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Power Generation and Nitrogen Removal of Landfill Leachate Using Microbial Fuel Cell Technology

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COLLEGE OF ENGINEERING

POWER GENERATION AND NITROGEN REMOVAL OF LANDFILL
LEACHATE USING MICROBIAL FUEL CELL TECHNOLOGY

By
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ABSTRACT

Microbial fuel cell (MFC) technology has been practiced in the treatment of landfill leachate. However, it is a big challenge for the usage of MFCs to treat landfill leachate with high ammonium content. The purpose of this study was to design and test two MFC reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor for the treatment of landfill leachate with high ammonium content in terms of power generation and nitrogen removal. Using the ammonium oxidation/MFC reactor, the landfill leachate collected from Leon County Landfill of Northwest Florida generated a power density of $8 \text{ mW}/\text{m}^2$ together with 92% of nitrogen removal. For the MFC/Anammox reactor, a power density of $12 \text{ mW}/\text{m}^2$ was achieved with 94% of nitrogen removal. Compared with the ammonium oxidation/MFC reactor, 50% more energy was generated because in the MFC/Anammox Reactor, nitrite served as the electron acceptor; while in the Ammonium Oxidation/MFC reactor, nitrate served as the electron acceptor. In this research, power generation was also found to be directly linked to the microbial species that were involved in organic decomposition, i.e., the greater the microbial concentration, the more power generated.

CHAPTER 1

INTRODUCTION

1.1 Motivation

Landfilling is recognized as one of the most cost effective, least polluting and safest means of disposing of solid urban waste (Alkalay et al., 1998). However, one of the challenges to be confronted during landfill operations is to handle the landfill leachate with high ammonium content (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. 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 and physicochemical treatment (Table 1). 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; Zhou et al., 2008). Both suspended processes and biofilm processes have been applied in full scale for nitrification and denitrification of wastewater with high nitrogen content as a means of nitrogen removal (Agdag and Sponza, 2008; Welander et al., 1997). A typical example is the 4-stage Bardenpho process, which consists of a sequence of anoxic and aerobic

zones with capacities of nitrification with pre- and post-denitrification (Ilies and Mavinic, 2001). However, conventional biological processes are complicated and very hard to manage and the results vary depending on the system management. Ammonia in landfill leachate can also be removed in a physicochemical way, i.e., by means of air stripping or magnesium ammonia phosphate ($MgNH_4PO_4 \cdot 6H_2O$, struvite or MAP) precipitation (Kabdasli et al., 2000; Kim et al., 2007). However, these physicochemical methods have their own limitations (Table 1.1).

Table 1.1: Summary of Nitrogen Removal Technologies

	Treatment Type		Advantage/Disadvantage		
Biological Nitrogen Removal	Suspended Process	Conventional Nitrification/Denitrification	High Efficiency/Complicated Management	Removal	Efficiency
		Anammox	Cost Effective and High Removal Efficiency/Complicated Management		
	Biofilm Process	Conventional Nitrification/Denitrification	High Efficiency/Complicated Management	Removal	Efficiency
		Anammox	Cost Effective and High Removal Efficiency/Complicated Management		
Physicochemical Nitrogen Removal	Air Stripping		Ammonia Removal Only		
	MAP Precipitation		High Efficiency/High Costs	Removal	Efficiency

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,b; 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 (HP Bennetto, 1983):

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, 2008; Pham et al., 2006; Raghavulu et al., 2009; Rhoads, 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 jin 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 electrochemi-

cally 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, *Rhodospirillum rubrum* 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 (2003). Remarkably, this bacterium is the first reported strain that can completely mineralize glucose to carbon dioxide while concomitantly generating electricity at 90% efficiency. During MFC operations, the anode is the electron acceptor recognized by the bacteria. Therefore, the microbial activity is strongly dependent on the redox potential of the anode (Lee et al., 2003; Lu et al., 2009; Manohar and Mansfeld, 2009; Rabaey et al., 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 MFCs (Jadhav and Ghangrekar, 2008). Oxygen is usually used as the electron acceptor 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). 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. 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 (Strous et al., 1997). 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. 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 (Strous et al., 1997). The key step for Anammox is to achieve stable nitrite accumulation through partial nitrification. 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 (Jetten et al., 1998). 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.

Microbial fuel cell (MFC) technology has advantages in treating landfill leachate since MFCs allow microorganisms to break down the organic components in the landfill leachate while simultaneously generating power (Pant et al., 2010). However, landfill leachate is difficult to deal with due to its high strength and complex composition. Prior research has demonstrated the feasibility of using MFCs for simultaneous leachate treatment and energy generation (Gálvez et al., 2009; Greenman et al., 2009; Puig et al., 2011). Especially, three MFCs fluidically connected in series were employed for en-

hanced electricity generation (Gálvez et al., 2009). Power generation from different leachate concentrations was also investigated. It was concluded that BOD_5 limited the process only at very small concentrations (Greenman et al., 2009). Most of prior studies focused on the production of electricity from the treatment of young leachate since in young landfills, leachate contains large amounts of biodegradable organic matter (Yilmaz et al., 2010). However, some open questions remain regarding the performance of MFCs in relation to the treatment of landfill leachate with the dynamics of nitrogen compounds for old landfills. To address this issue, MFCs have been reconfigured to treatment landfill leachate with nitrate as an electron acceptor to achieve both carbon and nitrogen removal (Clauwaert et al., 2007; Virdis et al., 2010, 2008). During the operation, ammonium was also possibly to be partially oxidized to nitrite to serve as the electron acceptor for power generation (Puig et al., 2011).

There is interest to incorporate Anammox into MFCs to handle municipal landfill leachate with high nitrogen content, which should be able to achieve power generation and nitrogen removal in a single unit. Since Anammox is an autotrophic process and can completely convert ammonium to nitrogen gas without the presence of organic matter, the organic compounds 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 content 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 MFC reactors, i.e., an ammonium oxidation/MFC reactor and a MFC/Anammox reactor, for power generation as well as nitrogen removal for landfill leachate. For the ammonium oxidation/MFC reactor, it was hypothesized that the electrons released from organic compound decomposition traversed from anode to cathode, where they were utilized by nitrate; while for the MFC/Anammox reactor, the released electrons were hypothesized to be consumed by nitrite.

1.2 Research Objective

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.

Figure 1.1: Sketch of Dual Chamber MFC

CHAPTER 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 (El-Fadel 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., 2008). 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., 2008). 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 (Méndez 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 (Méndez 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 physico-

chemical) 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 1.1). 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 fer-

ric 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; SH Roh, 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 *Rhodospirillum rubrum*, have been shown to generate electricity in mediator-less MFC systems (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 (Venkata 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*, *Desulfuromonadaceae*, *Alteromonadaceae*, *Enterobacteriaceae*, *Pasteurellaceae*, *Clostridiaceae*, *Aeromonadaceae*, and *Comamonadaceae* (Pham et al., 2008).

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 $3600\text{mW}/\text{m}^2$, 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 2.1) (Liu and Logan, 2004b).

Table 2.1: 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 (Venkata 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 2.1). After receiving electrons, the anode potential decreases to a level lower than that of the cathode in the cathodic chamber (Cheng et al., 2008, 2006b). The maximum power generation is closely related to the level of potential decrease in the anode.

Figure 2.1: 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 hexacyanoferrate or acidic perman-

ganate 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 (S Cheng, 2006; Zhao et al., 2006). Recently, potassium ferricyanide ($K_3[Fe(CN)_6]$) has been tested to be utilized as the electron acceptor in MFCs 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 Cation Exchange Membrane (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. Mem-

brane 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., less than 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 (Ganigué 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).

CHAPTER 3

MATERIAL 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. After collection, the leachate was stored in temperature-controlled containers at 4°C and immediately transported to the laboratory. The leachate was stored under refrigeration at 4°C. Based on the results of this research, the landfill leachate had a composition of COD of 18,435 mg/l, $NH_4^+ - N$ of 486.4 mg/l, and phosphorus of 189.1 mg/l. The conductivity of the landfill leachate was 74.2 mS/cm and pH was 8.41. Soil samples that were used for this research were also collected from this landfill site. Specifically, soil samples were collected 0.3 to 1 m below the surface, 30 to 100 m away from the landfill. 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°C until used in the experiments.

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

Continuous cultivation and enrichment of *S. putrefaciens* and *G. metallireducens* were carried out immediately in an anaerobic chamber after the sam-

ples 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; $FeCl_3 \cdot 6H_2O$, 250 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; $(NH_4)_6Mo_7O_{24}$, 0.03 mg/l; glucose, 200 mg/l; and ammonium 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 ($121^\circ C$ and 1 atm) for 20 min. Glucose was filter-sterilized and aseptically added to the autoclaved media. The serum bottle was equipped with CO_2 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 $35^\circ C$) 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 could 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) for 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 5109 cells/ml as determined by ATP assay (Chen and Strevett, 2001).

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 in Tallahassee had an average temperature of $20.5^\circ C$ and pH was in the

range of $6.05 \sim 8.02$. The wastewater was modified to have a composition of $COD_{of} \sim 60mg/l$, $NH_4^+ - N \sim 100 mg/l$, $NO_2^- - N < 1mg/l$, $NO_3^- - N < 1mg/l$ and total phosphorus of $0.18 \sim 0.74mg/l$ and used as the substrate. The alkalinity was adjusted by the addition of $KHCO_3$. During the inoculation, 1 liter 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 concentration of the bulk liquor in the reactor was in the range of $0.15 \sim 2mg/l$ and the ammonium concentration in the reactor was maintained at $\sim 100mg/l NH_4^+ - N$. After approximately one months adaptation, the inocula began working functionally with a bulk liquor suspended solid (SS) maintained at $\sim 1000mg/l$ and volatile suspended solid (VSS) at $820mg/l$. The sludge volume and sludge volumetric index of the bulk liquor were kept at 5% and 50 ml/g respectively, which was confirmed by experimental measurements.

3.4 Landfill Leachate Treatment in the 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 nitrogen content in this research. The laboratory scale ammonium oxidation/MFC reactor included an in-line ammonium oxidation column, followed by a conventional MFC reactor (Figure 3.1).

In the ammonium oxidation column, an air flow up to $4.72 ml/min$ was supplied. The MFC reactor was a custom-made dual-chamber mediator-less MFC (Pant et al., 2012). A graphite rod, without catalysts coated, was installed in the center of the inner chamber as the anode (1.0 cm ID, 4.0 cm length). 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 (geometrical effective area of $12.6 cm^2$, 30% wet proofing) was used as the cathode (1.0 cm ID, 4.0 cm length). Based on the growth simulation, it

was projected that *S. putrefaciens* had a coverage of $105 \sim 106 \text{ cell/cm}^2$ of the anode. 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 Ohms law. Synthetic polymeric nanoporous membrane (Ultrex CMI-7000, Membranes International Inc., Glen Rock, NJ) was used as the cation exchange membrane. Since ammonium was oxidized to nitrate, there was minimal chance for ammonium to pass through the cation exchange membrane 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. Electrons from organic decomposition were transported to the cathodic chamber. Consequently, nitrate was reduced to nitrogen gas.

Figure 3.1: Ammonium Oxidation/MFC reactor Setup

During operation, the reactor was operated with a pulse input of landfill leachate. The anolyte was kept in the anodic chamber until the power generation was low. Then it was transferred to the cathodic chamber. Though there was possibility of power loss owing to the electrons left in the anolyte being transferred to the cathodic chamber, this part of power loss can be minimal owing to the low concentrations of organic content. Once the experiments were completed, power generation from landfill leachate was also tested with oxygen supplied at a rate of 4.72 ml/min in the cathodic chamber for comparison purposes.

3.5 Landfill Leachate Treatment in the 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 3.2). 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 (1.0 cm $ID \sim 4.0$ cm length). In order to *S. putrefaciens* to efficiently transport electrons to the anode, it must adhere to the anode. With the attachment, biofilm would form. It should be noted that the multilayer biofilm formation was not encouraged to avoid the interference of mass transfer. For the cathodic chamber, carbon cloth (effective area of 12.6cm^2 , 30% wet proofing) was used as the cathode (1.0 cm $ID \sim 4.0$ cm length). The cathode was inoculated with *G. metallireducens*. The cathodic chamber was also inoculated with Anammox consortia. Again, measurements of voltage followed the same method as described above and the same synthetic polymeric nanoporous membrane was used. During the operation, collected landfill leachate was introduced to the anodic chamber for organic decomposition. The operation proceeded in the absence of oxygen, which was tested by resazurin (1 mg/l) as a redox indicator. The treated leachate was then introduced to the cathodic chamber, where low level oxygen was supplied. The dissolved oxygen of 0.6 mg/l was maintained by air-flow adjusted by a flow

rotameter. CO_2 -scrubbed air was used in this research. Since there was possibility for electrotrophs to utilize oxygen as the electron acceptor, the inlet of oxygen was ranged further away from the cathode. In addition, the low level of oxygen concentration would be consumed by ammonium quickly, thus minimizing the chance of being utilized by the electrotrophs. With the introduced low level of oxygen, ammonium was partially oxidized to nitrite. Consequently, nitrogen was removed from the system by means of Anammox. 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, ammoniums passing through the CEM from the anodic chamber to the cathodic chamber had minimal adversely impact on the reactor performance. In addition, the electrons form organic decomposition ensured that nitrate produced through over oxidation was reduced to nitrogen gas and removed.

Figure 3.2: MFC/Anammox Reactor Setup

CHAPTER 4

RESULTS AND DISCUSSION

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

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 5109 cells/ml as determined by ATP assay (Chen and Strevett, 2001). Agar plate using the same culturing media was used to culture the colonies. Based on the morphology, distinct colonies were selected for *S. putrefaciens* and *G. metallireducens* identification by polymerase chain reaction (PCR) analysis. For the selected colony, PCR was used to amplify specific regions of the DNA in the microorganisms 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. The colonies whose DNA codes matched the codes of *S. putrefaciens* and *G. metallireducens* were selected and enriched in 100 ml fresh culturing media.

Figure 4.1: Image of *S. putrefaciens*

Figure 4.2: Image of *G. metallireducens*

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 dissolved oxygen concentrations, AOB had a higher affinity for oxygen than NOB. Accordingly, partial nitrification could be achieved by maintaining low dissolved oxygen levels. In addition, high pH favored AOB rather than NOB. 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 4.1). Therefore, ammonium oxidation dominated over nitrite oxidation during the partial nitrification process.

Typically, aerobic AOB, such as *Nitrosospira* and *Nitrosomonas* are able to oxidize ammonium to nitrite, and aerobic NOB, such as *Nitrobacter* are able to oxidize the nitrite further to nitrate. NOB are more sensitive to the detrimental environmental conditions than AOB. The most important environmental parameters to obtain partial nitrification were found to be the temperature, pH and dissolved oxygen concentration in this research. Temperature above 25 led to an increase of the specific growth rate of both AOB and NOB. In this research, it was discovered that the optimal temperature was 35 for AOB and 38 for NOB. The Anammox consortia activity was 25-fold higher than AOB under anoxic conditions when using nitrite as the electron acceptor. From this research, the overall nitrogen balance gave a NH_4^+ to NO_2^- ratio of $1 : 1.31 \pm 0.06$ and a NO_2^- to NO_3^- ratio of $1 : 0.22 \pm 0.02$. Thus, Anammox should have a good potential for ammonium removal in the cathodic chamber.

Figure 4.3: Image of Anammox Consortia

4.3 Landfill Leachate Treatment in the Ammonium Oxidation/MFC Reactor

Ammonium oxidation was a function of dissolved oxygen. In this research, variable dissolved concentrations in the range of 2.0 mg/l to 8.5 mg/l were tested in the ammonium oxidation reactor. As shown in Figure 4.4, all the ammonium could be oxidized to nitrate within 8 hours at dissolved oxygen above 7.0 mg/l. In order to save the energy costs, dissolved oxygen level of 7.0 mg/l was selected for this research to ensure the ammonium in the landfill leachate was completely oxidized before the landfill leachate was introduced to the MFC. At the dissolved oxygen of 7.0 mg/l, nitrate production was observed to increase with the depletion of ammonium (Figure 4.5). Minimal nitrite was observed in the reactor, which was confirmed by the mass balance calculation of ammonium depletion and nitrate production.

Figure 4.4: Ammonia Depletion as a Function of Time

Figure 4.5: Ammonia Depletion and Nitrate Production at DO of 7 mg/l

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. To remove the remaining dissolved oxygen and eliminate its impact on the MFC performance, landfill leachate was degased with helium after ammonium oxidation to remove the excess of dissolved oxygen 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. Glucose and landfill leachate collected from Leon County Landfill were used as the carbon sources for this research. According to prior research, leachate concentrations limited the process only at very small concentrations (Greenman et al., 2009). In this research, landfill leachate was diluted to a BOD_5 value ~ 250 mg/l and total nitrogen of ~ 120 mg/l. For comparison purposes, glucose was also utilized in the reactors at 250 mg/l (~ 266 mg/l COD). Both glucose and landfill leachate were applied to the reactor at a rate of 200 ml/5 days.

The power generation was lower as compared with that of oxygen serving as the electron acceptor. When glucose was used as the carbon source, the ammonium oxidation/MFC reactor generated half of the power produced by that of oxygen serving as the electron acceptor ($\sim 30 mW/m^2$ of anode surface area versus $\sim 50 mW/m^2$) (Figure 4.6). Similar observation was made for the landfill leachate collected from Leon County Landfill ($\sim 8 mW/m^2$ versus $\sim 20 mW/m^2$). Ammonia removal was obvious for the ammonium oxidation/MFC reactor. With an input total N of $\sim 120 mg/l$, above 92% of nitrogen was removed with an effluent N concentration below 9 mg/l (Figure 4.7).

4.4 Landfill Leachate Treatment in the MFC/Anammox Reactor

Similar to the ammonium oxidation/MFC reactor, power was produced in the MFC/Anammox reactor with the same carbon source supplies (Figure 4.6).

Figure 4.6: Power Generation of the Ammonium Oxidation/MFC Reactor (A) and MFC/Anammox Reactor (B)

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 higher for both glucose and landfill (~ 35 mW/m² and ~ 12 mW/m² as compared with ~ 30 mW/m² and ~ 8 mW/m²). But when compared with those of oxygen serving as the electron acceptor (~ 50 mW/m² for glucose and ~ 20 mW/m² for landfill leachate), the power generation was still low. These results were comparable with those of prior studies (Sevda et al., 2013; Yang et al., 2012). For the ANAMMOX process, only 50% of the ammonium was needed to be converted to nitrite:

This reaction stoichiometry implied that no extra addition of base was required since the landfill leachate generally contained enough alkalinity (in the form of bicarbonate) to compensate for the acid production if only 50% of the ammonium was oxidized. The possibility to produce a 50/50 mixture of ammonium and nitrite was achieved in this research. The second step of Anammox was the conversion of nitrite to nitrogen with ammonium serving as the electron donor and nitrite serving as the electron acceptor under anoxic conditions:

Ammonia removal was obvious for MFC/Anammox reactor. Approximately 94% of nitrogen was removed with an effluent N concentration approximately 7.5 mg/l (Figure 4.7).

Figure 4.7: Nitrogen Removal of the Ammonium Oxidation/MFC Reactor (A) and the MFC/Anammox Reactor (B)

4.5 Landfill Leachate Decomposition and *S. putrefaciens* Growth

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

$$\frac{dS}{dt} = -\frac{1}{Y} \frac{u_m SX}{K_s + S} \quad (4.3)$$

$$\frac{dS}{dt} = \frac{u_m SX}{K_s + S} - \frac{bX}{K_s + S} \quad (4.4)$$

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

$$Y = -\frac{\Delta X}{\Delta S} \quad (4.5)$$

$$X = X_0 + Y(S_0 - S) \quad (4.6)$$

By substituting equation (6) into equation (3), organic depletion can be expressed as:

$$\frac{dS}{dt} = -\frac{1}{Y} \frac{u_m S [X_0 + Y(S_0 - S)]}{K_s + S} \quad (4.7)$$

The simulated half-saturation coefficient K_s (mg/l), growth yield coefficient Y (g biomass per g substrate), and maximum specific growth rate u_m (day⁻¹) based on organic decomposition (Figure 4.8) are listed in Table 4.3. *S. putrefaciens* had greater K_s values in the ammonium oxidation/MFC reactor than that of the MFC/anammox reactor for both leachate and glucose. *S. putrefaciens* also had greater Y values and m values in the ammonium oxidation/MFC reactor than that of the MFC/anammox reactor.

Figure 4.8: Organic Decomposition Profile

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

$$\epsilon A \Delta G_r + G_s = 0 \quad (4.8)$$

where ϵ is the efficiency of energy transfer; ΔG_r is the free energy released of electron-donor substrate converted for energy (e.g., respiration); δG_s is the energy required to synthesize cells which includes energy loss incurred in using the energy carrier; and A is the balance ratio between δG_r and δG_s . Cell synthesis is closely linked to energy generation in MFCs as demonstrated by Equation (4.8). Average power generation was plotted against microbial concentration simulated based on organic consumption (Figure 4.9). Power generation was found to increase with microbial concentrations. Energy production is also limited by the energy potential by the electron acceptors. The MFC/Anammox reactor generated more power than that of the ammonium oxidation/MFC reactor. This was because under the same operating conditions, more energy was released when organic compound decomposition was coupled with nitrite than that of nitrate. As shown in Table 4.3, nitrate and nitrite could retrieve 17.128 kcal and 22.304 kcal of energy per electron receiving, respectively.

Power generation from this research was comparable with prior research. In this research, 8 mW/m^2 and 12 mW/m^2 were produced in the ammonium oxidation/MFC reactor and MFC/Anammox reactor. In three series of MFCs, landfill leachate was discovered to produce 1.6–5.6 mW/m^2 power (Gálvez et al., 2009). If converted to per unit volume of the cathodic chamber, this research produced 51.4 mW/m^3 and 77.1 mW/m^3 in the ammonium oxidation/MFC reactor and MFC/Anammox reactor. As a comparison, 5.3 mW/m^3 –278.2 mW/m^3 was reported (Puig et al., 2011).

Figure 4.9: Power Generation and *S. putrefaciens* Concentration

Table 4.1: 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					$\times 10^5$	$\frac{cell}{ml}$				
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					$\times 10^4$	$\frac{cell}{ml}$				

Table 4.2: *S. putrefaciens* Growth Parameters

		$K_S(mg/L)$	Y (g/g)	$u_{max}(hr^{-1})$
Ammonium Oxidation/MFC Reactor	Glucose	162.5	0.76	0.84
	Leachate	214.6	0.65	0.60
MFC/Anammox Reactor	Glucose	147.4	0.98	0.72
	Leachate	201.5	0.87	0.55

Table 4.3: Gibbs Free Energy of Electron Acceptor Half Reactions

Reactions for Electron Acceptors	$\Delta G^0(kcal/e^-eq)$
$1/5 NO_3^- + 6/5 H^+ + e^- = 1/10 N_2 + 3/5 H_2O$	17.128
$1/3 NO_2^- + 4/3 H^+ + e^- = 1/6 N_2 + 2/3 H_2O$	22.304

CHAPTER 5

CONCLUSIONS

The high energy demand and nutrient removal requirements of landfill leachate treatment are warrant for alternative treatment technologies which use less energy for the efficient removal of organic and nutrient components during the treatment operations. 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. The proposed ammonium oxidation/MFC reactor and MFC/Anammox reactor can achieve power generation 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. In the ammonium oxidation/MFC reactor, approximately 8 mW/m^2 could be generated with simultaneous 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, approximately 12 mW/m^2 could be generated with simultaneous nitrogen removal. More power was generated by the MFC/Anammox reactor as compared with that of the ammonium oxidation/MFC reactor. This was because under the same operating conditions, more energy was released when organic compound decomposition was coupled with nitrite than that of nitrate.

APPENDIX A

FIGURES

Figure A.1: Batch MFC Reactor Setups

Figure A.2: Continuous MFC Reactor Setups

Figure A.3: Anaerobic Partial Nitrification Anammox Reactor Setup

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BIOGRAPHICAL SKETCH

Background

Experienced in managing and conducting a variety of engineering and scientific projects. Have diverse experience and project background for multi-party and multi-discipline projects.

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Publication

Lee, Yongwoo, et al. "Power Generation and Nitrogen Removal of Landfill Leachate Using Microbial Fuel Cell Technology." Environmental Technology just-accepted (2013): 1-36.

Membership

Tau Beta Pi