate) were decomposed. According to the following equation, raising the pH should favor electron release:

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

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

$$1/4O_2 + H^+ + e^- \rightarrow 1/2H_2O \qquad (12)$$

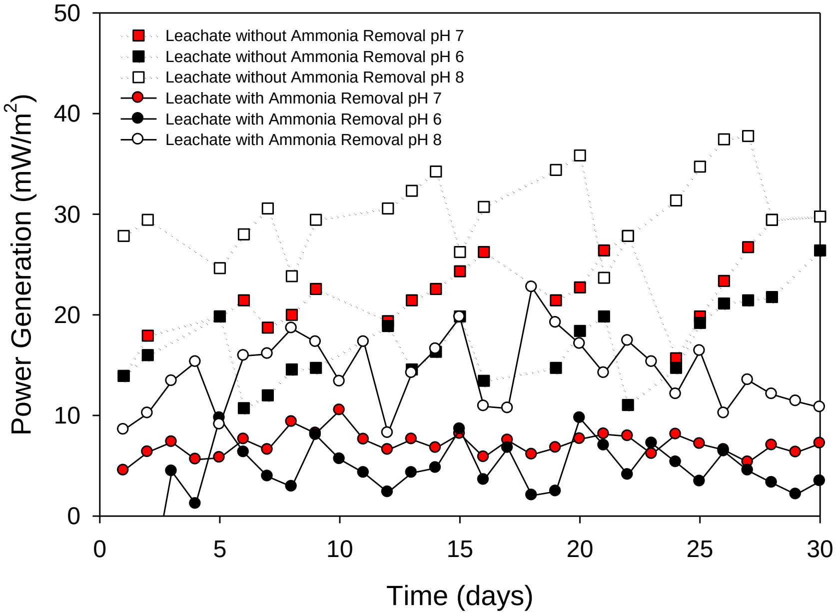

Figure 23. Impact of pH on Ammonium Oxidation/MFC Reactor Performance

Since the cathodic chamber is totally separated from the anodic chamber, for above experiments, pH variations were only examined at the anodic chamber. The pH of landfill leachate ranges from 3 to 10. However, the typical values are usually in the range of 6 to 8. Consequently, typical pH ranges of the soils nearby the landfills, i.e., pH 6 to pH 8 were investigated. Based on this research, there is a general trend that high pH favors the MFC performances since organic decomposition consumes alkalinity.

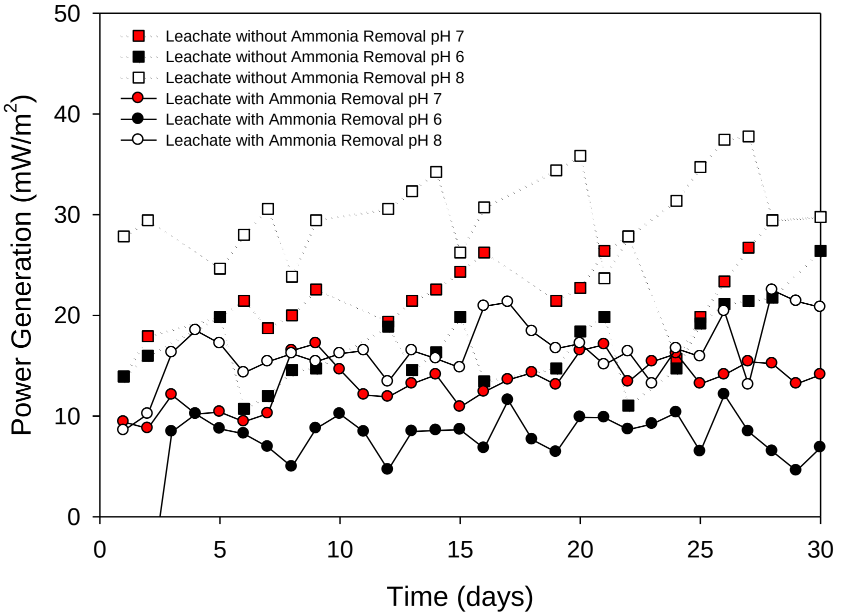

Figure 24. Impact of pH on MFC/Anammox Reactor Performance

4.6 Pulse Input and Power Generation

Factors that limit electricity generation in MFCs include organic compound oxidation at the anode, electron transfer from the microorganisms to the anode, presence of electrochemically active redox enzymes, external resistance of the circuit, proton transfer through the membrane to the cathode, and electron consumption at the cathode. Among these 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). Through the “loop-in” path, incompletely treated landfill leachate might get to the cathodic chamber, which would affect the MFC performance. To address this issue, pulse input of landfill leachate was tested in both the ammonium oxidation/MFC reactor and the MFC/Anammox reactor. For this part of experiments, the reactors were operated under static conditions until the power generation becomes low. The landfill leachate was then discharged and fresh leachate was introduced. During the static operation, organic components in the landfill leachate were fully utilized before fresh leachate was introduced. Therefore, more electricity was generated (Figure 25 and Figure 26).

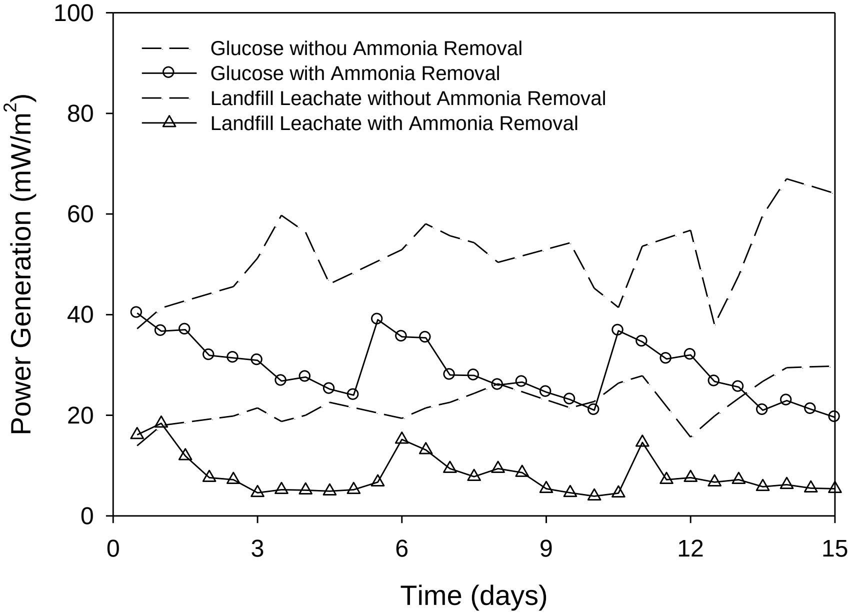

Figure 25. Power Generation of Pulse Input for Ammonium Oxidation/MFC Reactor

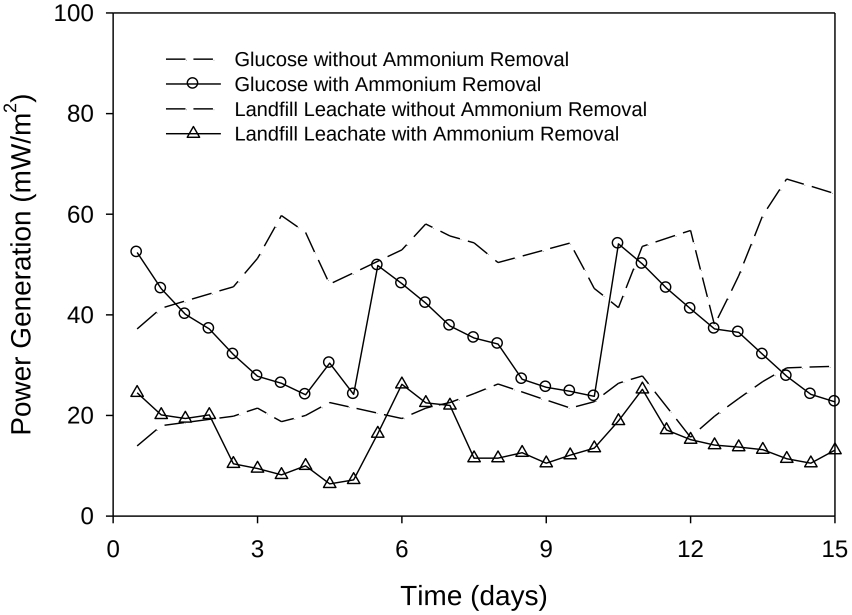

Figure 26. Power Generation of Pulse Input for MFC/Anammox Reactor

However, pulse input results in longer residence time. Residence time or static operation time is the mixing characteristics in the reactors. The residence time can be used to guide the continuous flow MFC reactor operations. It can also be used as simple tool to analyze flow property to develop a flow mathematical model, and to predict the performance of the MFC reactors. Flow characterization using the residence time test has been extensively studied for MFC reactors, although the analysis of non-ideal flow in reactors is often neglected or not considered properly in wastewater treatment processes (Dekker et al. 2009; Moon et al. 2005).

4.7 *S. putrefaciens* and *G. metallireducens* Attachment to CEM

Microbial biofouling, i.e., immobilized microbial cell colonization on abiotic surfaces, is important in a variety of applications (Herzberg et al.; Ramesh et al. 2007). The attachment of suspended microbial cells to a solid–liquid interface is the first step in microbial biofouling. Zobell and Allen (Zobell and Allen 1935) first recognized the role of microbial attachment in microbial fouling and Zobell (Zobell 1943) later found that microbial attachment was a time-dependent process, which could be enhanced by the formation of microbial slime as surface contact time increased. Marshall et al. (Marshall et al. 1971) described the initial microbial attachment to solid surfaces as a two-step process: Microbial adhesion begins with long-range, non-specific, reversible interactions between microorganisms and substrates, which is unstable and adsorbed microorganisms at this stage can be removed from surfaces by fluid shear before firm adhesion can occur. These long-range interactions are dependent on the physicochemical properties of microbial and substratum surface as well as on the intervening medium (Chen and Strevett 2001). Once microorganisms are in close proximity to a surface, they can establish short-range, irreversible interactions, which are also dependent on the physicochemical properties of microbial and substratum surface as well as on the intervening medium. These two processes together refer to initial adhesion (Gottenbos et al. 1999; Gottenbos et al. 2000). When microorganisms stabilize on a surface, they may slow down their metabolism and grow slowly as microcolonies, starting to secrete an exopolysaccharide matrix in order to cement themselves to the surface, which is a time-dependent biological process (Costerton et al. 1995; Fletcher 1994). This slimy layer of microorganisms embedded in a polysaccharide matrix is known as biofilm. All of the above stages depend on microbial physiological states. Many microbial species have evolved sophisticated mechanisms to adapt to variations in energy and nutrient availability, resulting in favorable surface physicochemical properties for biofouling (Chen and Strevett 2001).

In this research, attachment of dissimilatory Fe(III) reducing bacteria such as *S. putrefaciens* and *G. metallireducens* was investigated. In our prior research, we have demonstrated that the physiological stage of the microorganisms affected their attachment to solid surfaces (Chen and Strevett 2003). We have also proved that microorganisms at the late logarithmic growth stage had the most potential to attach. For this research, both *S. putrefaciens* and *G. metallireducens* were maintained at the late logarithmic growth stage and were able to utilize their specialized flagella to relocate and attach to the CEM in the solution. As shown in Figure 27, *S. putrefaciens* and *G. metallireducens* attachment to CEM followed a linear isotherm. Compared to *G. metallireducens*, more *S. putrefaciens* attached to the CEM. *S. putrefaciens* and *G. metallireducens* are motile bacteria that possess the ability to bias their random movement toward favorable living environment. The biased motion in response to environmental cues is accomplished through adjustment in their tumbling frequency (Ford and Lauffenburger 1991; Ford et al. 1991). When swimming in an upstream direction of increasing attractants or decreasing repellents, *S. putrefaciens* and *G. metallireducens* tumble less frequently (and thus swim longer) than when they swim in a downstream direction. Such biased random motion results in a net migration of the bacterial random trajectories toward attractants or away from repellents, a phenomenon termed “chemotaxis”. It has been recently shown that *S. putrefaciens* and *G. metallireducens* specifically expresses flagella and chemotactic motility under conditions variable electron acceptor (Childers et al. 2002).

With *S. putrefaciens* and *G. metallireducens* attachment to the CEM, MFC performances would be affected. *S. putrefaciens* could only transfer electrons to the anode and *G. metallireducens* could only release the electrons from the cathode when they attached to the anode and cathode. However, when they attached to the CEM, the electrons could not be transported efficiently, which affected the MFC performances.

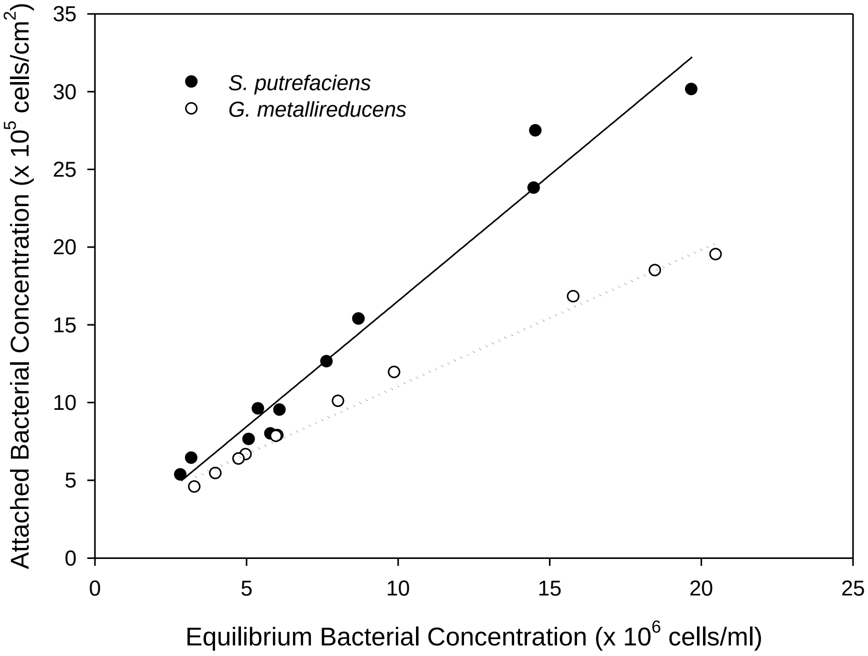

Figure 27. *S. putrefaciens* and *G. metallireducens* Attachment to CEM

4.8 Ammonium Diffusion through *S. putrefaciens* and *G. metallireducens*-Coated CEM

During the MFC operations, ammonium is able to pass through the CEM and get to the cathodic chamber from the anodic chamber. In this research, the diffusion of ammonium through the CEM with and without *S. putrefaciens* and *G. metallireducens* attachment was investigated. Electric forces were the driving force for this diffusion process. In addition to driving the migration of ions, electric forces could also induce an electroosmotic flow of the solution (Hsu et al. 2012). It is clear that the diffusion and migration of ammonium through the CEM are highly coupled processes and depend largely on the charge and diffusivity of NH_4^+ as well as the physical characteristics of the medium of CEM.

An equation for the flux of ammonium through the CEM separating the anodic chamber and the cathodic chamber can be derived based on several assumptions regarding the physical nature of the CEM (Karve and Dutt 2011; Miyake et al. 2011; Okada et al. 1996; Rigaud and Garybobo 1977):

$$(\frac{C_c^* - C_\infty}{C_A^* - C_\infty^0}) = \exp(-D_{eff} t) \qquad (13)$$

where C_c^* is the ammonium concentration outside the CEM in the cathodic chamber in the solution (mg/l); C_A^* is the ammonium concentration outside the CEM in the anodic chamber in the solution (mg/l); C_∞ is the ammonium concentration in the cathodic chamber in the solution (mg/l); C_∞^0 is the initial ammonium concentration in the anodic chamber in the solution (mg/l); t is the time; and D_{eff} is the effective diffusion coefficient (cm^2/min). The effective diffusion coefficient is the key parameter that describes the combined diffusion of ammonium through the CEM.

Ammonium passing through the CEM is an instantaneous process. To estimate the ammonium effective diffusion coefficient, it was assumed that ammonium was homogenously distributed over the CEM. All the parameters needed in equation 13, except the effective diffusion coefficient D_{eff}, were experimentally determined in the experiments. The ammonium concentrations from two chambers of two sides of the CEM were determined and used as input for the modeling. The only parameter fitted was the effective diffusion coefficient D_{eff}. The analysis of the diffusion problem with equation 13 indicated that the characteristic time scale for the diffusion was in the order of minutes; i.e., the diffusion process was completed within about 40 min. The estimated D_{eff} of ammonium though the CEM was 6×10^{-9} cm^2/min. This suggested that the CEM indeed consisted of a porous structure. The modeling results also indicated that the diffusion of ammonium was likely a solid-state diffusion, because the estimated diffusion coefficient (6×10^{-9} cm^2/min) was five to six orders of magnitude smaller than the diffusion coefficient of water molecules ($D = 1.2 \times 10^{-3}$ cm^2/min). The ammonium concentration outside the CEM in the cathodic chamber as a function of time is illustrated in Figure 28.

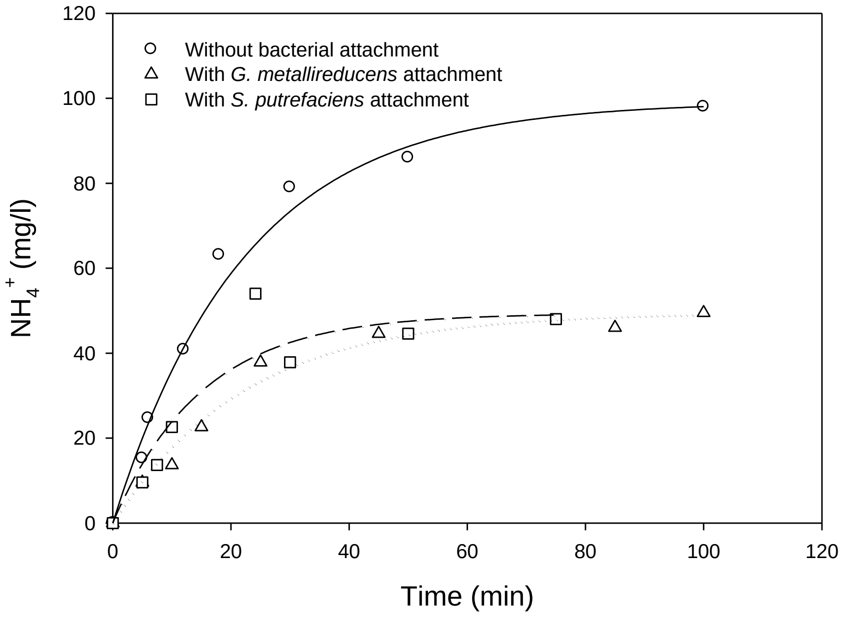

Figure 28. Ammonium Diffusion through CEM

With *S. putrefaciens* and *G. metallireducens* attached to the CEM, ammonium diffusion decreased accordingly (diffusion coefficient decreased to ~ 10^{-9} cm^2/min). Ammonium diffusion from the anodic chamber to the cathodic chamber only occurred in the MFC/Anammox reactor. Since ammonium would be looped into the cathodoc chamber even if it did not diffuse to the cathodic chamber through the CEM. Therefore, although *S. putrefaciens* and *G. metallireducens* attachment had impact on ammonium diffusion, it had minimal impact on the MFC/Anammox reactor performance.

4.9 *S. putrefaciens* and *G. metallireducens* Surface Properties and Interactions

S. putrefaciens, *G. metallireducens* and the CEM surface thermodynamic properties were calculated according to equation (7) based on their contact angles measured with diiodomethane, formamide and water (Table 4). γ^{LW} was found to be 42.6 mJ/m^2 and 45.2 mJ/m^2 for *S. putrefaciens* and *G. metallireducens*, which are in consistence with van Oss's prediction that γ^{LW} typically equals to 40 mJ/m^2 with minor variability for a considerable number of bacterial strains (van Oss 1994) (Table 5). *S. putrefaciens* and *G.*

metallireducens exhibited a monopolar surface, i.e., the γ^- was at least one order in magnitude greater than γ^+ (21.2 mJ/m^2 as compared to 0.81 mJ/m^2 for *S. putrefaciens* and 21.5 mJ/m^2 as compared to 1.94 mJ/m^2 for *G. metallireducens* (van Oss 1994). The CEM had a γ^{LW} value of 20.4 mJ/m^2. Similar to *S. putrefaciens* and *G. metallireducens,* the CEM also exhibited a monopolar surface (12.7 mJ/m^2 as compared to 1.06 mJ/m^2).

Table 4. Contact Angles of *S. putrefaciens*, *G. metallireducens* and CEM

	θ^{DII} (^{o})	θ^F (^{o})	θ^W (^{o})
S. putrefaciens	37.0 ± 0.5	26.0 ± 0.4	50.6 ± 0.5
G. metallireducens	39.7 ± 0.3	7.50 ± 0.3	45.7 ± 0.6
CEM	79.2 ± 1.0	67.6 ± 0.7	77.5 ± 1.1

^{Dii} Contact angles measured with diiodomethane.
^{F} Contact angles measured with formamide
^{W} Contact angles measured with water.

Table 5. Surface Properties of *S. putrefaciens*, *G. metallireducens* and CEM

	γ^{LW} (mJ/m^2)	γ^+ (mJ/m^2)	γ^- (mJ/m^2)
S. putrefaciens	42.6	0.81	21.2
G. metallireducens	45.2	1.94	21.5
CEM	20.4	1.06	12.7

5. Discussion

5.1 Landfill Organic Composition and Energy Generation

The high energy and nutrient removal requirements of landfill leachate treatment are warrant for alternative treatment technologies which require less energy for the efficient removal of organic and nutrient components during the treatment operations (Depountis et al. 2009; Fang et al. 2010). In the past two decades, high rate anaerobic processes are finding increasing applications for the treatment of landfill leachate, during which energy can be recovered in the form of methane gas. However, the utilization of methane is not very successful (Iza et al. 1992). When treating small quantities of low strength landfill leachte, the generated methane is usually flared. In addition, due to global environmental concerns, there is emergent interest in finding treatment technologies that can also remove nutrients during the landfill leachate treatment.

The proposed ammonium oxidation/MFC reactor and MFC/Anammox reactor can achieve power generation, organic compound decomposition and nitrogen removal from landfill leachate simultaneously. Using these two technologies, MFCs are capable of providing clean energy, apart from the effective treatment of landfill leachate. However, the commercialization of MFC applications has been halted due to the low power output, which is mainly due to the sluggish kinetics of organic compound oxidation and electron transfer from the microorganisms to the anode and release from the cathode to the electron acceptors (Erable et al. 2009). Among above factors, the most important one is the organic compound oxidation, which is a function of the 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. As shown in Table 6, glucose can release more energy than other organic compounds such as acetate, ethanol, lactate, etc. Since landfill leachate is a combination of variable compounds, the energy release would be different once they are applied in MFCs.

Table 6. Gibbs Free Energy of Organic Compound Half Reactions

Reactions for Organic Compounds	ΔG^0 (kCal/e^- eq)
Acetate: 1/8 CH_3COO^- + 3/8 H_2O = 1/8 CO_2 + 1/8 HCO_3^- + H^+ + e^-	-6.609
Ethanol: 1/12 CH_3CH_2OH + 1/4 H_2O = 1/6 CO_2 + H^+ + e^-	-7.592
Methanol: 1/6 CH_3OH + 1/6 H_2O = 1/6 CO_2 + H^+ + e^-	-8.965
Glucose: 1/24 $C_6H_{12}O_6$ + 1/4 H_2O = 1/4 CO_2 + H+ e-	-10.0
Lactate: 1/12 $CH_3CHOHCOO^-$ + 1/3 H_2O = 1/6 CO_2 + 1/12 HCO_3^- + H^+ + e^-	-7.873
Propionate: 1/14 $CH_3CH_2COO^-$ + 5/14 H_2O = 1/7 CO_2 + 1/14 HCO_3^- + H^+ + e^-	-6.664

Besides the organic compound oxidation, the energy production is also limited by the energy potential by the electron acceptors. The energy potential for different electron acceptors is listed in Table 7. As shown in Table 7, oxygen, nitrate and nitrite can retrieve 18.675 kcal, 17.128 kcal and 22.304 kcal of energy per electron receiving, respectively.

Table 7. Gibbs Free Energy of Electron Acceptor Half Reactions

Reactions for Electron Acceptors	ΔG^0 (kcal/e^- eq)
¼ O_2 + H^+ + e^- = ½ H_2O	18.675
1/5 NO_3^- + 6/5 H^+ + e^- = 1/10 N_2 + 3/5 H_2O	17.128
1/3 NO_2^{2-} + 4/3 H+ + e- = 1/6 N_2 + 2/3 H_2O	22.304

The energy release by coupling the organic compound oxidation and electron acceptation is summarized in Table 8. Around 6% more energy is released when oxygen is used as electron acceptor as compared with that of nitrate. The difference was not significant and was consistent with the redox potential values (+0.74 V for NO_3^-/N_2 as compared with +0.82 V for O_2/H_2O at pH 7). Therefore, nitrate is competitive to oxygen as an electron acceptor. Theoretically, even more power could be generated when using nitrite as the electron acceptor. However, the actual results may vary since not very high nitrite concentration can be maintained in the cathodic chamber. It should be pointed out in the MFC/Anammox reactor more power was generated when compared with that of the ammonium oxidation/MFC reactor. This is because under the same operating conditions, more energy was release when organic compounds are coupled with nitrite than that of nitrate (Table 8).

Table 8. Gibbs Free Energy of Organic Compound Oxidation

Organic Compounds	Oxygen (kcal)	Nitrate (kcal)	Nitrite (kcal)
Acetate	-25.284	-23.737	-28.913
Ethanol	-26.267	-24.72	-29.896
Formate	-27.64	-26.093	-31.269
Glucose	-28.675	-27.128	-32.304
Lactate	-26.548	-25.001	-30.177
Propionate	-25.339	-23.793	-28.968

5.2 *S. putrefaciens* and *G. metallireducens* Attachment and Interactions with CEM

S. putrefaciens as electrochemically active species in MFC applications has advantages over MFCs driven by other cultures, which include higher resistance against process disturbances, higher substrate consumption rates, smaller substrate specificity and higher power output (Rabaey et al. 2004; Rabaey and Verstraete 2005). Other electrochemically active species such as *Geobacteraceae, Desulfuromonas*, *Alcaligenes faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Proteobacteria*, *Clostridia*, *Bacteroides* and *Aeromonas* species have also been observed to be able to function in MFCs by means of molecular analysis. To render the anode more susceptible for receiving electrons from the organic compound oxidation, electrochemically active species are attached to the graphite anodes. However, these species are able to suspend in the anode solution and attach to the CEM and form biofilms. The same phenomena occurred to *G. metallireducens* in the cathode chamber.

The CEM separates the anode and cathode, which should have a high selectivity and high stability. Nafion has been widely used as the CEM and has the large advantage of being very selective for protons. However, this membrane contains sulfonic acid groups that are binding with ammonium present in the anode chamber solution. Therefore, synthetic polymeric nanoporous membrane of Ultrex CMI-7000 was used in this research. There is a potential attachment of *S. putrefaciens* and *G. metallireducens* to attach to the CEM. When *S. putrefaciens* and *G. metallireducens* stabilize on the CEM surface, they start to secrete an exopolysaccharide matrix to cement themselves to the surface and form a multilayer biofilm, which is a time-dependent biological process. *S. putrefaciens* and *G. metallireducens* attachment to the CEM surface after exopolysaccharide secretion is governed by attractive interactions between extracellular polymeric substances (ECPS) and the CEM surface. The major component of ECPS is polysaccharides, which are mainly composed of D-mannose, D-glucose and D-galactose. For *S. putrefaciens* and *G. metallireducens*, outer membrane surface is largely composed of lipopolysaccharides. Thus, interactions between lipopolysaccharides and CEM can be used to simulate interactions of attached the *S. putrefaciens* and *G. metallireducens* with the CEM.

The Lifshitz-van der Waals and Lewis acid-base interactions between *S. putrefaciens* and *G. metallireducens* and the CEM immersed in water at the equilibrium distance or closest approach, y_0 (assumed to be 1.57 Å) (van Oss 1994) were estimated by equation (14) and equation (15) assuming that the contact area is $2\pi Ry_0$ (Meinders et al. 1995):

$$\Delta G(y)_{132}^{LW} = 2\pi Ry_0 \Delta G_{y_0 132}^{LW} \qquad (14)$$

$$\Delta G(y)_{132}^{AB} = 2\pi Ry_0 \Delta G_{y_0 132}^{AB} \qquad (15)$$

where R is the hydrodynamic radius of the microorganisms of *S. putrefaciens* and *G. metallireducens*; y_0 is the equilibrium distance (assumed to be 1.57 Å) (van Oss 1994); $\Delta G_{y0132}{}^{LW}$ and $\Delta G_{y0132}{}^{AB}$ are the Gibbs energies of two parallel plates, 1 and 2, immersed in water 3 at the equilibrium distance and were calculated from equation (16) and equation (17) (Meinders et al. 1995):

$$\Delta G_{y_0\ 132}^{LW} = -2(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_2^{LW}})(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}}) \qquad (16)$$

$$\begin{aligned} \Delta G_{y_0\ 132}^{AB} = {} & 2\sqrt{\gamma_3^{+}}(\sqrt{\gamma_1^{-}} + \sqrt{\gamma_2^{-}} - \sqrt{\gamma_3^{-}}) \\ & + 2\sqrt{\gamma_3^{-}}(\sqrt{\gamma_1^{+}} + \sqrt{\gamma_2^{+}} - \sqrt{\gamma_3^{+}}) - 2\sqrt{\gamma_1^{+}\gamma_2^{-}} - 2\sqrt{\gamma_1^{-}\gamma_2^{+}} \end{aligned} \qquad (17)$$

In equation (16) and equation (17), *S. putrefaciens* and *G. metallireducens* were modeled as spheres having a radius at least one order less than that of the CEM. Therefore, a sphere-plate interaction configuration was adopted to simplify interactions between *S. putrefaciens* and *G. metallireducens* and the CEM at the equilibrium distance and the CEM was treated as a flat surface. For interactions between *S. putrefaciens* and *G. metallireducens* themselves, a sphere-sphere interaction configuration was assumed and thus the contact area is πRy_0 and the Gibbs energies can be calculated as:

$$\Delta G(y)_{131}^{LW} = \pi Ry_0 \Delta G_{y_0 131}^{LW} \qquad (18)$$

$$\Delta G(y)_{131}^{AB} = \pi Ry_0 \Delta G_{y_0 131}^{AB} \qquad (19)$$

where $\Delta G_{y0131}{}^{LW}$ and $\Delta G_{y0131}{}^{AB}$ are Gibbs energies of two plats immersed in water at the equilibrium distance and were calculated using equation (16) and equation (17) by substituting 2 for 1. When *S. putrefaciens* and *G. metallireducens* got close to the CEM, electrical double layers became compressed. In this study, interactions were evaluated at the equilibrium distance of 1.57 Å where electrical double layers of *S. putrefaciens* and *G. metallireducens* and the CEM were superimposed. Thus electrostatic (EL) interactions were ignored compared to van der Waals and Lewis acid/base interactions (Wu et al. 1995). The total interaction free energies between *S. putrefaciens* and *G. metallireducens* and the CEM are summarized in Table 9. The amounts of attached *S. putrefaciens* and *G. metallireducens* on the CEM surface were found to be proportional to the corresponding ΔG_{132}^{TOT} value. Compared to *G. metallireducens*, *S. putrefaciens* had negatively greater ΔG_{132}^{TOT} value, and correspondingly, more *S. putrefaciens* attached to the CEM.

Table 9. *S. putrefaciens* and *G. metallireducens* Interactions with CEM

	ΔG_{132}^{LW} (kT)$^{+}$	ΔG_{132}^{AB} (kT)	ΔG^{TOT} (kT)
S. putrefaciens	-67.9	-1862.7	-1794.8
G. metallireducens	-75.1	-1701.9	-1626.8

$^{+}$ k is the Boltzmann constant (1.38×10^{-23} J/K) and T is absolute temperature (K). At 25°C, 1 kT = 4.11×10^{-21} J.

At the end of the experiments, the CEM was taken out from the MFCs and *S. putrefaciens* and *G. metallireducens* showed a significant morphological difference on the CEM surface. With the attachment of *S. putrefaciens* and *G. metallireducens* on the CEM, cation exchange decreased accordingly and reduced proton migration would be expected due to the physical barrier of the biofilm. The biofouling of *S. putrefaciens* and *G. metallireducens* on the CEM had the potential to cause adverse effects on mass transport through the membrane. During MFC operations, ammonium was able to diffuse through the CEM to the cathodic chamber from the anodic chamber. Based on this research, Ultrex CMI-7000 was found to be permeable to ammonium, even at a low concentration. For the ammonium oxidation/MFC reactor, most ammonium was oxidized to nitrate before leachate was introduced to the anodic chamber. Therefore, minimal ammonium diffused through the CEM. However, for the MFC/Anammox reactor, ammonium diffused to the cathodic chamber and was partially oxidized to nitrite and subsequently reduced to nitrogen gas.

For the ammonium oxidation/MFC reactor, the observation of increased ammonium lost from the anodic chamber indicated that ammonium was able to be transported across the CEM to maintain charge balance. The loss of ammonium from the anodic chamber cannot be due solely to diffusion. Another reason for ammonium loss without a commensurate increase in nitrite and nitrate could be biodegradation due to nitrification and denitrification, or to ANAMMOX. *Nitrosomonas europaea* was detected on the cathode, but not on the anode. These results suggested that nitrification could be occurring by AOB on the cathode, supported by ammonium diffusion through the CEM. It is unlikely that AOB contributed directly to current generation. There were no AOB on the anode, and the addition of a nitrification inhibitor did not affect voltage. Ammonium was preferentially transported (compared to protons) across the CEM due to its higher concentration in the landfill leachate. For the MFC/Anammox reactor, ammonium diffusion was not a big problem since ammonium would be partially oxidized to nitrite in the cathodic chamber.

5.3 Comparison of Ammonium Oxidation Oxidation/MFC Reactor and MFC/Anammox Reactor

In the ammonium oxidation/MFC reactor, organic compounds are oxidized in the anodic chamber and the freed electrons are consumed by nitrate in the cathodic chamber. Nitrate production is based on the ammonium oxidation before landfill leachate is introduced into the MFC. In the MFC/Anammox reactor, organic compounds are oxidized in the anodic chamber and the freed electrons are consumed by nitrite in the cathodic chamber. Nitrite is produced in the cathodic chamber through partial ammonium oxidation. This process also helps energy conservation from anaerobic ammonium oxidation with nitrite serving as the electron acceptor. The Anammox consortia activity is 25-fold higher than aerobic nitrifying bacterial oxidation of ammonium under anoxic conditions when using nitrite as the electron acceptor. The main product of Anammox is N_2, but about 10% of the N-feed (nitrite and ammonium) can be converted to nitrate. From our prior research, the overall nitrogen balance gave a NH_4^+ to NO_2^- ratio of 1:1.31 ± 0.06 and a NO_2^- to NO_3^- ratio of 1:0.22 ± 0.02. Thus, Anammox should have a good potential for ammonium removal in the cathodic chamber. The 10% nitrate can be denitrified to nitrogen gas by combining with the electrons released from the cathode by *G. metallireducens*. Compared to the ammonium oxidation/MFC reactor, the biomass yield for the MFC/Anammox reactor is very low, and consequently, little sludge is produced. However, the low biomass yield also necessitated an efficient system for sludge retention, and long start-up times were required to obtain a sufficient biomass concentration. Power generation, organic removal, nitrogen removal and operation cost comparison of the ammonium oxidation/MFC reactor and the MFC/Anammox reactor is summarized in Table 10.

Table 10. Comparison of Ammonium Oxidation Oxidation/MFC Reactor and MFC/Anammox Reactor

	Ammonium Oxidation/MFC Reactor	MFC/Anammox Reactor
Power Generation	Average	Higher
Organic Removal	Average	Average
Nitrogen Removal	Average	Higher
Operation Cost	Average	Higher

As summarized in Table 10, the MFC/Anammox reactor had obvious advantages over the ammonium oxidation/MFC reactor. However, although the power generation was higher for the MFC/Anammox reactor than the ammonium oxidation/MFC reactor, the power density needed to be further improved, especially for landfill leachate with low organic to nitrogen ratios. The nitrogen removal through both reactors was satisfactory. It should be noted that the operation costs for the MFC/Anammox reactor should be higher and the complex management is required owing to partial nitrification requirements.

MFC technology has been progressing rapidly in the past few years, with potentially higher and higher power density generation. However, most of the studies to date have been conducted at laboratory scales, and many technological and economic barriers remain to be overcome prior to large-scale applications of the MFC technique. Therefore, the development of MFCs is still in its infancy and the power density needs to be further improved before MFC are implemented in large-scale field applications.

6. Conclusions

High concentrations of organic matter and nitrogen present in landfill leachate are undoubted to require high capital investment and consume a substantial amount of energy for the effective treatment. MFCs, by which electricity can be directly generated from organic substances in the leachate, represent a fully novel process in reproducing energy from the leachate treatment and reducing the overall treatment cost. In this research, besides complex organic pollutants present in leachate being utilized as substrate for electricity generation, it is also demonstrated that nitrogen can be efficiently removed simultaneously. In the ammonium oxidation/MFC reactor, around 10 ~ 25 mW/m^2 could be generated with simultaneous organic and nitrogen removal. Although power obtained here is relative low, but several breakthroughs have been made with an achievement of increasing the power generation. It is believed that these technologies can also be used to recycle electricity with high power output from the leachate. For the MFC/Anammox reactor, around 15 ~ 40 mW/m^2 could be generated with simultaneous organic and nitrogen removal. This process may even utilize the potential power through ammonium oxidation. Therefore, higher power generation was observed as compared to that of the ammonium oxidation/MFC reactor.

Although these two systems have unique merits as stated above, there are still several problems in their applications in practical practices. The most important one is the attachment of *S. putrefaciens* and *G. metallireducens* to the CEM which may interfere with proton transfer from the anodic chamber to the cathodic chamber. This could be realized by developing the more efficient configuration of MFCs, improving the attachment of the strains to the anode and cathod, avoiding the possible attachment of *S. putrefaciens* and *G. metallireducens* to the CEM. Moreover, the CEM can be treated to decrease the *S. putrefaciens* and *G. metallireducens* attachment. Electrons produced in the anodic chamber flow through the external electrical circuit to the cathode to generate electrical current. At the cathode, the electrons and protons combine to reduce the terminal electron acceptor, in this research, nitrate and nitrite. While electrons move externally, protons diffuse from the anode to the cathode via the CEM to complete the internal circuit. Considering that ammonium can also diffuse through the CEM, which competes with protons to balance the system balance, measurements that can enhance proton diffusion should be taken to improve the power production.

Under anaerobic conditions, *S. putrefaciens* oxidized organic compounds that serve as the carbon source in the anodic chamber. *S. putrefaciens* had a maximum specific growth rate of 0.0089 day^{-1}, yield coefficient of 0.412 g/g and half saturation coefficient of 172.1 mg/l for the landfill leachate used in this research. *S. putrefaciens* also had a higher attachment potential to the CEM than *G. metallireducens* because *S. putrefaciens* had negative greater interaction free energy with the CEM. With *S. putrefaciens* and *G. metallireducens* attachment to the CEM, ammonium diffusion through CEM was a kinetic process, which increased with the increase of the reaction time. The characteristic time scale for the diffusion was in the order of minutes. The effective diffusion coefficient D_{eff} was experimentally determined to be 6×10^{-9} cm^2/min.

7. Recommendation

To enhance both the ammonium oxidation/MFC reactor and MFC/Anammox reactor performance, it is recommended that the CEM be treated to reduce the possible attachment of *S. putrefaciens* and *G. metallireducens*, which may interfere with proton transfer from the anodic chamber to the cathodic chamber. Especially, it is highly recommended ammonium be partial oxidized to nitrite for the landfill leachate before it is introduced to the MFC/Anammox reactor. This can eliminate the possibility of ammonium diffusion through the CEM from the anodic chamber to the cathodic chamber, which competes with proton transfer.

The retention time of these systems would likely be reduced in the future to possibly increase their treatment capacities. These new system designs will need to be evaluated not only in terms of power generation, but also carbonaceous and nitrogen removal from the landfill leachate. The current study has clearly established that both *S. putrefaciens* and *G. metallireducens* attachment to the CEM and ammonium diffusion can affect the treatment efficiency. Therefore, both of these mechanisms will need to be considered in future studies. It should be noted that if properly designed and managed, the MFC/Anammox reactor can perform much better than the ammonium oxidation/MFC reactor in terms of power generation and nitrogen removal.

8. Future Work

The conversion of organic waste to energy is considered an essential part of a sustainable global energy portfolio. While the use of MFCs for wastewater treatment is in its infancy, MFC applications beyond electricity production have been practiced. MFCs are used to power cathodic reduction reactions for bioremedial or industrial processes. Since electricity is not being harvested, the biologically generated current is used to stimulate microbial metabolism on a cathode, these systems are not considered fuel cells, but are called bioelectrical reactors (BERs). An external power source usually provides the reducing equivalents in these systems, but a biological anode may be used. Cathodes have served as electron donors for bacterial reduction of bioremediation targets such as uranium, perchlorate, chlorinated solvents, and nitrate. This technology could be applied to remediate other contaminants including toxic metals, dyes, pesticides, and herbicides.

BERs in which reducing equivalents are produced at the anode may also yield industrially important chemicals such as hydrogen peroxide, sulfur, and butanol. Using BERs to produce fuels such as propanol and butanol from organic waste is very appealing. In this process, organic waste with sugar contents too low to allow ethanol production would be microbially fermented in the absence of an electron acceptor into volatile fatty acids (VFA). These VFA can be fed to the cathodic compartment, where bacteria would use the electrons supplied from the cathode to reduce VFA into propanol and butanol. This process uses hydrogen rather than MFC cathodes as the source of reducing equivalents. Specific research hurdles include evaluating the use of current rather than hydrogen for reducing equivalents, fine-tuning concentrations of VFA and electrons for favorable thermodynamic conditions, and developing methods for separating the desired end-products from the reactor liquor.

In addition to powering BERs, MFCs can also be modified to produce hydrogen gas. With transportation fuels accounting for up to 25% of global fossil fuel consumption, alternative, sustainable fuel sources are needed. Microbial electrolysis cell (MECs), like MFCs, are 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 voltage 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. We have interests in these topics by improving the reactor design based on the research achievements from our current research.

9. Student Training

Two graduate students, Pawan Subramaniam and Yongwoo Lee were trained in this project. Pawan and Yongwoo were very active and productive in this research. So far, Pawan has published four technical journal papers in leading technical journals based on the work sponsored by the Hinkley Center for Solid and Hazardous Waste management. Yongwoo is currently working on a manuscript to be published. In addition, they have presented their research work at national conferences. Pawan holds a Master of Science Degree from Florida State University and was a Ph.D. candidate during this research. Yongwoo holds a Bachelor of Science Degree from Florida State University and currently is a Master of Science student in the Department of Civil and Environmental Engineering at FAMU-FSU College of Engineering.

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10.1 Peer-Reviewed Journal Paper Publications

1. Subramaniam, P. K., Martin, L., Grasel, P. and Chen, G., *Iron Reduction and Adsorption on Shewanella putrefaciens nearby Landfills in Northwest Florida*, Journal of Environmental Science and Engineering, 2010, 4, 60-69.
2. Subramaniam, P. K., Martin, L., Grasel, P. and Chen, G., *Landfill Leachate Treatment and Energy Generation Using Microbial Fuel Cell*, International Journal of Environmental Engineering, in press.

10.2 Conference Presentations

1. Subramaniam, P., and Chen, G. (Presented 2009, November). *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. (Presented 2010, November). *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.
3. Subramaniam, P., and Chen, G. (Presented 2011, November). *Iron Reduction and Adsorption on Shewanella putrefaciens nearby Landfills in Northwest Florida*. 97th Annual American Society of Microbiology Southeastern Branch Conference, Gainesville, FL: American Society of Microbiology Southeastern Branch.

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