# Comparison of Onsite Biological and Physicochemical Systems for the Treatment of Landfill Leachate with High Ammonium Content

September 27, 2011

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Report # 1032022

# FINAL REPORT

September 1, 2010 to August 31, 2011

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**COMPLETION DATE:** August 31, 2011

**PHONE NUMBER:** 850-410-6303

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**KEY WORDS:** Landfill Leachate, Ammonium, MAP Precipitation, ANAMMOX, and Northwest Florida.

## ABSTRACT (1 page only)

One of the challenges to be confronted during landfill operations is the handling of leachate with high ammonium content. Current biological ammonium removal processes either are complicated and difficult to operate, or not cost-effective. Low-cost and low-maintenance onsite systems for the treatment of landfill leachate with high ammonium content are in need, especially for landfills located in low population areas where landfills are smaller and often far from a wastewater-treatment plant and lack trained personnel.

The objective of this study is to compare onsite biological and physicochemical systems for the treatment of landfill leachate with high ammonium content. Laboratory-scale anaerobic ammonium oxidation (ANAMMOX) and magnesium ammonium phosphate (MAP) precipitation systems were evaluated in this research. Organic and ammonium removal efficiency for leachate collected from landfills in Northwest Florida was compared for the ANAMMOX and MAP precipitation processes. It was discovered that the initial ammonium content in the landfill leachate was a significant variable in ammonium removal for both cases. Through ANAMMOX treatment, up to 97% ammonium removal efficiency was achieved. By MAP precipitation without filtration, up to 80% of ammonium was removed. With filtration, up to 95% of ammonium was removed. Compared to the physicochemical system, the featured biological system removed ammonium from the landfill leachate more efficiently.

# EXECUTIVE SUMMARY

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**Objective:**

One of the challenges to be confronted during landfill operations is the handling of leachate with high ammonium content. Current biological ammonium removal processes either are complicated and difficult to operate, or are not cost-effective. Low-cost and low-maintenance onsite systems for the treatment of landfill leachate with high ammonium content are in demand, especially for landfills located in sparsely populated areas where small landfills exist and often are located far from a wastewater treatment plant and lack trained personnel. The objective of this study is to compare onsite biological and physicochemical systems for the treatment of landfill leachate with high ammonium content. Laboratory-scale anaerobic ammonium oxidation (ANAMMOX) and magnesium ammonium phosphate (MAP) precipitation systems were evaluated in this research. Organic and ammonium removal efficiency for leachate collected from the Leon County Landfill was compared for the ANAMMOX and MAP precipitation processes. The more favorable system was recommended and optimal operation parameters were identified.

**Methodology:**

ANAMMOX is an autotrophic process and can 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 has a lower oxygen demand and reduced $CO_2$ emission when compared to the conventional nitrification/denitrification process. Ammonium in landfill leachate can also be removed by MAP precipitation. In Northwest Florida, iron content is a common problem associated with landfill leachate. Iron can subsequently be removed using MAP precipitation. Landfill leachate typically has a high phosphorous content. For MAP precipitation applications, magnesium salts are a major economic constraint. Most importantly, the purity of precipitated MAP can be high, which makes it possible for the precipitated MAP to be recovered. The commercialization of the purified MAP may offset the operation and chemical costs of the landfill leachate treatment. Though MAP precipitation has advantages in treating landfill leachate, all soluble or volatile organic compounds cannot be removed in this process. For onsite applications, microbial fuel cell technology can be combined with MAP precipitation for the removal of the volatile organic compounds remaining in the landfill leachate, resulting in the generation of energy.

**Results:**
Laboratory-scale ANAMMOX and MAP precipitation experiments were conducted. The laboratory-scale ANAMMOX reactor consisted of three sequencing continuously stirred tank reactors (CSTRs) with a working volume of 500 ml each. 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 and was operated at 37-42°C using a temperature-controlled water bath. The key step for ANAMMOX was to achieve partial nitrification and obtain stable nitrite accumulation. It was discovered that the initial ammonium content in the landfill leachate was a prominent factor in ammonium removal. Up to 97% of ammonium can be removed through ANAMMOX treatment.

For MAP precipitation, magnesium ($MgCl_2 \cdot 6H_2O$) and phosphate ($NaH_2PO_4 \cdot 2H_2O$) addition was made to the landfill leachate. The chemical addition was made under flash-mixing conditions and was continuously stirred by a single mechanical blade for 5 minutes. After mixing, the leachate was transformed to the settling tank for MAP precipitation for 20 minutes. As in the case of ANAMMOX, the initial ammonium content in the landfill leachate significantly affected ammonium removal. By MAP precipitation, up to 80% of ammonium can be removed. With a further filtration process, up to 95% of ammonium can be removed. Compared to the physicochemical system, biological system provided more efficient ammonium removal.

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# 1. Introduction

## 1.1 General

There is worldwide consensus that landfilling is the most cost effective, least polluting and safest means of disposing of solid urban waste (Carrieri et al. 1998; Garcia et al. 1996; Gouveia and do Prado 2010; Mannarino et al. 2010). However, one of the challenges during landfill operations is the management of leachate with high ammonium content. The main source of ammonium in the landfill leachate is protein, which is hydrolyzed to generate ammonium-nitrogen in the leachate (Kabdasli et al. 2000b; Li et al. 1999; Urtiaga et al. 2007; Yusof et al. 2010; Yusof et al. 2011). The release of soluble nitrogen from municipal solid waste into landfill leachate continues over a long period compared to that of soluble carbon compounds as the hydrolysis of the polypeptide chain 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 (Domingues et al. 2011; Molins-Legua et al. 2006). Therefore, nitrogen is usually removed from landfill leachate such as 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, subsequently, anoxic denitrification of nitrate via intermediate stages to nitrogen gas (Ma et al. 2009; Peng et al. 2008; Peng et al. 2007; Vaboliene and Matuzevicius 2007). Suspended solid processes have been applied in full-scale for nitrification and denitrification of high-nitrogen content wastewater as a means of nitrogen removal (Agdag and Sponza 2008; Huo et al. 2008). For the treatment of high ammonium content landfill leachate, 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 hard to manage and the results vary depending on the level of system management. New methods such as suspended carrier biofilm processes have also been studied for landfill leachate nitrogen removal, even at low temperatures (Welander and Henrysson 1998; Welander et al. 1997). These processes are reliable, but they generally require a high capital investment.

Currently, there is a need for low-cost and low-maintenance and energy-generating onsite systems to treat landfill leachate with high ammonium content. This is especially the case in low-population areas where smaller landfills are prevalent and are often far from available wastewater collection and treatment systems, and lack trained personnel.

## 1.2 Literature Review

Recently, a novel process called anaerobic ammonium oxidation (ANAMMOX) has been proposed to treat municipal landfill leachate with high concentrations of ammonium (Shivaraman 2003). ANAMMOX is a microbiological mediated exergonic process in which ammonium is converted to nitrogen gas under anaerobic conditions with nitrite serving as the electron acceptor (Ganigue et al. 2007; van Dongen et al. 2001). 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*" (Nitisoravut and Chamchoi 2007; Strous et al. 1997). ANAMMOX is an autotrophic process and can convert ammonium to nitrogen gas without the presence of organic matter (Chamchoi et al. 2008; Mikhailovskaya 2008). Thus, ANAMMOX not only eliminates the need for complex compromises between organic carbon removal and nitrogen removal, but also has a reduced oxygen supply need and has a lower $CO_2$ emission compared to the conventional nitrification/denitrification process (de Araujo et al. 2010; Nedwell et al. 2009; Op den Camp et al. 2009). For the ANAMMOX process, partial nitrification occurs in which nitrite is accumulated. Also, a high organic content may interfere with partial nitrification and the subsequent ANAMMOX process (de Araujo et al. 2010; Kartal et al. 2010; Nedwell et al. 2009; Op den Camp et al. 2009; Song et al. 2011; Zheng et al. 2011). If these two issues can be addressed, ANAMMOX can be a promising means to handle landfill leachate with high ammonium content. 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 partial nitrification to obtain stable nitrite accumulation. 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 favors ammonium oxidizers rather than the nitrite oxidizers (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 nearly the entire load of ammonium into nitrite. Varying the dissolved oxygen concentration in the reactor is also a possible way for enhancing nitrite accumulation (Biswas et al. 2010).

Landfill leachate with high ammonium content can also be treated in a physicochemical way, i.e., by means of magnesium ammonium phosphate (MAP, $MgNH_4PO_4\ 6H_2O$ or struvite) precipitation (Tunay et al. 2004; Yetilmezsoy and Sapci-Zengin 2009). In addition to ammonium removal, MAP precipitation also helps the removal of some other pollutants such as suspended solids and heavy metals. Since suspended solids attribute greatly to the organic load of landfill leachate, MAP precipitation can thus remove organic matter by means of co-precipitation of suspended solids. In Northwest Florida, iron content is a common problem associated with landfill leachate. Iron can thus also be removed from landfill leachate through MAP precipitation. Landfill leachate usually has a high phosphorous content. For MAP precipitation applications, magnesium salts are the major economic constraint (Giesen 1999; Lee et al. 2003).

In order to reduce the cost, MAP may be used as a recycling material for the removal-recovery of ammonium (Stefanowicz et al. 1992a; Stefanowicz et al. 1992b). Ammonium elimination from MAP to magnesium phosphate (MP) and ammonium incorporation into MP to regenerate MAP has been investigated. Most importantly, the purity of precipitated MAP can be high, which makes it possible for the precipitated MAP to be recovered. The commercialization of the purified MAP may offset the operation and chemical costs of the landfill leachate treatment. Though MAP precipitation has advantages in treating landfill leachate, all soluble or volatile organic compounds cannot be removed in this process. For onsite applications, microbial fuel cell technology can be combined with MAP precipitation for the removal of the volatile organic compounds remaining in the landfill leachate, resulting in energy generation.

Although physicochemical treatment technologies such as air-stripping (Cheung et al. 1997; Ozturk et al. 2003), adsorption and membrane separation processes (Chianese et al. 1999; Di Palma et al. 2002) have been proven to be applicable for the treatment of landfill leachate with high ammonium content, MAP precipitation has obvious advantages over others in ammonium removal from landfill leachate since the precipitated MAP can be recovered and used as a fertilizer. The fertilizer value of MAP was demonstrated in the 1960s in Germany and the United States (Schuiling and Andrade 1998). MAP is only slightly soluble in water and soil solutions and slow-release MAP has been found to be a highly effective source of phosphorus, nitrogen and magnesium for plants through both foliar and soil applications. Especially, the usage of MAP may be particularly advantageous when soluble fertilizers are inefficient, low soluble salts are required in the root zone, or a long residual effect is required. In addition, when properly granulated, MAP can be applied to the soil at rates exceeding those of conventional fertilizers without danger of burning plant roots (Ponce and De Sa 2007). MAP has also been commercially used for container plants as well as turf, tree seedlings, ornamentals, vegetables and flower boards since less frequent applications of MAP are required. Improved growth of grass, fruit and various high-value crops has been observed when they are fertilized with MAP as compared with conventional soluble fertilizers (Rothbaum and Rohde 1976).

Most importantly, among the physicochemical treatment methods, MAP precipitation has been proved to be most effective for the removal of high ammonium concentrations from the landfill leachate because of its high reaction rate and removal efficiency (Barnes et al. 2007; Booker et al. 1999; de-Bashan and Bashan 2004; Kabdasli et al. 2000b; Shu et al. 2006; Turker and Celen 2007). For instance, Tünay et al. (Tunay et al. 2004) studied the MAP precipitation on raw leather tanning wastewater with an initial ammonium concentration of 200 ~ 650 mg N/l and obtained a removal efficiency over 95%. MAP usually precipitates at the stoichiometric ratio of $Mg^{2+}$: $NH_4^+$: $PO_4^{3-}$ = 1:1:1 according to following reaction (Booker et al. 1999):

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow \tag{1}$$

In practice, crystalization experiments have shown that the precipitation of MAP reduces the pH of the solution, and therefore $HPO_4^{2-}$ will take place in the reaction rather than $PO_4^{3-}$ as follows (Nelson et al. 2003):

$$Mg^{2+} + NH_4^+ + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + H^+ \tag{2}$$

Based on the above equation, when 1 g of $NH_4^+$ is removed, 13.6 g $MgNH_4PO_4$ $6H_2O$ should be theoretically formed as precipitated MAP sludge.

### 1.3 Objectives

The objectives of this study are to compare the onsite applications of ANAMMOX (biological process) and MAP precipitation (physicochemical process) for the treatment of landfill leachate. Landfill leachate is rich in dissolved organic matter, inorganic macrocomponents ($Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, $NH_4^+$, $Fe^{2+}$, $Mn^{2+}$, $Cl^-$, $SO_4^{2-}$ and $HCO^{3-}$), heavy metals ($Cd^{2+}$, $Cr^{3+}$, $Cu^{2+}$, $Pb^{2+}$, $Ni^{2+}$ and $Zn^{2+}$), and xenobiotic organic compounds. For this research, organic matter and ammonium were the focus. For ANAMMOX, a preceding anaerobic process can easily handle the heavy organic load of the landfill leachate. In addition, the anaerobic process maintains an ideal operating condition for the subsequent partial nitrification and ANAMMOX processes where low or no dissolved oxygen is required. For MAP precipitation, ammonium can be more quickly removed since MAP precipitation is a physicochemical process. In addition, the recovered ammonium can be potentially uused as a fertilizer. Specific objectives of this research project are as follows:

1. Laboratory scale landfill leachate treatment by means of ANAMMOX: Leachate collected from the Leon County Landfill is to be treated in a laboratory-scale anaerobic-partial nitrification-ANAMMOX reactor. For this treatment process, partial nitrification is a significant factor for nitrite accumulation. Therefore, factors that may impact the nitrite accumulation such as the dissolved oxygen concentration and alkalinity are to be investigated.

2. Laboratory scale landfill leachate treatment by means of MAP precipitation: Leachate collected from the Leon County Landfill is to be treated in a laboratory-scale MAP

precipitation reactor. The success of the MAP precipitation depends on two main factors: the molar ratio of Mg:N:P and the pH value of the solution in the reactor. For this investigation, impact factors on MAP precipitation and subsequent ammonium removal, including molar ratio of $Mg^{2+}$/ $NH_4^+$/$PO_4^{3-}$, pH and reaction time, are to be assessed. The effluent is then introduced to a microbial fuel cell reactor for the removal of volatile organic compounds.

3. System comparison: The two onsite treatment systems are compared in terms of ammonium and organic removal. The more favorable system is to be recommended and operation parameters are to be identified.

# 2. Background

## 2.1 Landfill Leachate Production and Characteristics

Land disposal of solid wastes has been practiced for centuries, dating back to prehistoric times (Heath and Lehr 1987; Quinlan and Aley 1987). Most landfills receive municipal domestic, industrial, and agricultural wastes. With the increase of human population, the amount of generated waste has increased rapidly. Consequently, land space for waste disposal has become a critical issue for urban management (Heath and Lehr 1987; Quinlan and Aley 1987). Solid waste in a landfill is degraded through aerobic and anaerobic processes. Stabilization of the wastes is a complex and variable event due to the site-specific characteristics of each landfill. Landfill leachate and gas are the major degradation products generated from landfill stabilization (Eden 1994; Huang et al. 2008a; Johnson 1983; Kim et al. 2009; Watzinger et al. 2006). Landfill leachate is formed from the contact of water with refuse. The water, mainly from precipitation, dissolves soluble organics and inorganics including some toxic compounds if present in the landfill material (Kim et al. 2009). Landfill gas is generated due to the anaerobic biological degradation of organic material (Johnson 1983).

Rainfall is the main factor in the generation of leachate. The precipitation percolates through the waste and absorbs dissolved and suspended components from the biodegrading waste through several physical, chemical and biological reactions (Varank et al. 2011). Other contributors to leachate generation include groundwater inflow, surface water runoff, and biological decomposition (Jaskelevicius and Lynikiene 2009; Rule 1979; Teirumnieks et al. 2005). Liquid fractions in the waste will also add to the leachate as well as moisture in the cover material. Moisture can be removed from the landfill by water consumed in the formation of landfill gas, water vapor removed in the landfill gas, and leachate leaking through the liner (Masada 1998; Pivato and Raga 2006). Since the short-term leachate quantity depends heavily on precipitation, it is sometimes hard to predict. Long-term leachate quantity is not as difficult to predict. Leachate quality is also difficult to predict because each landfill is unique and the wastes vary widely. The major factors that affect leachate quantity and quality include the type of disposed waste, hydrogeological and climactic conditions, age of the landfill, phase of waste decomposition, and chemical and physical properties of the precipitation (Fatta et al. 1998; Galvez et al. 2010; Kilmer et al. 1999; Zhao et al. 2006a). Leachate quantity and quality are site specific. In arid regions, leachate quantity can be negligible to non-existent. While in areas of wet climate, nearly 100 % of precipitation can become leachate. Once the adsorptive capacity of the waste field capacity has been achieved, continuous leachate flow will occur (Visvanathan et al. 2011).

The characteristics of leachate vary according to the operational stage of the landfill and the climatic features of the location of the landfill. Leachates from old sites are usually highly contaminated with ammonium resulting from the hydrolysis and fermentation of nitrogen-containing fractions of biodegradable refuse substrates (Kjeldsen et al. 1998; Lei et al. 2009). As stabilization of the waste proceeds, the accumulating concentration of ammonium is also influenced by washout as leachate is collected and removed for offsite treatment. However, in bioreactor landfills with leachate containment, collection, and in-situ recirculation to accelerate decomposition of readily available organic fractions of the wastes, leachate ammonium nitrogen concentrations may accumulate to much higher levels when compared to traditional landfills (He et al. 2011; Kumar et al. 2011). Recirculation of leachate will produce stabilized leachates containing relatively low concentrations of degradable carbon compounds but high concentrations of ammonium; therefore, COD and BOD will be removed, but ammonium concentrations will climb (Diaz 1999; He et al. 2006; Swati et al. 2007).

## 2.2 Nitrogen and Environmental Impact

Anthropogenic activities have adversely impacted the natural nitrogen cycle off balance. As a consequence, a number of environmental media have become overloaded with nitrogen compounds (Magnani et al. 2007). Commonly, agricultural and domestic wastewaters contain high concentrations of nitrogen, which can affect public health and have harmful ecological impacts. The major effect of excess nitrogen in the ecosystem is eutrophication, resulting in the overabundance of photosynthetic blue-green bacterial and algae (Gleisberg et al. 1976; Kontas et al. 2004). These occurrences are generally associated with the disposal of municipal wastewater and fertilizer application to agricultural lands (Bloetscher et al.

2005). Nitrogen can exist in different oxidation forms such as organic nitrogen, ammonium, nitrite, and nitrate. Ammonium is extremely toxic to fish and many other aquatic organisms and it is also an oxygen-consuming compound, which can deplete the dissolved oxygen in water (Passell et al. 2007; Paungfoo et al. 2007). Nitrite is a potential public health hazard in water consumed by infants (Murawska-Cialowicz et al. 2000). In human beings' body, nitrite can oxidize ferrous iron to form methemoglobin, which binds oxygen less effectively than normal hemoglobin, resulting in a decrease in oxygen levels and leading to shortness of breath, diarrhea, vomiting, and in extreme cases even death, especially for kids (Power et al. 2007). Although nitrate itself is not toxic, its conversion to nitrite is a concern to public health.

A major source of nitrogen pollution of surface water is urban wastewater. Therefore, modern wastewater treatment systems have been designed to remove nitrogen compounds more efficiently. Different processes have been developed for this purpose, of which biological denitrification is considered to be an efficient and cost-effective process (Randall and Cokgor 2001). Increasing numbers of wastewater treatment plants are now operated with denitrification as an essential step in the treatment system. Biological nitrogen removal is generally used for elimination of nitrogen from wastewater, which usually includes ammonification, nitrification, and denitrificaion. In ammonification, microorganisms decompose the organic nitrogen and produce ammonium. In nitrification, microorganisms oxidize the ammonium compounds to nitrite and then to nitrate. In denitrification, nitrate is converted to nitrogen gas and released to the atmosphere. It has been demonstrated that nitrification and denitrification are important nitrogen removal mechanisms in a wastewater treatment process since ammonium is especially abundant in wastewater streams, although nitrate or nitrite can also be present (Zhou 2007). While denitrification is generally a stable process once it is initiated, various factors such as external pH, the presence of oxygen and the availability of carbon and nitrogenous compounds may disturb the development of denitrifying cultures (Blackburne et al. 2008; Blaszczyk et al. 1985; Kim et al. 2003).

### 2.3 Treatment of Landfill Leachate with High Ammonium Content

Landfill leachate stream can be compared to a complex wastewater stream with varying characteristics. Landfill leachate characteristics not only vary because of the different kinds of waste present, but also vary according to the landfill age (Kulikowska and Klimiuk 2008). Usually landfill leachate from old landfills is rich in ammonium nitrogen due to the hydrolysis and fermentation of the nitrogenous fractions of the biodegradable wastes. Leachate from young landfills typically contains high dissolved solids content as well as high concentrations of organic matter compared to domestic wastewater (Khattabi et al. 2002). Leachate is usually handled in two ways: single pass leachate and recirculating leachate (Haydar and Khire 2006; Khire and Haydar 2007). For single-pass leachate, the liquid stream is collected, stored in a lagoon or tank, and treated either on-site or off-site before discharge to a receiving stream or subsequent treatment system. For the recirculating case, leachate is collected and recycled by reintroducing the leachate into the landfill. Compared with single-pass leaching, re-circulating leachate can enhance the landfill moisture content and increase resource recovery and site reutilization opportunities (McCreanor and Reinhart 1999; Qu et al. 2004; Reinhart 1996; Reinhart et al. 1996).

At landfills where leachate recirculation is practiced, ammonium concentrations of leachate may accumulate to much higher levels than those of conventional single-pass leaching, which trigger numerous problems to the environment including surface water eutrophication (Gobler and Boneillo 2003). Other damaging impacts resulting from nitrogenous discharges include reduction of chlorine disinfection efficiency, dissolved oxygen depletion in receiving waters, adverse public health effects, and a reduction in suitability for reuse (Hatano et al. 2005; Hondeveld et al. 1999; Schepers et al. 1991; Zavilopulo et al. 2005). Due to the toxic effects that ammonium produces, the ammonium level must be treated to an acceptable level of <10 mg/l before it is discharged (Adeyemo et al. 2008).

Leachate with a high ammonium and organic concentration is a major challenge for of landfill managers. There are many different leachate treatment options, including complex and expensive ex-situ physical-chemical and biological processes (Djelal et al. 2010; Xie et al. 2010; Yabroudi et al. 2010; Yang and Tsai 2011). Studies have shown that recirculation of leachate can produce stabilized leachates containing relatively low concentrations of degradable carbon compounds but high concentrations of ammonium (McCreanor and Reinhart 2000; Qu et al. 2004). Since carbon compounds are being removed in-situ, consideration has also been given to treating leachate ammonium in-situ in two stages: aerobic nitrification and subsequent anoxic denitrification.

### 2.3.1 Nitrification

Ammonium is one of the most important components in wastewater which has to be removed before effluents can be discharged. This is mostly achieved by complete oxidation to nitrate, and subsequent reduction of the nitrate to dinitrogen gas under anoxic conditions at the expense of organic compounds (Zeng et al. 2004). Conversion of nitrogen to the appropriate form for nitrogen removal is controlled by several biochemical reactions and nitrification is widely used to remove ammonium from wastewater as the first removal step by biological oxidation (Azevedo et al. 1995; Li et al. 2009; Yusof et al. 2010; Yusof et al. 2011). This biochemical reaction is part of the nitrogen cycle occurring in nature. In this cycle, aerobic biochemical reactions result in the oxidation of ammonium to nitrite, and then nitrite to nitrate. The overall biochemical process of oxidation of $NH_4^+$ to $NO_2^-$, then finally to $NO_3^-$ is known as nitrification. Nitrification is performed by the group of bacteria known as nitrifiers. Nitrification is assumed to be performed in two steps. First ammonium is converted to nitrite by *Nitrosomonas* and then nitrite is converted to nitrate by *Nitrobacter* (Montras et al. 2008). The overall reactions are written as:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \qquad (3)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (4)$$

$$NH_4^+ + 2.0\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+ \qquad (5)$$

The stoichiometric oxygen demand is 3.43 mg $O_2$ per mg of ammonium nitrogen converted to nitrite nitrogen and 1.14 mg $O_2$ per mg $NO_2$ converted to nitrate nitrogen. Thus, the overall demand is 4.57 mg $O_2$ per mg $NO_3$ formed. Since there is some buildup of autotrophic biomass, the total actual oxygen consumption is reported to be in the range of 4.2 – 4.3 mg/mg. For design calculations, an overall oxygen demand of 4.3 mg $O_2$ per mg $NO_3$ formed is widely used.

Nitrification is sensitive to pH. *Nitrosomonas* activity is inhibited by low concentrations of $HNO_2$, the result being a buildup of ammonium nitrogen (Zhou et al. 2008). The inhibition begins at 0.8 to 2.8 mg/l $HNO_2$. Free ammonia inhibits *Nitrobacter* starting at 0.1 to 10 mg/l $NH_3$, leading to a buildup of nitrite nitrogen. Higher concentrations of free ammonia also inhibit *Nitrosomonas*. Since the concentrations of $HNO_2$ and $NH_3$ depend on pH and the $NO_2$ or $NH_4^+$ of the mixed liquor, a graph indicating the range of inhibitory conditions has been developed (Figure 1) (Neufeld and Knowles 1999).

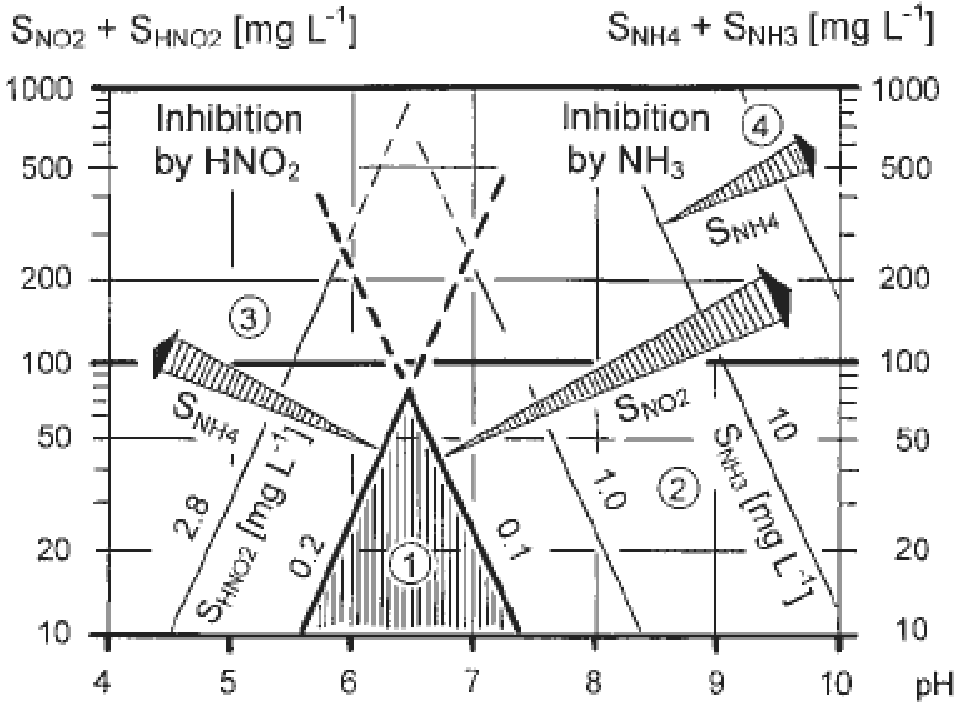

Figure 1. Inhibition of Nitrification by Free Ammonia and Nitrous Acid

It is evident that at low values of $NH_4^+$ and $NO_2$ and a pH of 6 to 7, as it is usual when treating municipal wastewater, inhibition does not occur (shaded area of Figure 1). When treating high strength wastewater such as that from rendering plants, disturbances are frequently observed, due to a buildup of nitrite (Pambrun et al. 2004).

### 2.3.2 Denitrification

From an engineering point of view, denitrification can be considered as heterotrophic respiration using nitrate instead of dissolved oxygen as the electron acceptor . Nitrate is finally converted to gaseous nitrogen by numerous reduction steps.

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (6)

Since nitrous oxide ($N_2O$) is a greenhouse gas, some concern about biological nitrogen removal has arisen (Green et al. 2002). A simplified overall reaction of the conversion of nitrate to molecular nitrogen may be written as:

$2\ NO_3^- + 2\ H^+ \rightarrow N_2 + H_2O + 2.5\ O_2$ (7)

If a substrate of acetate is added, the reaction becomes:

$5\ CH_3COOH + 8\ NO_3^- \rightarrow 4\ N_2 + 8\ HCO_3^- + 2\ CO_2 + 6\ H_2O$ (8)

The above equations indicate that for one mole of nitrate denitrified, one mole of alkalinity is gained and 1.25 moles of $O_2$ equivalent becomes available for heterotrophic respiration, which is equal to 0.645 mg of oxygen equivalent gained per mg of $NO_3^-$ to be denitrified. If only nitrite has to be denitrified, 1.7 mg of oxygen equivalent is gained per mg of $NO_2$ denitrified.

### 2.3.3 Disadvantage of Traditional Nitrogen Removal Technology

Ammonium is one of the most important nitrogen compounds in the surface water and other ecosystems since organic nitrogen can be converted to ammonium through hydrolysis and mineralization (Caffaz et al. 2006). Traditionally, ammonium removal is often achieved using nitrification/denitrification systems. In such systems, nitrifying bacteria oxidize ammonium to nitrate under aerobic conditions, and nitrate is subsequently reduced to dinitrogen gas under anoxic conditions (Zeng et al. 2004). Nitrifying bacteria are mainly autotrophic and derive energy from the oxidation of ammonium or nitrite. The oxidized nitrogen compounds can also be used as alternative electron acceptor by denitrifying bacteria. The denitrifiers are mostly heterotrophic and need reduced organic compounds as energy and carbon sources. If not enough organic compounds are available, addition of an exogenous carbon source, such as methanol, is often required to achieve complete denitrification. The introduction of oxygen into wastewater for the oxidation of ammonium requires a large amount of energy. Furthermore, the amount of organic compounds present is the wastewater is often limited, therefore, the addition of organic compounds such as methanol is required. Due to the long sludge age required for nitrification, large reactors are also needed. Some of these limitations might be circumvented by the application of two recently developed biological processes of ANAMMOX.

### 2.3.4 ANAMMOX

Recent studies have shown that it is possible to have substantial nitrogen losses in reactors with a low dissolved oxygen concentration and with low amounts of organic compounds present in the wastewater (Feng et al. 2007). The nitrogen loss could not be accounted for by the amount of organic compounds present in the wastewater. It is very likely that, in these systems, an autotrophic denitrification process is taking place and the bacteria capable of ANAMMOX might be involved in these processes (Cema et al. 2006). 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). The ANAMMOX includes partial nitrification of ammonium to nitrite by fast-growing nitrifiers, and denitrification of nitrite to dinitrogen gas using ammonium as electron donor. In this way nitrogen is removed with a minimum of organic compounds and energy (Hwang et al. 2005; Liu et al. 2005). Combined, these oxygen-limited systems could be described by:

$$1NH_3+1.5O_2 \rightarrow 1NO_2^-+H_2O+H^+ \qquad (9)$$

$$1\ NH_3+1.32\ NO_2^-+H^+ \rightarrow 1.02\ N_2+0.26\ NO_3^-+2\ H_2O \qquad (10)$$

$$1\ NH_3+0.85\ O_2 \rightarrow 0.11\ NO_3^-+0.44\ N_2+0.14\ H^++1.43\ H_2O \qquad (11)$$

Since ANAMMOX bacteria are reversibly inhibited by low (0.5% air saturation) concentrations of oxygen, the process must occur under oxygen-limiting conditions. The aerobic ammonium oxidizers will have to remove virtually all of the oxygen from the liquid. However, aerobic ammonium oxidizers are known to produce $N_2O$ and NO at low-oxygen conditions. The combined ANAMMOX process, in one-reactor system, may thus lead to a variety of nitrogen based compounds.

Ammonium oxidation has been operated without any biomass retention (Tsushima et al. 2007). This means that the sludge age equals the hydraulic retention time. In such a system, the effluent concentration is only dependent on the growth rate of the bacteria involved, and independent of the influent concentration. However, the organisms involved have a low affinity for ammonium. In practice this will lead, by application in a chemostat, to effluents with relatively high ammonium concentrations. Therefore, the ANAMMOX process is most suited to treat wastewater with a high ammonium concentration (>500 mg N/l), where the effluent quality is not critical (Bekmezci et al. 2011; Melicz 2003). The ANAMMOX process on sludge digestion effluents is performed at 30 - 40 ℃ in a chemostat without any biomass retention, therefore the dilution rate can be set at such a rate that ammonium oxidizers grow fast enough to stay in the reactor, while the nitrite oxidizers are washed out (Tokutomi et al. 2011; van Dongen et al. 2001).

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

$$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O \qquad (12)$$

This stoichiometric reaction implies that no addition of base is necessary since sludge liquor resulting from anaerobic digestion will generally contain 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 as electron donor under anoxic conditions:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O \qquad (13)$$

The bacteria catalyzing the ANAMMOX reaction are autotrophic, this means that nitrite can be converted to dinitrogen gas without the use of organic compouds or the addition of an external organic compound source.

ANAMMOX is a novel, promising, low-cost alternative to conventional denitrification systems. Furthermore, if the ANAMMOX process is combined with a preceding nitrification step, only part of the ammonium needs to be nitrified to nitrite while the ANAMMOX process combines the remaining ammonium with this nitrite to yield dinitrogen gas (Imajo et al. 2004; Kuenen 2008). This will reduce oxygen demand in the nitrification reactor and lead to a second reduction in cost. The biomass yield of the ANAMMOX process is low; consequently, little sludge is produced. The low sludge production is the third factor that contributes to the substantially lower operation costs compared to conventional denitrification systems (Hua et al. 2009; Mikhailovskaya 2008). However, the low biomass yield also demands efficient sludge retention. These characteristics make the ANAMMOX process an interesting option to remove ammonium from concentrated, warm effluents of sludge digestion dewatering facilities.

During the oxidation of ammonium to nitrate, nitrite is produced as an intermediate product. Theoretically, nitrite can rarely exist at a high concentration in terrestrial and aquatic environments. However, it has been reported that nitrite can be accumulated in ecosystems (Peng and Zhu 2006; van Dongen et al. 2001). Soil nitrite accumulation was reported for situations where high concentrations of urea, either by the addition of chemical fertilizers or by urine, were added to the soil (vandeGraaf et al. 1997). Buildup of nitrite was also observed in the pore space of some estuarine sediments as well as in some treatment plants (Strous et al. 1998; Vandegraaf et al. 1995). High nitrite concentration was attributed to a lower affinity for oxygen of the nitrite oxidizers as compared to ammonium oxidizers (Jetten et al. 1997). In the presence of sufficient nitrite and ammonium, anaerobic ammonium oxidation (ANAMMOX) would occur (Caffaz et al. 2006). The ANAMMOX process is strictly anaerobic, and is inhibited by high concentrations of oxygen and phosphates (Strous et al. 1999). ANAMMOX presents significant potential for the treatment of ammonium-rich wastewater since ANAMMOX is a microbiologically mediated exergonic process (Strous et al. 1997).

### 2.3.5 MAP Precipitation

Many widely used physicochemical processes can be applied in the treatment of leachate with high ammonium content, such as air stripping, membrane separation processes, chemical precipitation (Gavrilescu and Schiopu 2010; Novelo et al. 2009; Quemeneur et al. 2001). Currently, the most common method for eliminating a high concentration of $NH_4^+$–N (>3000 mg/l) in wastewater treatment is the air stripping process (Novelo et al. 2009). However, a major concern about ammonia air stripping is the release of $NH_3$ into the atmosphere which has the potential to cause severe air pollution if ammonia cannot be properly absorbed with an absorbent such as $H_2SO_4$ or HCl. The other concern is calcium carbonate scaling of the stripping tower when lime is used for pH adjustment. Since the leachate from an aged landfill usually contains a high alkalinity just as a strong pH buffering system, the pH variation before and after stripping will consume a large amount of alkali (20 g/l of caustic soda is required to increase pH) and acid (10 ml/l of 37% hydrochloric acid is required to absorb stripped $NH_3$) (Cheung et al. 1997; Guo et al. 2010; Hao et al. 2010). A significant amount of carbonate precipitate is usually formed in the process and may cause severe operation and maintenance problems. Moreover, a large stripping tower will be needed due to foaming when $NH_4^+$–N in raw leachate is stripped.

For the above reasons, other effective alternatives to eliminate $NH_4^+$–N in leachates need to be ascertained. Recently, chemical precipitation of ammonium by forming MAP precipitation has been studied widely (Akkaya et al. 2010; Li and Zhao 2002; Ra et al. 2011; Sertyesilisik et al. 2009). It is a useful technology to remove ammonium from leachate. The precipitation of $NH_4^+$–N by forming MAP precipitate has been practiced for different types of wastewater such as tannery effluent in leather industries, digester supernatant in wastewater treatment plants, wastewater from coke plants and nitrogen production and also sludge liquor (Espiell et al. 2003; Huang et al. 2008b; Kabdasli et al. 2000a; Li et al. 2007; Ra et al. 2011). These studied results indicated efficient removals of $NH_4^+$–N. Cost estimates indicated that the cost of the precipitation process is similar to the cost of nitrification and denitrification of 1 $m^3$ of domestic wastewater, and similar to or about 20% more expensive than that of air stripping. Chemical precipitation as MAP can form virtually or completely insoluble compounds that can be easily separated from the water phase (Li and Zhao 2002; Stumpf et al. 2008). MAP is a white crystalline substance consisting of magnesium, ammonium and phosphorus in equal molar concentrations. For example, its insoluble form of $MgNH_4PO_4$ $6H_2O$ with a low solubility of 0.023 g/100 ml-$H_2O$ at 0 ℃, is well known in analytical chemistry.

# 3. Materials and Methods

## 3.1 Landfill Leachate and Soil Sample Collection

Landfill leachate was collected from leachate sumps at the Leon County Landfill, located in Tallahassee, FL (Figure 2). The Leon County Landfill accepts Class III commercial and residential wastes through Marpan Recycling, which include yard trash, construction and demolition (C&D) debris, processed tires, asbestos, carpet, cardboard, paper, glass, plastic, furniture other than appliances, and other materials approved by the Florida Department of Environmental Protection (FDEP) (Figure 3). Yard debris (leaves and limbs) and waste tires are accepted through the Solid Waste Management Facility (Figure 4). In addition, the Leon County Landfill receives electronic waste such as miscellaneous electronics, computers and peripherals, televisions, video game systems, handheld electronics, and cell phones as well as household hazardous waste and stryofoam TM (packaging foam only), etc.

Figure 2. The Leon County Landfill under Operation

Figure 3. The Leon County Landfill Receiving Commercial and Domestic Waste

Figure 4. The Leon County Landfill Receiving Yard, Construction and Demolition Waste

The collected samples were stored in temperature-controlled containers and immediately transported to the laboratory. Based on the sample analytical results, the leachate had concentrations of COD up to 20,000 mg/l, $NH_4^+$-N up to 500 mg/l, and phosphorus up to 200 mg/l (Figure 5).

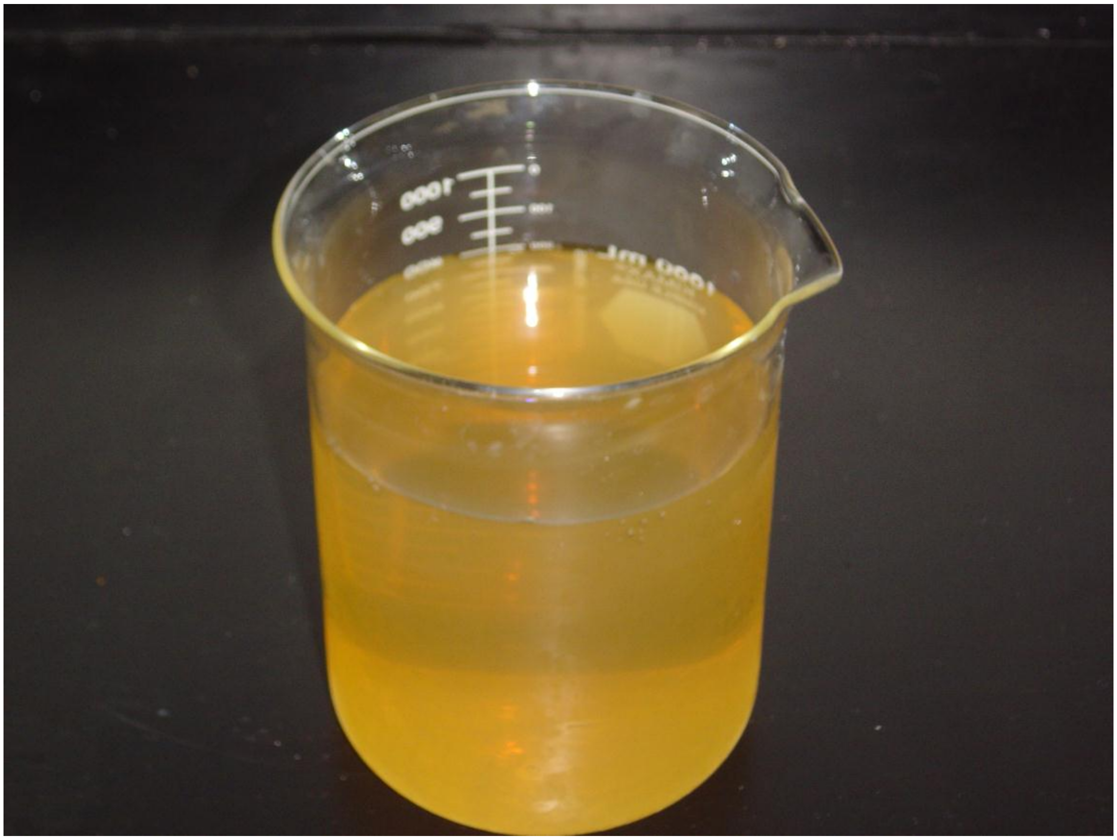

Figure 5. Leachate Collected from the Leon County Landfill

Soil samples were also collected from this landfill site. Specifically, soil samples were collected 1 to 3 feet below land surface, 100 to 300 feet away from the landfill (Figure 6). 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 they were used in the experiments.

Figure 6. Soil Profile at the Leon County Landfill

The soil varied slightly in soil type: 40% clay, 30% sandy clay, and 30% highly clayey sand. The analytical results indicated that the soil had a pH between 6.0 and 7.2 and was strongly humic with humus content between 3.5 and 7%. The soil hydraulic properties are summarized in Table 1. The saturated conductivity, $K_s$, decreased with the increase of soil depth, while the residual water content, $\theta_r$, and the saturated water content, $\theta_s$, were in the similar range for soil samples collected from different depth. There was also a general trend that the inverse of the air-entry potential, $\alpha$, and the parameter related to pore size distribution, n, decreased with the increase of soil depth. A sieve analysis was performed to characterize the soil size distribution. Briefly, 600 ~ 700 g soil was weighed and placed in a drying oven for approximately 10 days at 30 ℃. After the samples were determined to be thoroughly dry, the dry weight was recorded. During drying, all samples were broken up with a pestle and mortar. A stack of sieves was arranged from top to bottom in the respective order of decreasing sieve size openings, i.e. sieve number 4, 10, 20, 40, 60, 100, 140, and 200. A pan was placed after the 200 sieve. The dry soil samples were placed on a sieve shaker for 15 minutes. The total weight of the soil samples retained on each sieve was then determined (Table 2). Based on the sieve analysis, approximately 75.43% of the particles were found to be smaller than 0.425 mm, i.e., passing through the number 40 sieve (Table 2). Approximately 5.22% of the particles were found to be smaller than 0.075 mm, i.e., passing through the number 200 sieve (Figure 7).

**Table 1. Soil Hydraulic Properties**

| | Sample Depth | | | | |
|---|---|---|---|---|---|
| | 0 - 20 cm | 20 - 40 cm | 40 - 60 cm | 60 - 80 cm | 80 - 100 cm |
| $K_s$ (cm/hr)* | 2.98 – 58.3 | 3.50 – 52.7 | 2.74 – 31.4 | 0.76 – 32.1 | 0.35 – 17.2 |
| $\theta_s$ | 0.47 | 0.51 | 0.42 | 0.35 | 0.31 |
| $\theta_r$ | 0.11 | 0.09 | 0.07 | 0.06 | 0.06 |
| $\alpha$ ($m^{-1}$) | 8.3 | 6.7 | 8.2 | 7.1 | 6.3 |
| N | 1.41 | 1.27 | 1.45 | 1.32 | 1.08 |

* $K_s$ is the saturated hydraulic conductivity, $\theta_r$ is the residual water content ($cm^3/cm^3$), $\theta_s$ is the saturated water content ($cm^3/cm^3$), $\alpha$ is the inverse of the air-entry potential ($m^{-1}$); and n is the parameter related to pore size distribution (-).

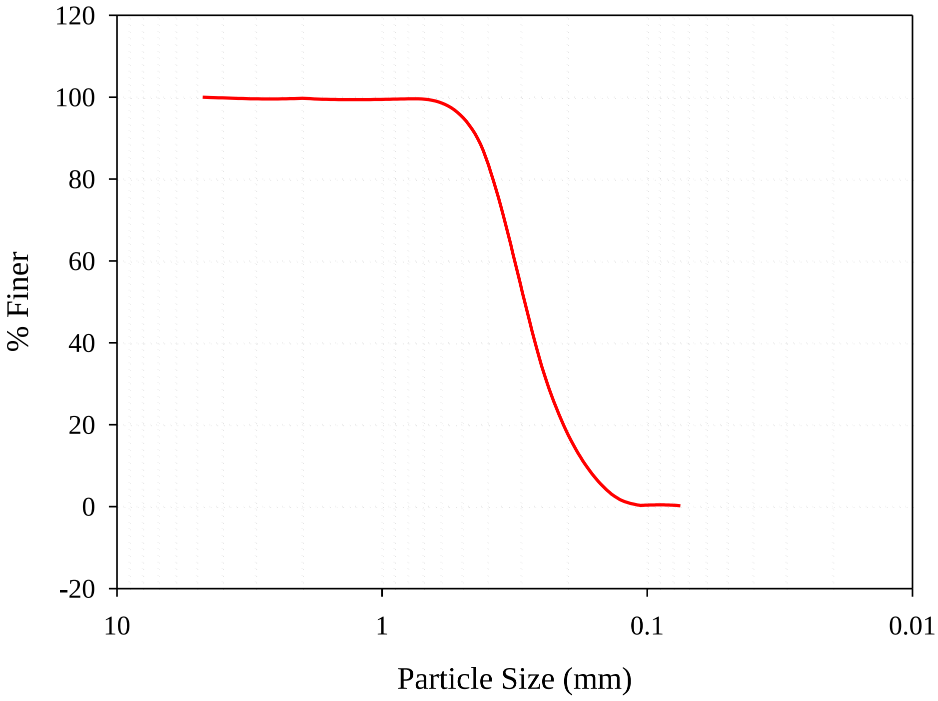

Figure 7. Soil Particle Size Distribution from the Leon County Landfill Soil Sample

**Table 2. Soil Size Distribution**

| Sieve # | Grain Size (mm) | Mass of Soil Retained (g) | % of Mass Retained | Cumulative Retained (%) | % Finer |
|---|---|---|---|---|---|
| 4 | 4.750 | 0.1 | 0.018 | 0.018 | 99.98 |
| 10 | 2.000 | 7.4 | 1.345 | 1.363 | 98.63 |
| 20 | 0.850 | 38.2 | 6.943 | 8.306 | 91.69 |
| 40 | 0.425 | 89.3 | 16.23 | 24.53 | 75.43 |
| 50 | 0.250 | 134.0 | 24.35 | 48.89 | 51.11 |
| 100 | 0.150 | 141.3 | 25.68 | 74.57 | 25.43 |
| 140 | 0.106 | 70.0 | 12.72 | 87.29 | 12.70 |
| 200 | 0.075 | 41.2 | 7.488 | 94.78 | 5.216 |
| Pan | N/A | 28.7 | 5.216 | 100 | 0 |
| | | 550.2 | 100.000 | | |

## 3.2 ANAMMOX Experiments

The laboratory scale anaerobic-partial nitrification-ANAMMOX reactor consisted of three sequencing batch CSTRs with a working volume of 500 ml each (Figure 8 and Figure 9). 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 37-42°C using a temperature-controlled water bath. Depending on the retention time, leachate was pumped from one reactor to the subsequent reactors. Throughout the experiments, the bulk liquid was sampled and measured for COD, $BOD_5$, ammonium, nitrite, and nitrate.

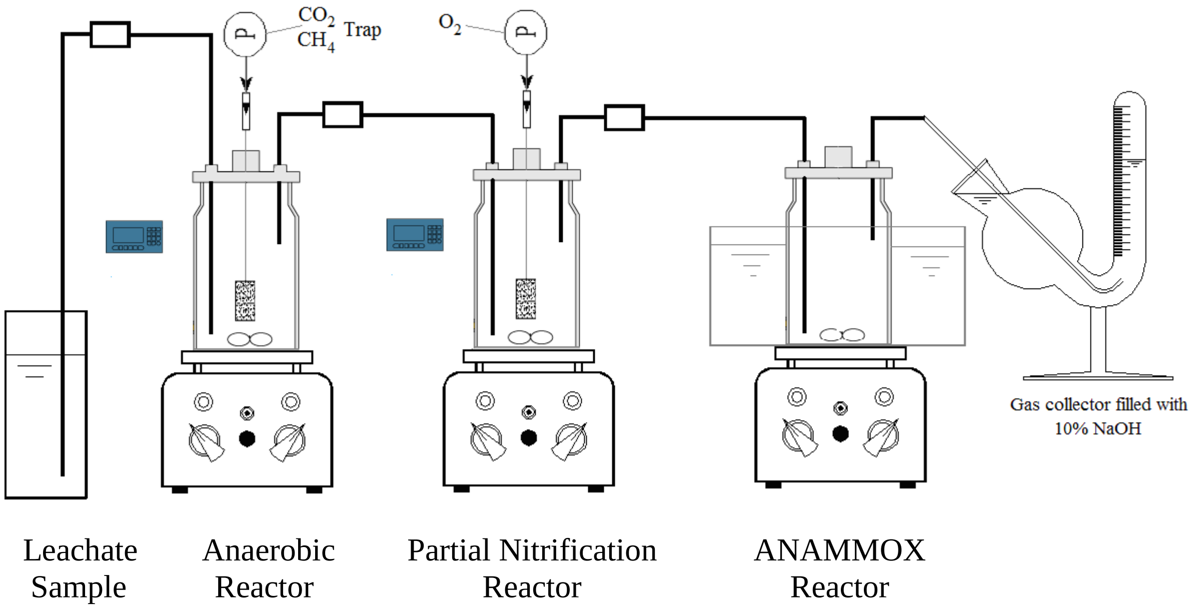

Figure 8. ANAMMOX Experiment Process Illustration

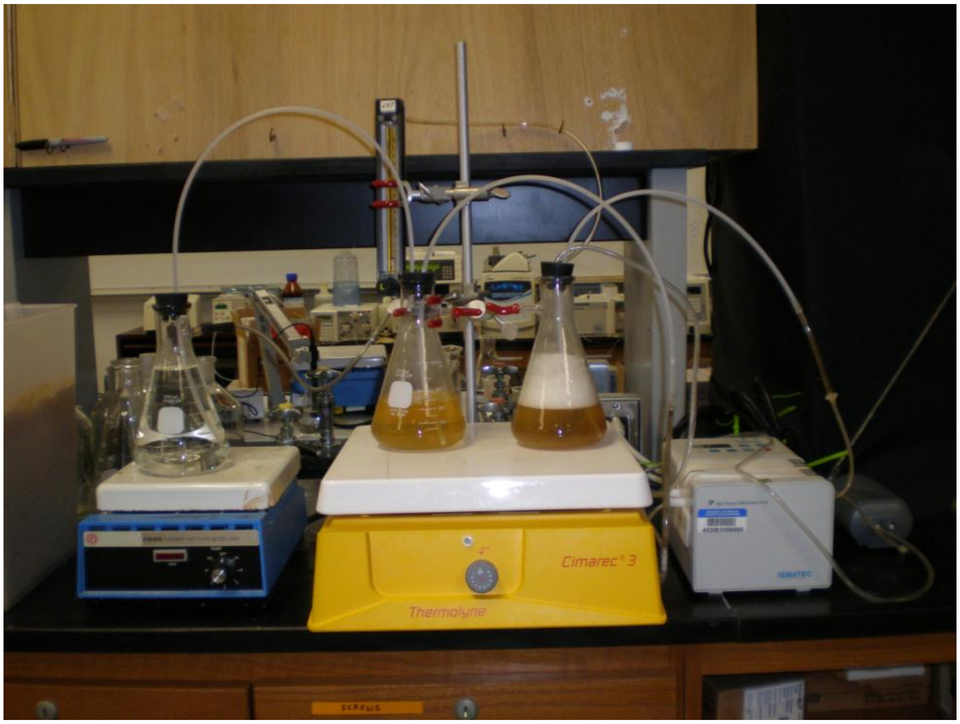

Figure 9. ANAMMOX Experiment Laboratory Setup

Reactions of ANAMMOX are an autotrophic process and no organic matter is required. The key step for above experiments is to achieve partial nitrification and obtain stable nitrite accumulation. Dissolved oxygen and alkalinity are important factors for partial nitrification. Depending on the dissolved oxygen and alkalinity of the leachate, it is possible to convert a fraction or nearly the entire load of ammonium into nitrite. The effect of dissolved oxygen and alkalinity on partial nitrification and subsequent ANAMMOX was investigated in this research.

The inocula for the anaerobic reactor were collected from the anaerobic digester from the Thomas P. Smith Water Reclamation Facility in Tallahassee, FL. The inocula for partial nitrification in the second reactor were cultured from the conventional nitrifying sludge in the sedimentation tank of the biological nitrogen removal system from the Thomas P. Smith Water Reclamation Facility. Specifically, 1 liter of regurgitant sludge was inoculated into the reactor. The dissolved oxygen 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 one month of adaptation, the inocula were able to work functionally with a bulk liquor suspended solids concentration maintained at ~ 1,000 mg/l. The inocula for ANAMMOX were cultured similarly as above with 50 mg/l $NH_4^+$-N and 200 mg/l $NO_2^-$ in the absence of dissolved oxygen.

Prior to conducting the experiments, a start-up phase was necessary. The start-up phase took about 2 weeks with no leachate addition. During the start-up period, the reactors were fed with glucose mineral medium containing mineral salts, sodium thioglycollate, $NaHCO_3$ with a COD concentration of 5,000 mg/l and an organic loading rate of 4 $kg/m^3$/day. After the start-up period, the COD concentration of leachate was steadily increased to 20,000 mg/l.

During the experiments, the sequential reactor system was operated at a constant flow rate of 2 liters per day. Sludge retention time in the reactors was adjusted to 15 days by wasting of mixed biomass from the reactors. The sequential reactor system was operated at dissolved oxygen concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.30, 0.40, 0.50 and 0.60 mg/l to achieve a stable nitrite accumulation for ANAMMOX. Five different levels of alkalinity of 200, 300, 400, 500 and 600 mg/l were examined in these experiments. For each experiment, influent and effluent COD, $BOD_5$, ammonium, nitrite, nitrate, nitrogen gas production, pH, oxidation/reduction potential (ORP) and dissolved oxygen were monitored throughout the course of the experiments. The COD in the influent and effluent samples were determined by the closed reflux colorimetric method. $BOD_5$ measurements followed the standard methods for wastewater treatment. Nitrogen gas production was measured using a liquid displacement method by passing the gas through distilled water containing 2% $H_2SO_4$ (w/v) and 10% NaCl (w/v). Dissolved oxygen concentration, pH and ORP of the bulk liquid were measured directly by a DO meter (WTW Oxi 315i, Cellox 325, Germany), pH meter (Waterproof pH Tester 10, Oakton Instruments, Vernon Hills, IL USA) and ORP meter (Waterproof ORP Tester 10, Oakton Instruments, Vernon Hills, IL USA), respectively.

Bulk liquid sample (25 ml) was collected regularly from the reactors each day. The samples were settled for 30 min and the supernatant (about 15 ml) was filtered for further measurements. Ammonium, nitrite and nitrate concentrations were determined colorimetrically by means of nesslerization, N-(1-naphthyl)-ethylendiamine dihydrochloride, and thymol spectrophotometry (Mccrady 1966). At the end of the experiments, sludge volume index of the bulk liquor was determined after 30 min of settling by measuring the volume occupied by the mass of sludge, suspended solids and volatile suspended solids concentrations were measured following the standard methods for wastewater treatment (Mccrady 1966), and the amounts of aerobic ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and ANAMMOX bacteria in the reactors were determined by the method of most probable number (MPN) (Mccrady 1966).

### 3.3 MAP Precipitation Experiments

The laboratory-scale MAP precipitation treatment process consisted of two CSTRs, each with a working volume of 1 liter (Figure 10). The MAP reaction tank was equipped with pH, DO and ORP probes and is continuously stirred by a single mechanical blade. In most cases, to remove all available nutrients such as ammonium and phosphorus from the water phase through MAP precipitation, magnesium is deficient and needs to be added. Two types of magnesium, $Mg(OH)_2$ and $MgCl_2$, are generally used in MAP precipitation. Compared to $Mg(OH)_2$, $MgCl_2$ is more widely used because it dissociates faster than $Mg(OH)_2$, resulting in a shorter reaction time. If MAP precipitation is used to remove ammonium only, $H_3PO_4$ or $NaH_2PO4$ are usually added. The required alkaline condition of MAP precipitation can be achieved by alkaline addition or pre-aeration (Battistoni et al. 1998). Even considering pH adjustment, it has been demonstrated that the addition of $Mg(OH)_2$ is not effective (Fujimoto et al. 1991). For this research, magnesium and phosphate were added as $MgCl_2$ $6H_2O$ and $Na_2HPO4 \cdot 12H_2O$ and pH was adjusted with lime. Both chemical addition and pH adjustment were made under flash-mixing conditions.

It has been demonstrated that the reaction time has an impact on MAP precipitation (Stumpf et al. 2008). For the experiments, ammonium removal by MAP precipitation and sedimentation was first tested as a function of reaction time. MAP was observed to be formed in the reaction tank and settled in the sedimentation tank. It was discovered that after 5 minutes of reaction and 20 minutes of sedimentation, ammonium removal became stable

(Figure 11). For the experiments, the leachate was first introduced to the reaction tank. After chemical addition and 5 minutes of mixing and reaction, the leachate was transferred to the sedimentation tank for MAP precipitation. After 20 minutes of sedimentation, the supernatant was pumped to the filter at a flow rate of 0.56 ml/min.

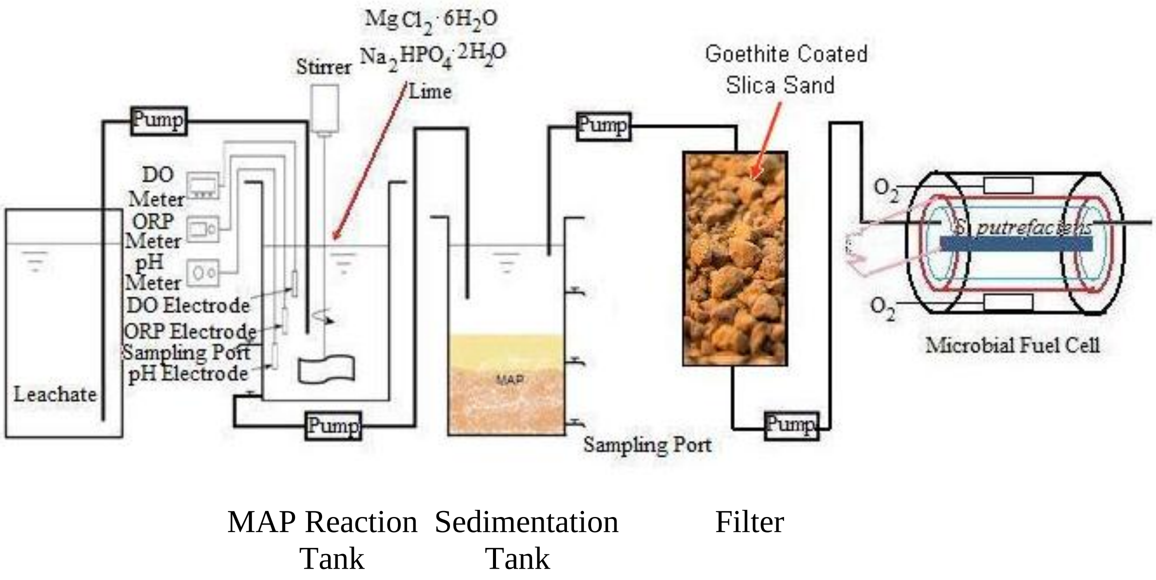

Figure 10. MAP Precipitation Experiment Illustration

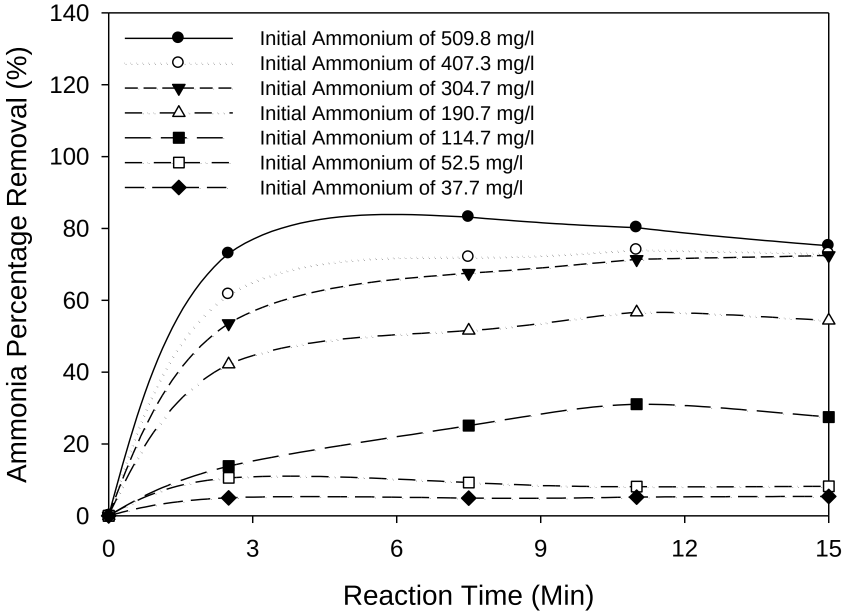

Figure 11. Ammonium Removal after Sedimentation as a Function of Reaction Time

Ammonium removal through MAP sedimentation was found to be relatively inefficient. Consequently, the effluent was introduced to a filter to provide further removal of unsettled micro-scale MAP. A filter (acrylic 2.5 cm × 15 cm column, Kimble-Kontes, Vineland, NJ) was added to the treatment train following the sedimentation tank to remove unsettled MAP (Figure 12). The filtration experiments were conducted using goethite-coated silica sand as the filtering material.

Goethite, a frequent and abundant form of iron oxide in soil and sediments, is an important component influencing phosphorous adsorption in natural aquatic environments or in the soil. Goethite has been used extensively in phosphorus adsorption studies in part because it is stable and can be easily produced in the laboratory. Goethite was prepared as described by Schwertmann et al. (Schwertmann and Cornell 1991). Briefly, 1.0M ferric nitrate was mixed with 1.0M KOH (1:9, v/v) and aged for 21 days at 25$^oC$. This suspension was then washed extensively with de-ionized water via centrifugation. The rinsed solid was re-suspended in 0.4 M HCl. After washed and dialyzed with de-ionized water, it was freeze-dried to obtain crystalline goethite. The obtained goethite was then coated on silica sand following the method of Schwertmann et al. (Schwertmann et al. 1985) and Scheidegger et al. (Scheidegger et al. 1993). Specifically, the goethite was mixed with silica sand (1:5, w/w) in 0.01M $NaNO_3$ solution (pH 7.5) and shaken for 48 hrs. Coated silica sand was then washed with 0.1M $NaNO_3$ (pH 7.0) via centrifugation. After being rinsed with de-ionized water, the coated silica sand was oven-dried at 110$^oC$. Goethite coated on the sand surface was determined by dissolving coated silica sand in $HNO_3$ (95%) and HF (40%) (2:1, v/v). The specific surface area of goethite-coated silica sand was measured by a surface area analyzer and was found to be 318 $m^2/g$.

The filter column was oriented vertically and sealed at the bottom with a custom frit to permit the flow of water and retain the filtering medium (Figure 13). Goethite-coated silica sand was packed in the column through $CO_2$ solvation to eliminate air pockets. Prior to the experiments, approximately 100 pore volumes of nano-pure de-ionized water were eluted through the column by a peristaltic pump to stabilize the column. During the experiments, supernatant from sedimentation tank was pumped into the column at a flow rate of 0.56 ml/min. After the filtration, the leachate was transferred to the sedimentation tank for MAP precipitation. Suspended solids were co-precipitated at this stage. After ammonium and suspended solid removal, the treated leachate was introduced to the microbial fuel cell, where volatile organic compounds were removed. For the microbial fuel cell, a highly porous graphite electrode was used as the anode, which was inoculated with *Shewanella putrefaciens*.

Figure 12. Filtration Experiments after MAP Precipitation

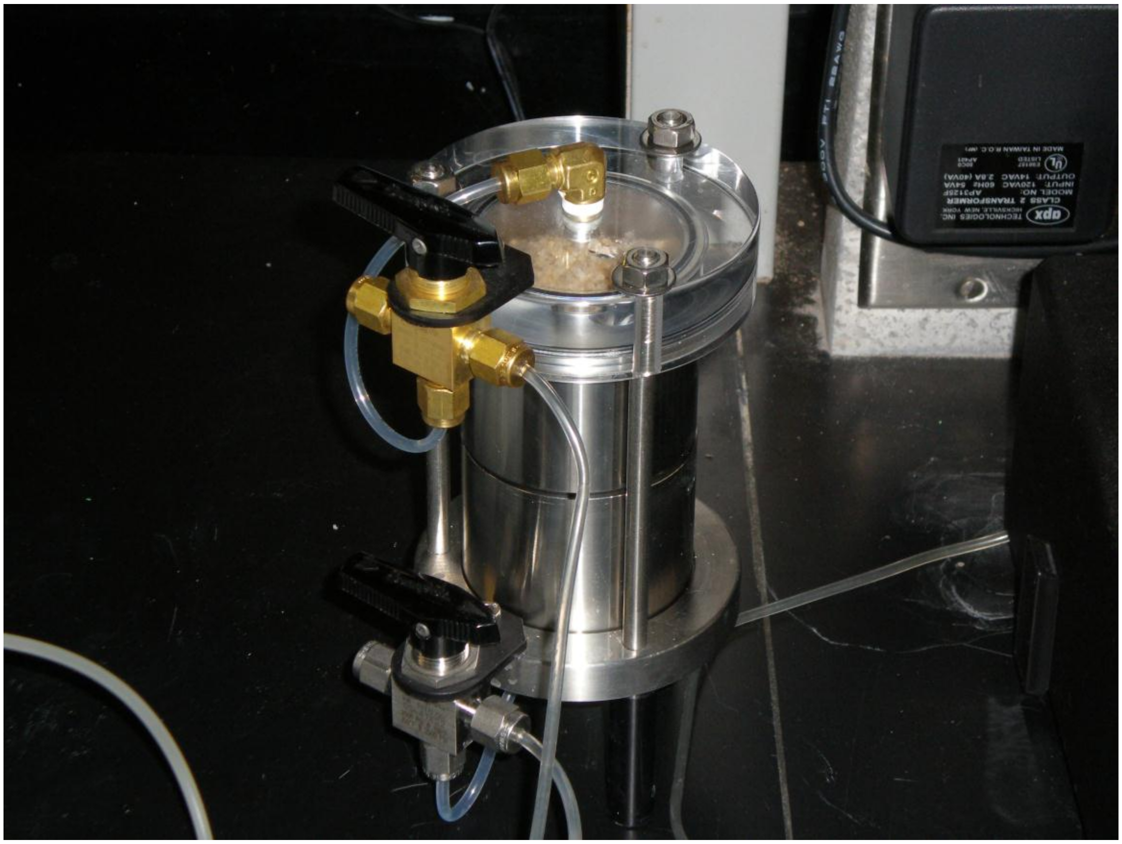

Figure 13. Filtration Column Assembly

A dual-chamber, continuous microbial fuel cell was attached to the MAP precipitation system after filtration (Figure 14). Graphite rods, without coated catalysts, were installed in the center of the inner chambers as the anodes. The anodes were inoculated with the cultured *Shewanella putrefaciens*. Carbon cloth (effective area of 12.6 $cm^2$, 30% wet proofing), coated with platinum catalysts (0.15 $mg/cm^2$, 5% Pt) served as the cathode. In the cathode chamber, $O_2$ served as the electron acceptor. The anodes and cathodes were connected through digital multimeters. Synthetic polymeric nanoporous membranes were used as the cation-exchange membrane (CEM).

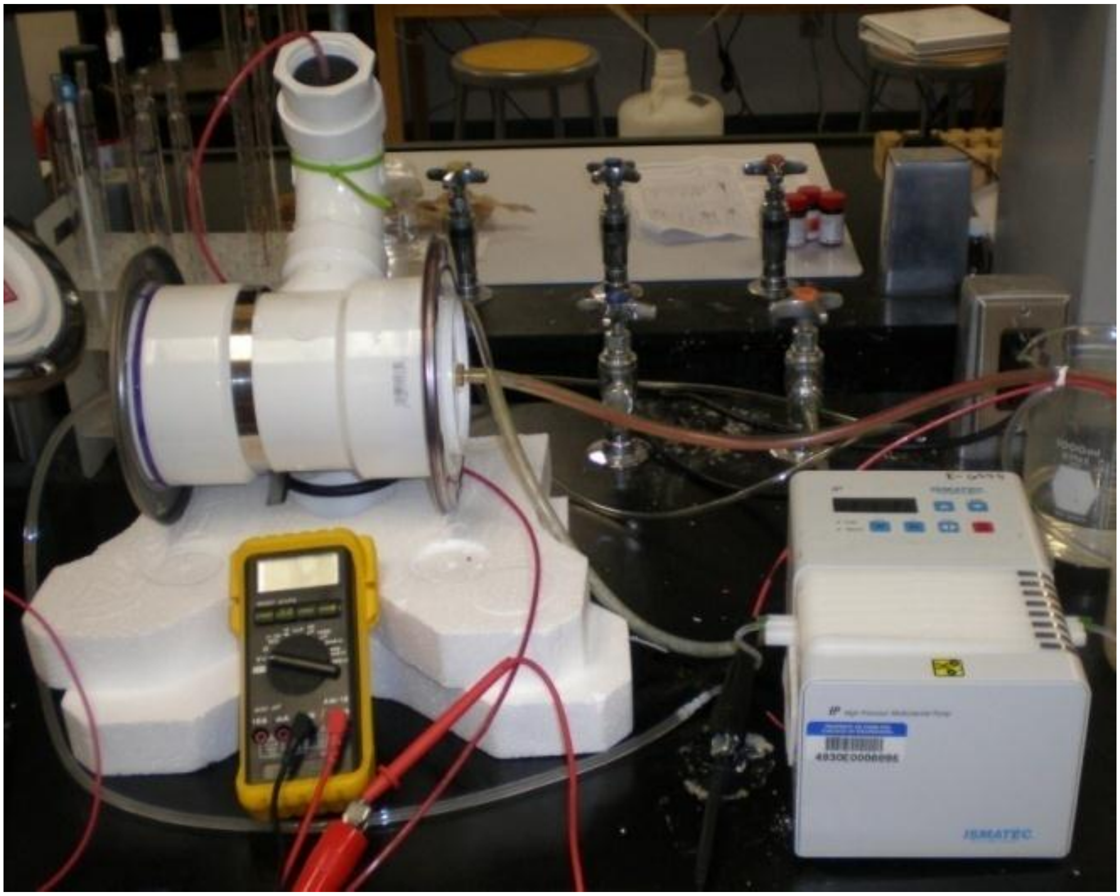

Figure 14. Continuous Microbial Fuel Cell Attached to MAP Precipitation Process

### 3.4 *Shewanella putrefaciens* Culturing

Electrochemically active *Shewanella putrefaciens* was cultured using collected soil samples as the inocula. Continuous cultivation and enrichment were carried out immediately in an anaerobic chamber after the samples were transported back to the laboratory. Specifically, 10 mg soil was transferred into a 250 ml serum bottle containing 100 ml sterilized culture media (Figure 15). The media had a composition (mg/l) of $KH_2PO_4$, 160; $K_2HPO_4$, 420; $Na_2HPO_4$, 50; $NH_4Cl$, 40; $MgSO_4·7H_2O$, 50; $CaCl_2$, 50; $FeCl_3·6H_2O$, 0.5; $MnSO_4·4H_2O$, 0.05; $H_3BO_3$, 0.1; $ZnSO_4·7H_2O$, 0.05; $(NH4)_6Mo_7O_{24}$, 0.03; glucose, 200; and ammonium chloride, 60. The pH of the media was adjusted to 7.4 with 1 M HCl or 1 M NaOH, after which the media were sterilized by autoclaving (121$^o$C and 1 atm) for 20 min. Glucose was filter-sterilized and aseptically added to the autoclaved media. 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.

Figure 15. *Shewanella putrefaciens* Culturing

A series of four culture enrichments were conducted in order to achieve the desired concentration of *Shewanella putrefaciens*. For the initial culture enrichment, 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 a black precipitate at the bottom and on the wall of the serum bottle could be observed. Then, 10 ml of enriched culture were transferred into 100 ml fresh culture media with approximately 50 mg/l $Fe^{3+}$ for the second-phase culture enrichment. 10 ml of enriched culture from the second phase were transferred into 100 ml fresh culture media with approximately 50 mg/l $Fe^{3+}$ for the third-phase culture enrichment and 10 ml of enriched culture from the third-phase were transferred into 100 ml fresh culture media with approximately 50 mg/l $Fe^{3+}$ for the fourth-phase culture enrichment. After the fourth-phase enrichment was completed, bacterial cells were harvested by centrifugation (6000 *g*, 15 min) and washed twice with fresh, anoxic $NaHCO_3$ buffer (0.05 M) under an extra-pure nitrogen atmosphere. The 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 $5\times10^9$ cells/ml. *Shewanella putrefaciens* was identified by polymerase chain reaction (PCR) analysis. Once *Shewanella putrefaciens* was screened out, it was enriched in 100 ml fresh culture media with approximately 50 mg/l $Fe^{3+}$.

### 3.5 $NH_4^+$, $Mg^{2+}$ and $PO_4^{3-}$ Molar Ratio on MAP Precipitation

In practice, MAP solubility product ($pK_s$) ranges from 9.94 to 13.26 (Ohlinger et al. 1998; Shin and Lee 1998; Stumm and Morgan 1996), thus its insoluble form can be easily formed and simply separated from the water phase. Still, there are several factors that affect the efficiency of ammonium removal by means of MAP precipitation, which include pH, stoichiometry of precipitating ions as well as reaction and settling time (Li and Zhao 2003). Among these factors, pH is the most important factor since it determines MAP solubility. Although $H^+$ concentration does not directly enter the ion-activity product equation, $MgNH_4PO_4$ $6H_2O$ precipitation is highly pH dependent because the activities of both $NH_4^+$ and $PO_4^{3-}$ are pH dependent (Nelson et al. 2003). It has been reported that the MAP solubility decreases with the increase of pH (Marti et al. 2008). pH also affects the mechanism and extent of interference of some cations such as calcium and iron. Therefore, a pH range of 7.0 to 10 was employed in the experiments to allow a multi-faceted evaluation. Stoichiometry of magnesium, phosphate and ammonium is important from the standpoint of MAP solubility, but it is also of importance for the formation of other precipitates such as MP, which significantly affects MAP precipitation. Therefore, variable $NH_4^+/Mg^{2+}/PO_4^{3-}$ molar ratios were practiced to provide optimum MAP precipitation results. Based on the theoretical calculations and experimental observations, $NH_4^+/Mg^{2+}/PO_4^{3-}$ molar ratios of 1:1:0, 1:1:0.5, 1:1:0.75, 1:1:1, 1:1:1.1, 1:1:1.25, 0:1:1, 0.5:1:1, 0.75:1:1, 1.1:1:1, and 1.25:1:1 were tested in this research.

Standard procedures were followed during the execution of the experiments and measurements obtained from instrumentation were made in accordance with procedures provided by the manufactures. The instrumentation was calibrated regularly and whenever work conditions changed. Data acceptability was determined in terms of precision, accuracy, representativeness, completeness, and comparability. Relative standard deviation, variance, and confidence interval were applied to test the accuracy and eliminate systematic and random errors.

# 4. Results

## 4.1 ANAMMOX Experiments

### 4.1.1 Partial Nitrification

After approximately one month of adaptation, the inocula for partial nitrification became effective with a bulk liquor suspended solids concentration maintained at ~ 1000 mg/l and volatile suspended solids at ~ 820 mg/l. The sludge volume and sludge volumetric index of the bulk liquor were kept at 5% and 50 $mL \cdot g^{-1}$ respectively. The partial nitrification was then operated at DO concentrations of 0.03, 0.07, 0.13, 0.15, 0.19, 0.30, 0.40, 0.50 and 0.60 mg/l to achieve a stable nitrite accumulation. For these processes, the influent ammonium concentration was diluted to 100 mg/l $NH_4^+$-N. For each DO concentration level, pH, oxidation/reduction potential (ORP) and DO profiles were monitored throughout the course of the experiments. At the same time, 25 ml bulk liquid was sampled regularly from the reactor. The sample was settled for 30 min and the supernatant (about 15 ml) was further filtered for measurements. Based on the experimental results, DO of 0.3 mg/l was chosen for the continued research to investigate the effect of alkalinity on partial nitrification. Five different levels of alkalinity were examined in these experiments with variable ammonium input concentrations.

At all DO concentration levels, nitrite was accumulated with only a small amount of ammonium converted to nitrate (Figure 16). The average nitrite accumulation ratio was about 95% with specific ammonium removal rate up to 0.86 kg $NH_4^+$-N/kg VSS per day and volumetric ammonium removal rate up to 0.70 kg $NH_4^+$-N/$m^3$ per day. The ammonium depletion rate and nitrate production rate (slope of lines in Figure 17 and Figure 16) increased with the increase of the DO concentration, whereas, the increase of nitrate production with the increase of the DO concentration was not obvious (Figure 16). The increase of ammonium depletion rate (Figure 17) and nitrite production rate (Figure 18) with the increase of the DO concentration was more pronounced at low DO concentrations (i.e., from 0.03 mg/l to 0.19 mg/l) than at high DO concentrations (i.e., from 0.30 mg/l to 0.60 mg/l). In fact, the increased ammonium depletion rate and nitrite production rate with the increase of the DO concentration was minimal after the DO concentration reached 0.30

mg/l. It was thus assumed that oxygen was no longer the limiting substrate when the DO concentration was above 0.30 mg/l. Since the following ANAMMOX process is strictly anaerobic, the dissolved oxygen concentration should be controlled below 0.30 mg/l in order to avoid impeding the ANAMMOX process and to maintain high partial nitrification efficiency at the same time.

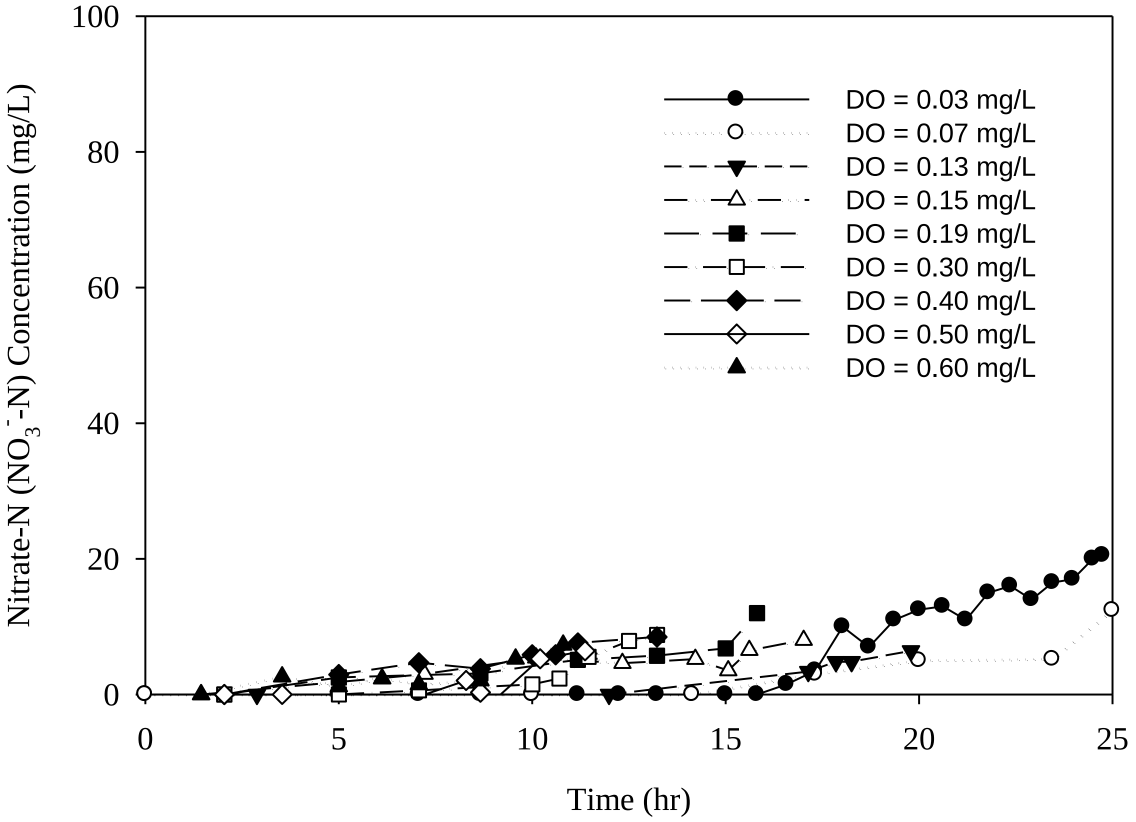

Figure 16. Nitrate Generation during Partial Nitrification

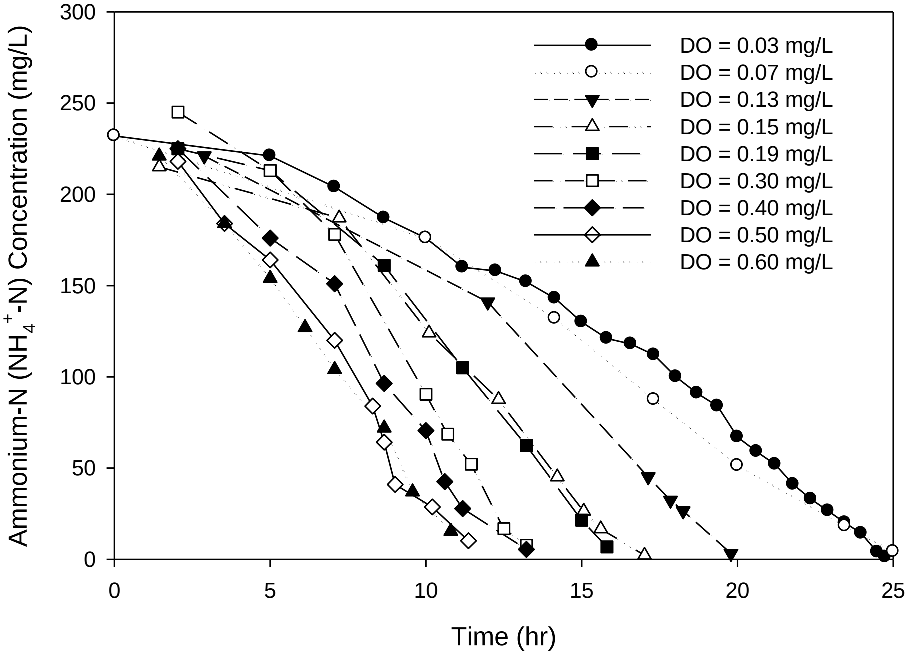

Figure 17. Ammonium Depletion during Partial Nitrification

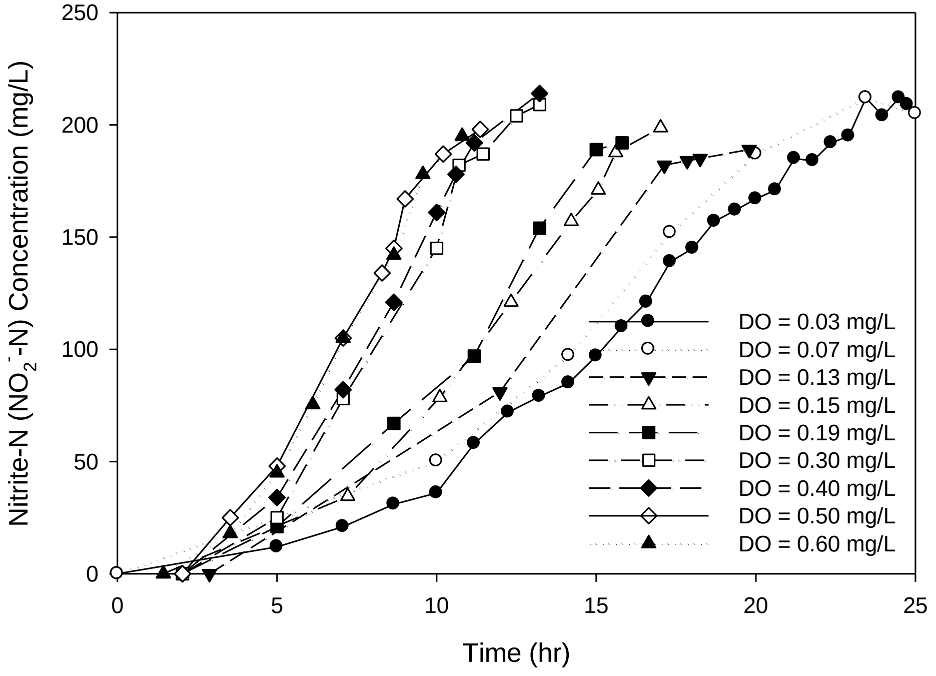

Figure 18. Nitrite Generation during Partial Nitrification

In addition to the DO concentration, partial nitrification was also closely related to pH (Figure 19) and ORP (Figure 20). At the beginning of the experiments, oxygen supply and consumption were able to be stabilized and achieve equilibrium rapidly when ammonium was not limited. Consequently, the DO concentration could stay at a constant level (Figure 21). Since $H^+$ was produced during the process of partial nitrification, pH decreased with the proceeding of the reaction owing to the accumulation of $H^+$. The decrease was more pronounced for higher DO concentrations. When the ammonium concentration dropped below the restrictive substrate concentration (~ 20 mg/l), the nitrification reaction decreased rapidly owing to the lack of the substrate and so did the oxygen consumption rate. Accordingly, an abrupt jump of in the DO profile was observed (DO sharp jump inflexion) (Figure 21). Similar observation was made to nitrite (Figure 22). For all the DO level, the sharp jump inflexion occurred after 10 hours.

It was also noted that the pH had a similar behavior as that of DO when ammonium became limited. In other words, an abrupt jump in the pH profile was also observed at the same point where the abrupt jump in the DO profile was observed (Figure 19). Once ammonium became the limiting substrate, the production of $H^+$ stopped. On the other hand, air stripping of carbon dioxide resulted in the decrease of $H^+$ and a consequent pH increase. As compared to the DO profile, the jump in the pH profile was more moderate since the pH increase was controlled by the air stripping of carbon dioxide, a separate reaction process of partial nitrification.

As in the case of DO and pH, a similar trend was observed for ORP (Figure 20). Since ORP was directly related to the ratio of the hydrogen ion $[H^+]$ and the hydroxyl ion $[OH^-]$ concentrations, the pH jump triggered the ORP increase when ammonium was limited. Based on these observations, the sharp jump inflexion in the DO profile can be used as an indicator for the completion of partial nitrification. The sharp jump inflexion in the DO profile was easy to be observed, making it a perfect indicator as the ending point of partial nitrification.

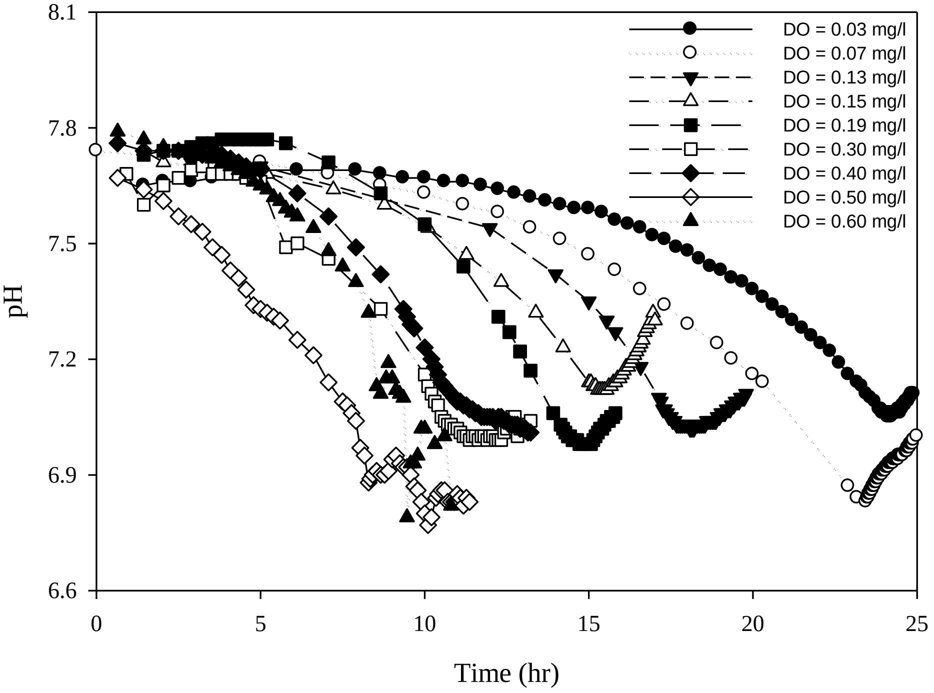

Figure 19. pH Profiles under Different DO Concentrations

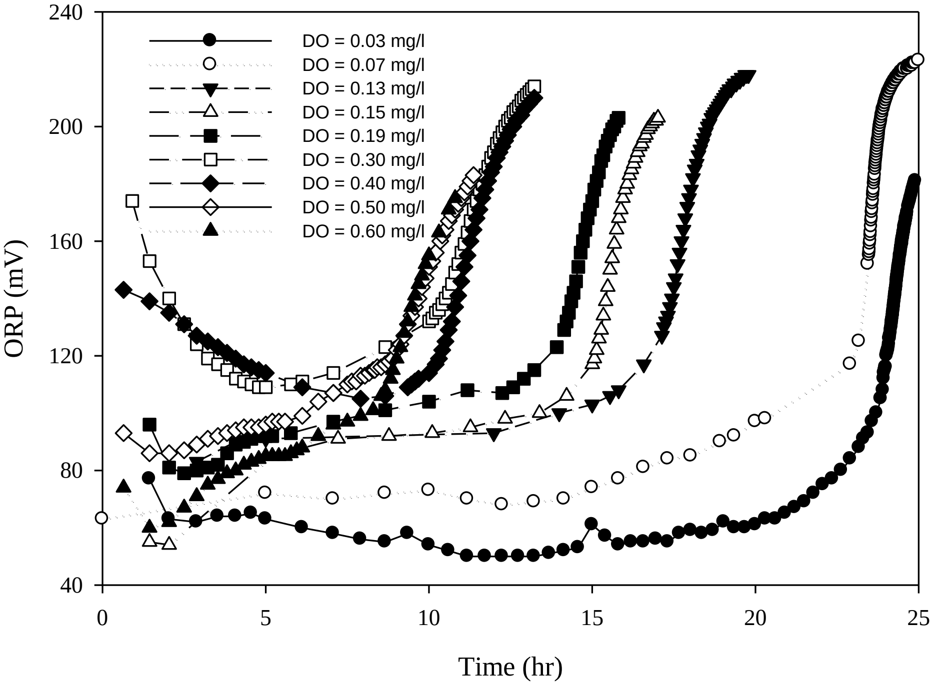

Figure 20. ORP Profiles under Different DO Concentrations

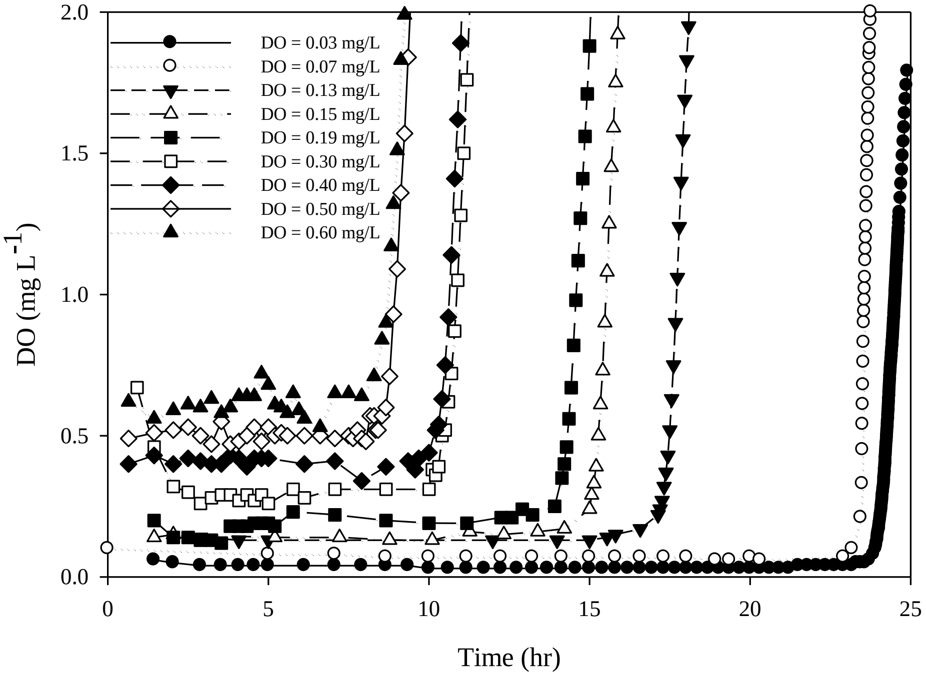

Figure 21. DO Profile during Partial Nitrification

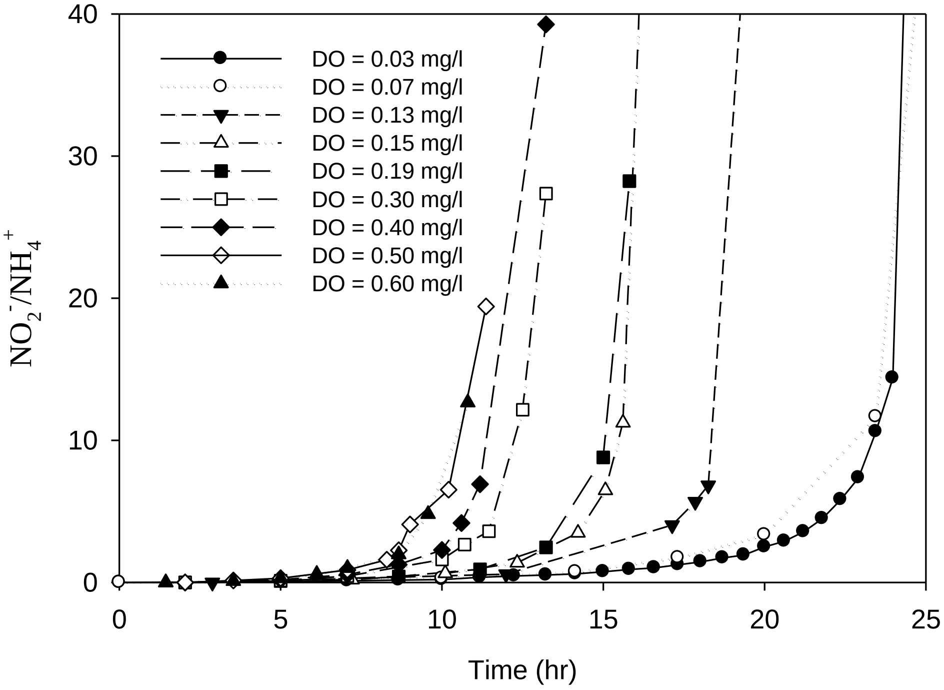

Figure 22. Nitrite to Ammonium Ratio during Partial Nitrification

Throughout the course of the experiments, the sludge remained in a stable condition. At the end of the experiments, the amounts of the AOB and NOB in the mixed liquid suspended solids were assayed via the MPN method, which were $0.95 \times 10^6$ cells/ml and $2.5 \times 10^4$ cells/ml respectively (Table 3) with an AOB/NOB ratio of 38. 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 | $0.95\times10^{6}$ 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 | $2.5\times10^{4}$ cell per ml | | | | | | | | |

### 4.1.2 Effect of Alkalinity on Partial Nitrification

Biological activity usually is highly dependent upon alkalinity. Insufficient alkalinity leads to the accumulation of $H^+$, thus prohibiting partial nitrification. The accumulation of $H^+$ (as reflected by the decrease in pH) was more obviously observed for low alkalinity than high alkalinity (Figure 23 with input ammonium of 100 mg/l $NH_4^+$-N). With the increase of alkalinity, the system buffering capacity increased and consequently the decrease of pH with respect to the proceeding of the reaction became moderate. The $NO_2^-/NH_4^+$ ratio also increased with the increase of system alkalinity (Figure 24). The increase of $NO_2^-/NH_4^+$ ratio with the increase of alkalinity was more pronounced for low ammonium concentrations than high ammonium concentrations since higher alkalinity had better buffering capacity. To achieve a stable nitrite accumulation, a high alkalinity was required.

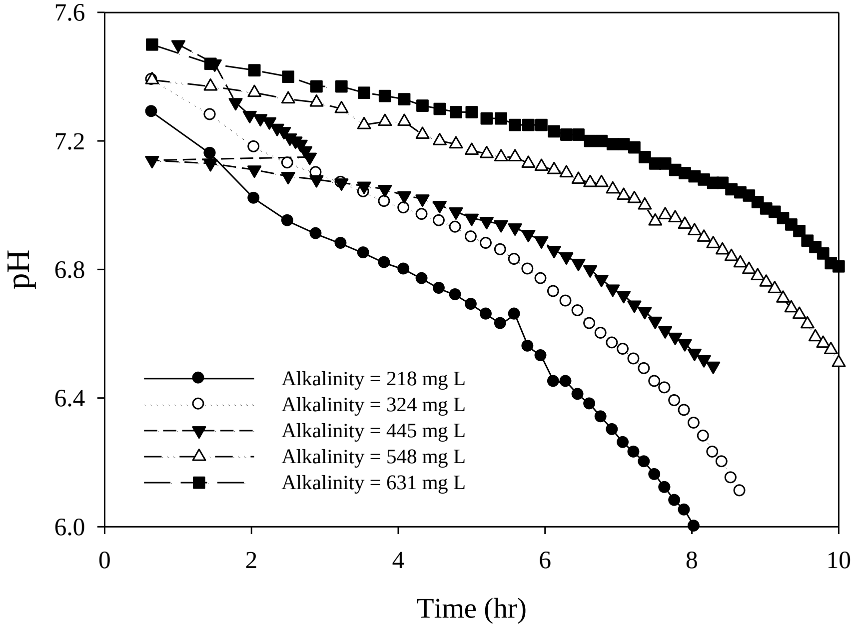

Figure 23. pH Profiles under Different Alkalinity Conditions

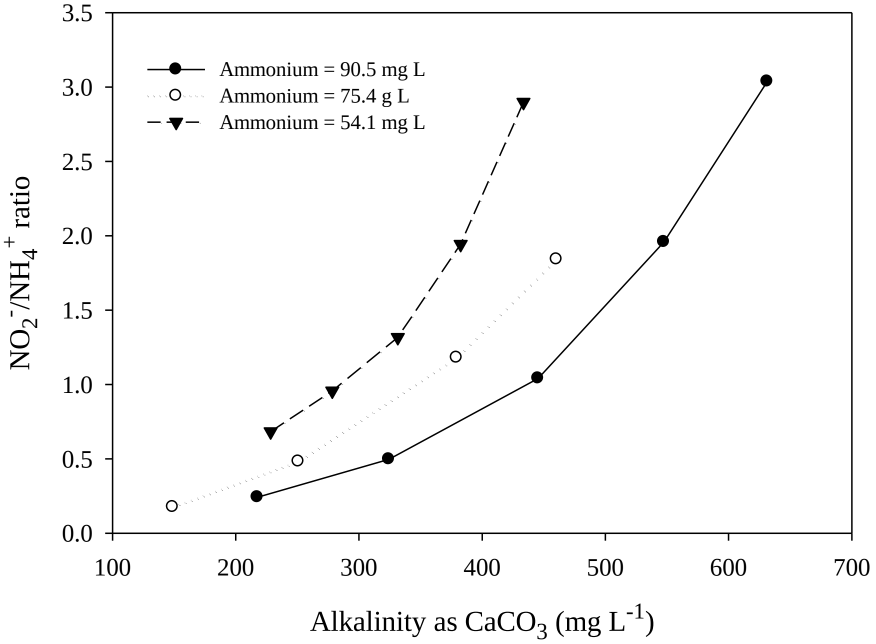

Figure 24. Nitrite to Ammonium Ratio as a Function of Alkalinity

### 4.1.3 Applicability of Partial Nitrification for ANAMMOX

ANAMMOX has been experimentally demonstrated to be responsible for high nitrogen removal at low oxygen and organic carbon concentrations (Mulder et al. 1995; Mulder et al. 2001). The microbial nature of this process was further verified and nitrite was identified as the preferred electron acceptor (vandeGraaf et al. 1997). The batch ANAMMOX experiment further examined the applicability of the partial nitrification effluent from this study for the ANAMMOX process. The ANAMMOX reaction was conducted at ~ 35°C. Stable and continuous bubbles were observed. At the end of the ANAMMOX experiments, the bulk liquid was sampled and measured for ammonium, nitrite, and nitrate. About 73.1% ammonium and 99.4% nitrite was removed with 52.7 ml nitrogen produced. The results of the ANAMMOX experiments demonstrated that the effluent from the partial nitrification was suitable as the substrate for the ANAMMOX process.

The ANAMMOX experiments were operated at a DO concentration of 0.30 mg/l and alkalinity of 50.1 to 90.5 mg/l as $CaCO_3$ until the DO sharp jump inflexion was observed. The effluent was then mixed with input ammonium at a ratio of 1: 1.31 and transferred into the ANAMMOX reactor with a gas tight rubber plug. The reactor had a working volume of 1 L and was connected to a gas collector that was filled with 10% NaOH. The reactor was inoculated with 40 g ANAMMOX wet biomass and was continuously mixed with a magnetic stirrer. For the ANAMMOX process, ANAMMOX bacteria consume ammonium and nitrite at a ratio of 1: 1.31:

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (14)$$

Approximately 0.26 mol of nitrate is produced during ANAMMOX (Tran et al. 2006; Wang and Jing 2005; Zhu et al. 2006). Owing to the presence of produced nitrate, denitrification cannot be avoided, which was observed in the experiments. Based on the above equation, ammonium and nitrite should react at a ratio of 1: 1.31, whereas, ammonium and nitrite reacted at a ratio of 1: 0.99 in the experiments (2.31 mmol ammonium to react with 2.36 mmol nitrite). In addition, more nitrogen was produced than theoretical estimations, which was attributed to the denitrification of nitrate to nitrogen gas. Based on the equation, 0.60 mmol nitrate should be produced. On the contrary, the production of nitrate cannot be detected owing to the denitrification.

Nitrogen was observed to be produced during the ANAMMOX experiment. As shown in Figure 25, nitrogen production was a function of both initial ammonium concentration and the solution alkalinity. With the increase of initial ammonium concentration, more nitrogen was produced. Initially, with the increase of solution alkalinity, nitrogen production increased. However, with the continuous increase of alkalinity, nitrogen production decreased. The optimal alkalinity was 280 mg/l, 378 mg/l and 445 mg/l as calcium carbonate corresponding to initial ammonium concentration of 54.1 mg/l, 75.4 mg/l and 90.5 mg/l, respectively.

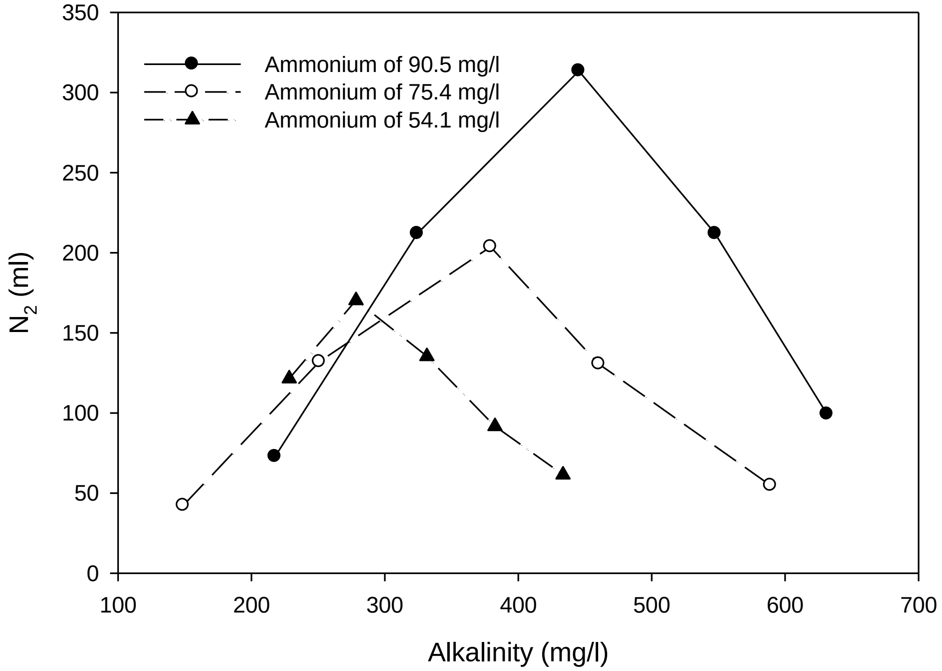

Figure 25. Nitrogen Production as a Function of Alkalinity

Nitrogen production was also a function of dissolved oxygen (Figure 26). The effect of dissolved oxygen on the ANAMMOX process or nitrogen production followed the same trend as that of alkalinity. The optimal dissolved oxygen was 0.12 mg/l, 0.14 mg/l and 0.15 mg/l corresponding to initial ammonium concentration of 54.1 mg/l, 75.4 mg/l and 90.5 mg/l, respectively.

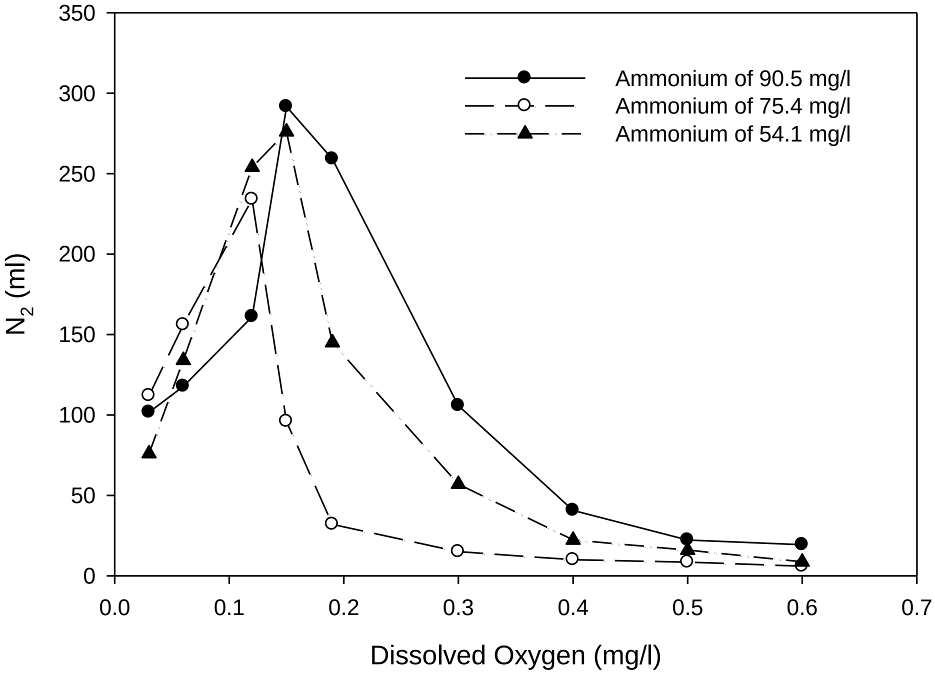

Figure 26. Nitrogen Production as a Function of Dissolved Oxygen

## 4.2 MAP Experiments

### 4.2.1 Impact of pH on MAP Precipitation

MAP experiments were conducted using batch reactors (Figure 27). After flash-mixing, MAP precipitation was easily observed (Figure 28). MAP precipitation was an obvious function of pH. MAP solubility product decreased with the increase of pH until pH reached 10, after which MAP solubility product increased with the continue increase of pH (Figure 29). At pH 10, MAP had the minimum solubility product of $10^{-9.8}$. The decrease of solution product with the increase of pH was attributed to the fact that activities of both $NH_4^+$ and $PO_4^{3-}$ are pH dependent. According to Equation (2), the increase of pH favored MAP precipitation. However, when pH reached 10, ammonium reacted with $OH^-$ to release $NH_3$, resulting in the formation of MAP.

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O \tag{15}$$

In addition, high pH also resulted in $Mg(OH)_2$ precipitation, which interfered with MAP formation. If the pH was greater than 10.5, $Mg_3(PO_4)_2$ might be formed, which was insoluble in strong alkali solution.

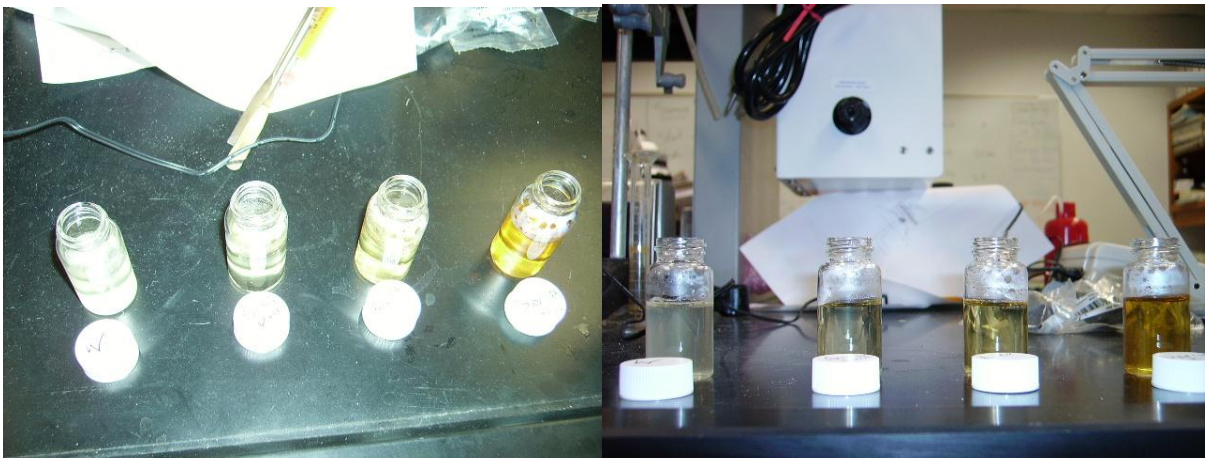

Figure 27. MAP Precipitation Experiments

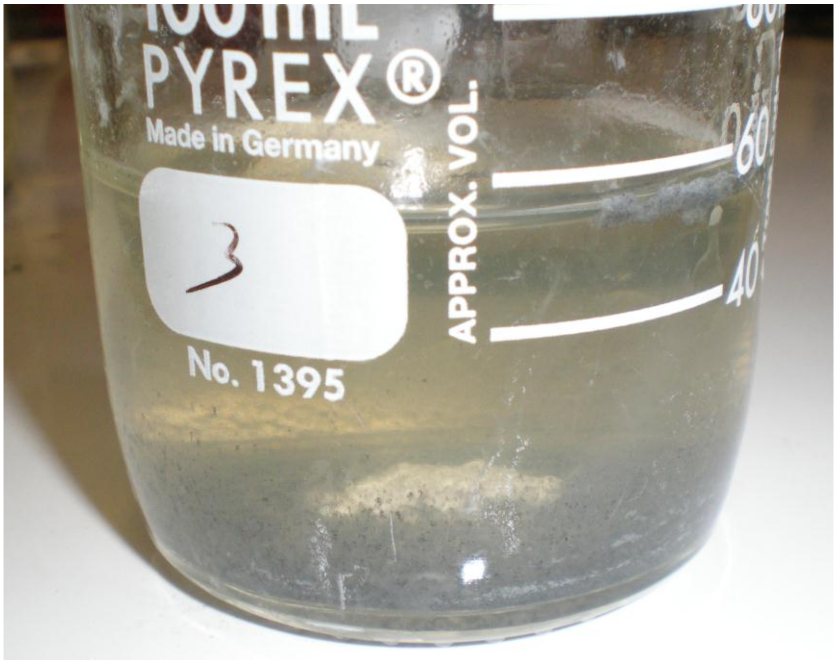

Figure 28. MAP Precipitation Observation

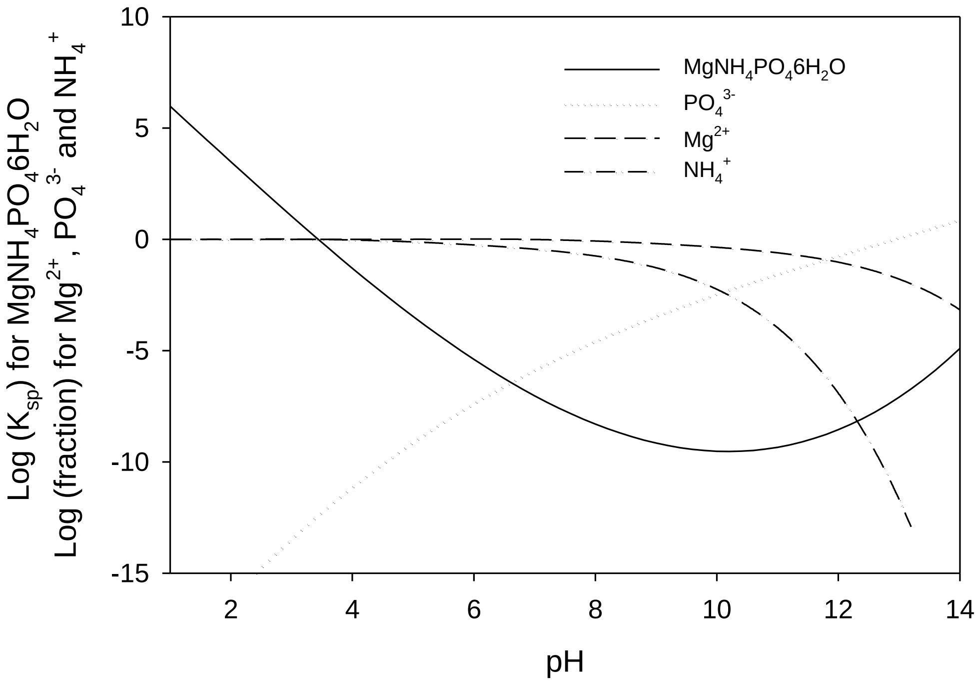

Figure 29. MAP Solubility Product as a Function of pH

### 4.2.2 Ammonium Removal

MAP can efficiently precipitate ammonium and phosphorous from leachate. However, after sedimentation, the efficiency was not satisfactory for ammonium removal, especially for leachate with low initial ammonium content (Figure 30 and Figure 31). Overall, leachate with high ammonium content had much better ammonium removal results. Stoichiometry also had an influence on ammonium removal which was more pronounced for leachate with high ammonium content than that of low ammonium content. $PO_4^{3-}$ and $Mg^{2+}$ had similar impacts on stoichiometry (Figure 30 and Figure 31). When $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ were provided at 1:1:1 ratio, the effect of initial ammonium content on ammonium removal can be easily observed. By plotting ammonium removal of $Mg^{2+}/NH_4^+/PO_4^{3-}$= 1:1:1 against initial ammonium concentration, ammonium removal was found to increase linearly with initial ammonium concentration (Figure 32). The low ammonium removal efficiency through MAP precipitation and sedimentation was attributed to the fact that micro-scale MAP does not settle efficiently. To improve the ammonium removal efficiency, the effluent was introduced to the filter column.

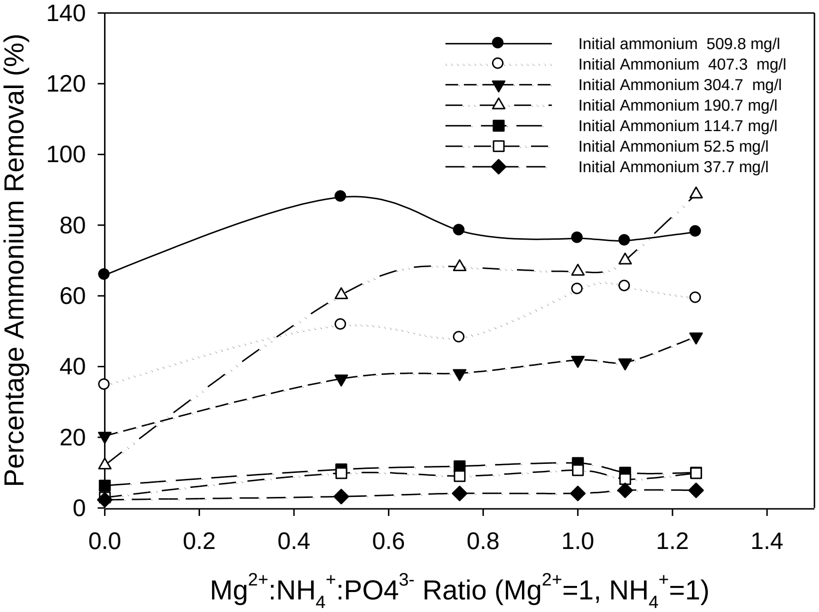

Figure 30. Ammonium Removal and $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ Stoichiometry I

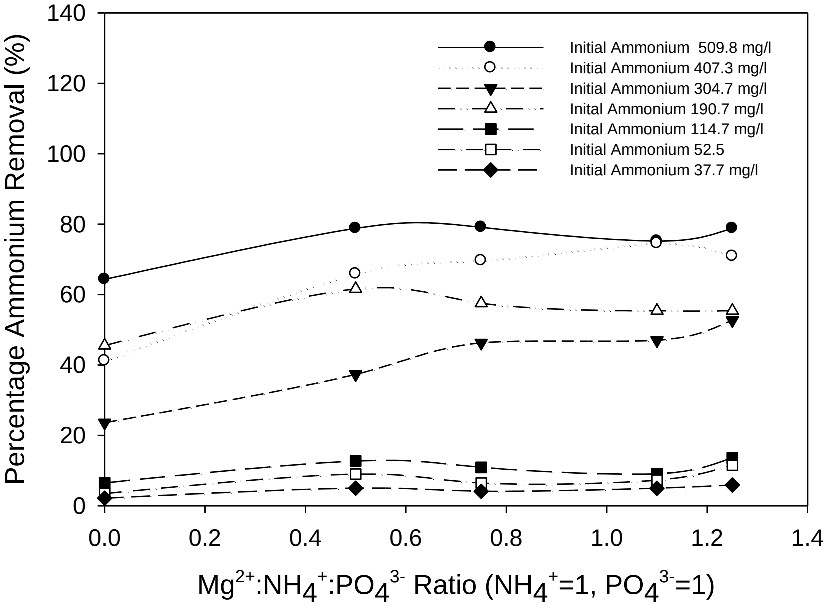

Figure 31. Ammonium Removal and $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ Stoichiometry II

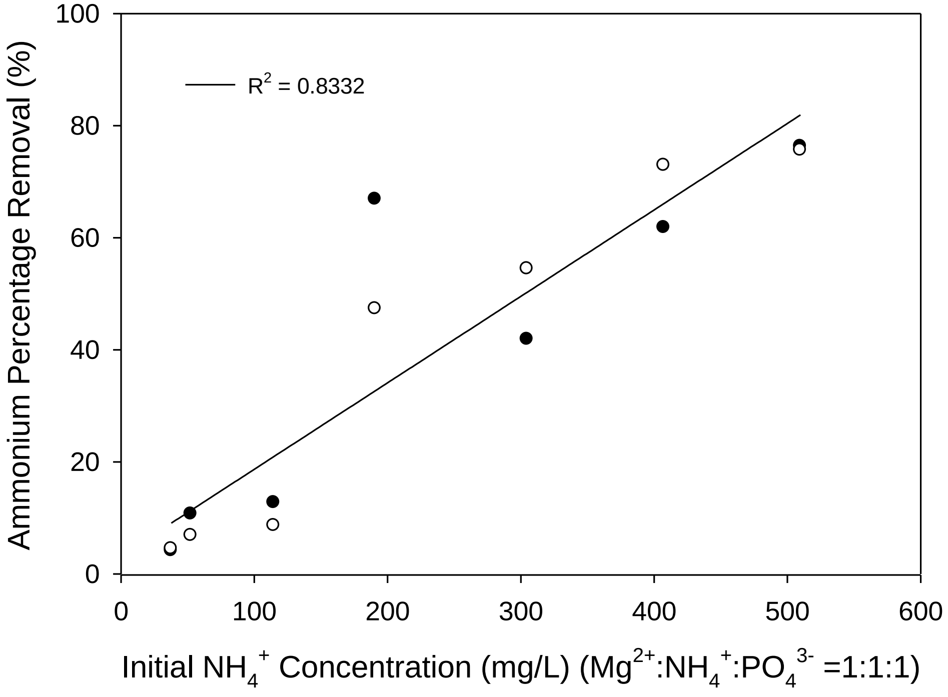

Figure 32. Ammonium Removal as Function of Initial Ammonium Concentration

The filter was arranged to provide for the removal of MAP precipitate that did not settle in the sedimentation tank. After filtration, greater ammonium removal efficiency was achieved (Figure 33 and Figure 34). The ammonium removal efficiency reached above 80% for the leachate investigated in this study regardless of the initial ammonium content when $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ were provided at 1:1:1 ratio. Ammonium removal increased linearly with the increase of initial ammonium concentration (Figure 35). The higher ammonium removal efficiency for filtration was attributed to the fact that the micro-scale MAP that did not settle in the sedimentation reactor could be easily removed during filtration.

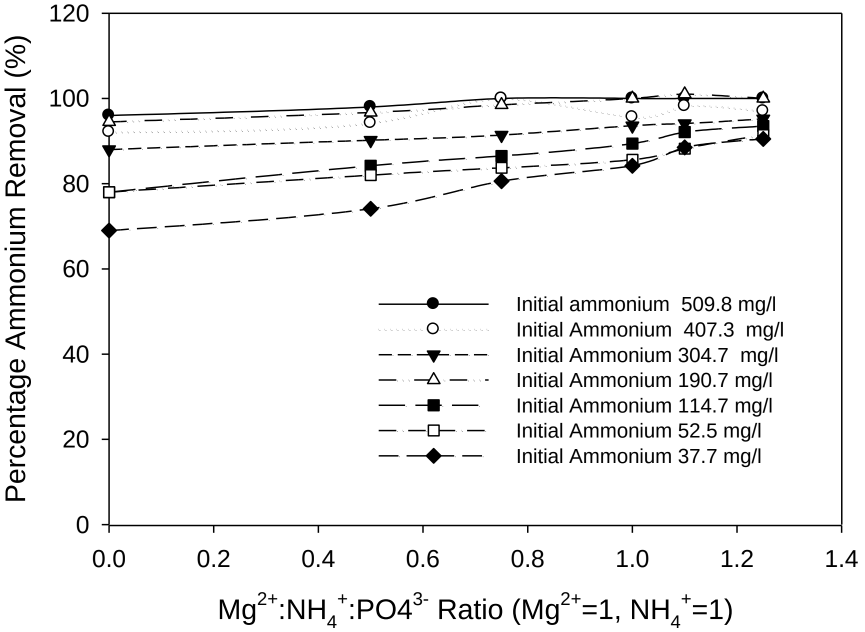

Figure 33. Ammonium Removal and $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ Stoichiometry after Filtration I

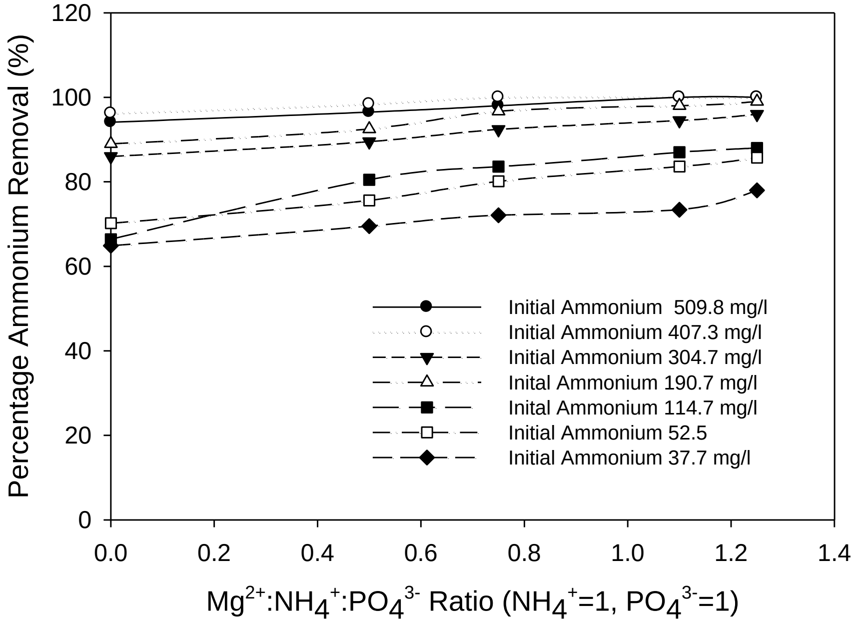

Figure 34. Ammonium Removal and $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ Stoichiometry after Filtration II

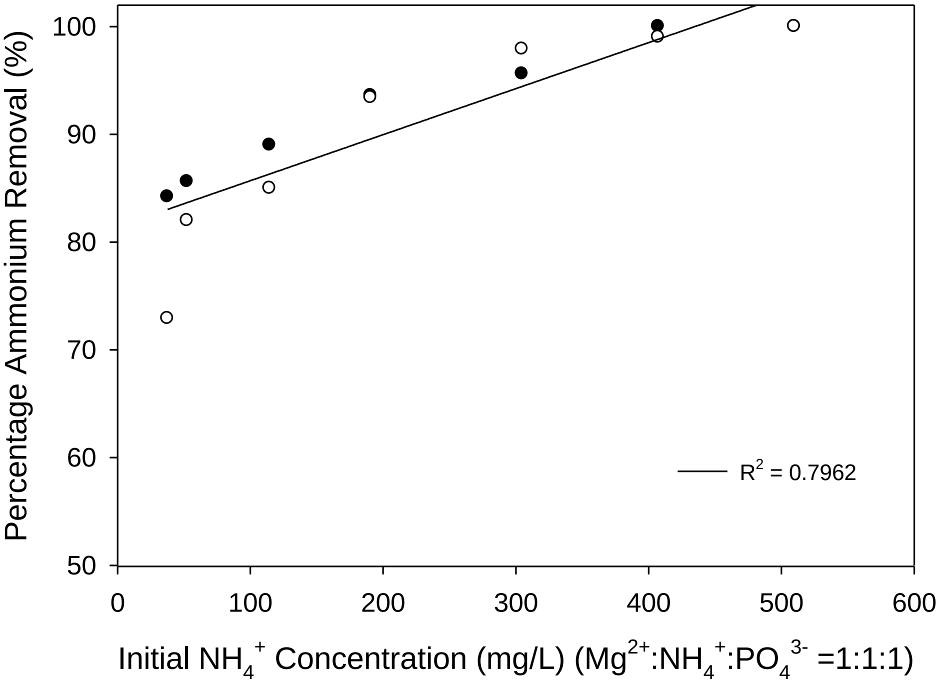

Figure 35. Ammonium Removal as Function of Initial Ammonium Concentration

### 4.2.3 Impact of pH on Ammonium Removal

pH had a great influence on leachate ammonium removal, i.e., ammonium removal increased with the increase of pH until pH 10, after which, ammonium removal decreased with the increase of pH. For leachate with an initial ammonium content of 190.7 mg/l, the maximum ammonium removal achieved 84% after sedimentation and 98% after filtration at pH 10 (Figure 36). pH impacted ammonium removal since it determined MAP solubility. Although $H^+$ concentration did not directly enter the ion-activity product equation, $MgNH_4PO_4$ $6H_2O$ precipitation was highly pH dependent because the activities of both $NH_4^+$ and $PO_4^{3-}$ were pH dependent (Nelson et al. 2003).

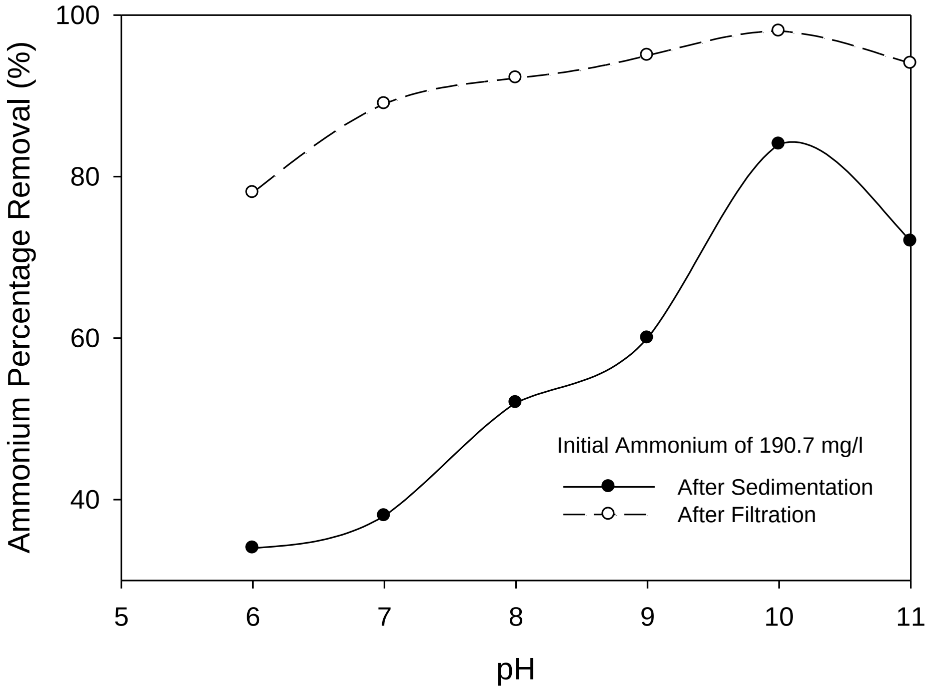

Figure 36. Ammonium Removal as Function of pH

Some research reported that the MAP solubility decreased with the increase of pH (Marti et al. 2008). The decrease of solution product with the increase of pH was attributed to the fact that activities of both $NH_4^+$ and $PO_4^{3-}$ were pH dependent. Based on the theoretical calculation, MAP solubility product decreased with the increase of pH until pH reached 10.2, after which MAP solubility product increased with the continuing increase of pH (Figure 29). At pH 10.2, MAP had the minimum solubility product of $3 \times 10^{-10}$.

### 4.2.4 Phosphorus Removal by Goethite-Coated Silica Sand

The filter was used to remove unsettled MAP in the sedimentation tank. In addition, the filter with goethite-coated silica sand serving as the filtering medium can also remove the residual phosphorous in the effluent, which was especially important when excess $Na_2HPO_4 \cdot 12H_2O$ was added. When a low dose of $Na_2HPO_4 \cdot 12H_2O$ was added, no phosphorous was observed after filtration. However, when a high dose of $Na_2HPO_4 \cdot 12H_2O$

was added, phosphorous was able to escape the filter. At $Mg^{2+}$: $NH_4^+$: $PO_4^{3-}$ = 1:1:1.2, the effluent was measured for phosphorous concentration under different pH conditions. Phosphorous was found to escape the filter, which was a function of solution pH. Phosphorous concentration in the effluent increased with the increase of pH, i.e., the higher the pH, more phosphorous escaped the filter (Figure 37). Therefore, a low pH is preferred for phosphorous adsorption in goethite-coated silica sand.

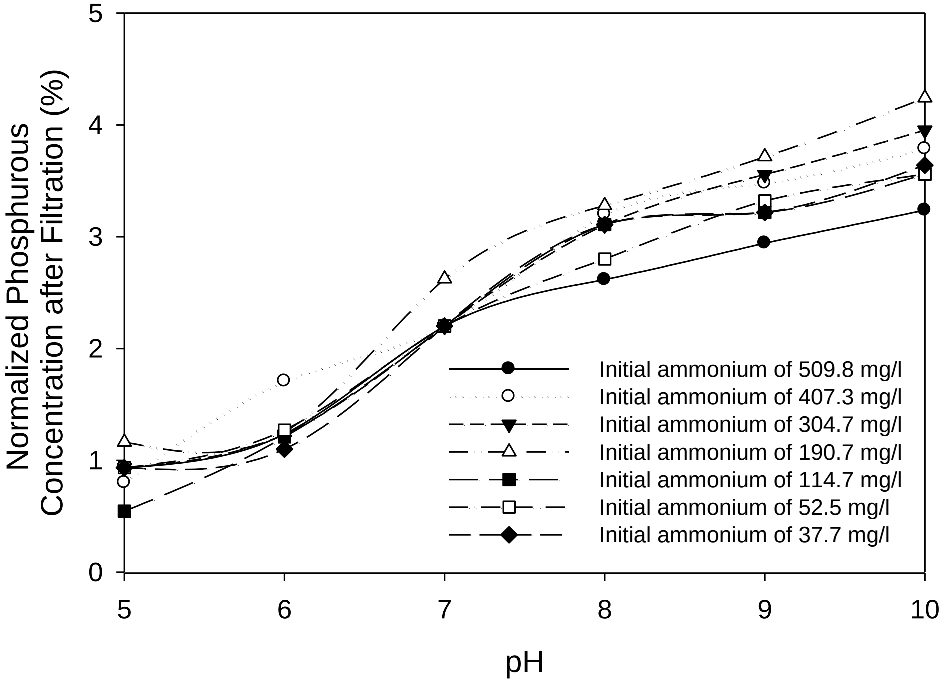

Figure 37. Normalized $PO_4^{3-}$ Concentration after Filtration

The mechanism of phosphate adsorption onto ferric oxides was generally dominated by ligand exchange in which two singly coordinated hydroxyl groups or water molecules were replaced by a single phosphate anion resulting in the formation of a bidentate, binuclear complex (Beck et al. 1999; Ioannou and Dimirkou 1997; Jones et al. 2006; Wang et al. 2005). Since $H_2O$ was a more mobile ligand than $OH^-$, sorption was therefore favored at lower pH. Four key characteristics that impacted phosphorus adsorption on the goethite surface included the easiness of hydroxyl release, the specificity toward binding sites, hysteresis, and the surface charges (Hinsinger et al. 2009; Mora et al. 2009; Zhao et al.

2006b). After adsorption, the phosphate surface complexes were very stable and resulted in slow exchange rates and an apparent irreversibility (hysterisis) of phosphorus adsorption.

### 4.2.5 Power Generation

A dual-chamber, continuous microbial fuel cell was used to investigate the efficiency of power generation using a *Shewanella putrefaciens* culture. Graphite rods, without coated catalysts, were installed in the center of the inner chambers as the anodes. The anodes were inoculated with the cultured *Shewanella putrefaciens*. Carbon cloth (effective area of 12.6 $cm^2$, 30% wet proofing), coated with platinum catalysts (0.15 $mg/cm^2$, 5% Pt) served as the cathode. In the cathode chamber, $O_2$ served as the electron acceptor. The anodes and cathodes were connected through digital multimeters. Synthetic polymeric nanoporous membranes were used as the cation-exchange membrane (CEM). During the operation, effluent after MAP sedimentation and filtration was continuously supplied to the microbial fuel cell. Continuous power in the range of 10 to 50 $mW/m^2$ was generated (Figure 38).

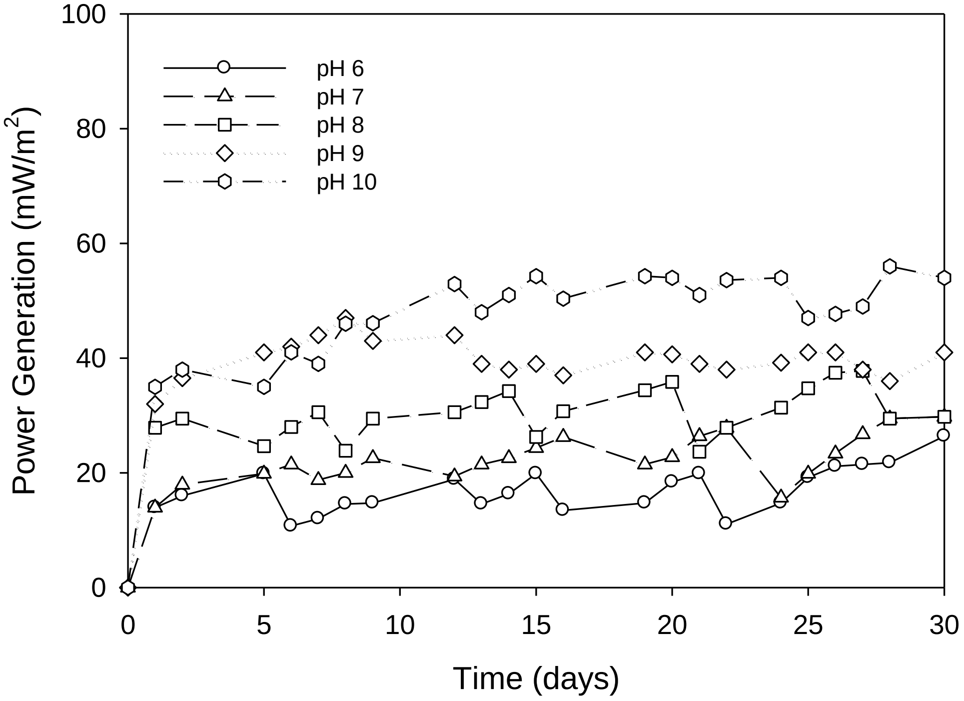

Figure 38. Power Generation from Attached Microbial Fuel Cell

It was discovered that the power generation was a function of pH. High pH (i.e., pH 10) generated more power as compared to low pH (i.e., pH 6). As high as 50 mW/m$^2$ of power was generated at pH 10. It should be noted that pH control was achieved at the anode chamber where organic compounds were decomposed. According to the following equation, raising the pH should favor electron release:

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^- \qquad (16)$$

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

$$1/4O_2 + H^+ + e^- \rightarrow 1/2H_2O \qquad (17)$$

# 5. Discussion

## 5.1 ANAMMOX for Treating Landfill Leachate

Because ANAMMOX operates with minimal sludge retention time, a substantially smaller reactor volume is needed for ANAMMOX than is currently required for conventional nitrification and denitrification. In addition, the ANAMMOX process allows for both an approximately savings of twenty five percent (25%) in oxygen transfer energy and forty percent (40%) in carbon feed for denitrifying bacterial growth compared to conventional processes.

For the system design, ANAMMOX reaction tanks can be >30% smaller than those of conventional processes. In the partial nitrification reactor, ammonium is converted mainly to nitrite by oxidation at a minimal sludge retention time and at temperatures between 30 - 40℃. In this mode of operation, the reactor selects *Nitrosomonas* over *Nitrobactor* by by washout of *Nitrobactor*. The nitrite is then anoxically converted to nitrogen gas in the ANAMMOX reactor. The reactor pH is controlled by the production of alkalinity from the denitrification process. Ammonium removal efficiencies of greater than 90% can be easily achieved. This is 90% reduction in the recycle flow. The effluent can be controlled to between 85-95% ammonium removal through adjustments to the solids retention time, pH, and dissolved oxygen.

## 5.2 Comparison of ANAMMOX with Traditional Nitrification/Denitrification Processes

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification. As nitrification and denitrification are carried out under different conditions and by different microorganisms, experience shows that these processes have to be separated in time or space to function effectively (Sumino et al. 2006). Because the organic carbon present naturally in the wastewater is quite limited, the complete removal of nitrogen from wastewaters that contain a high nitrogen concentration requires a large amount of an added carbon source for denitrification. A relatively low-cost electron donor methanol is commonly used for denitrification in the absence of oxygen. Therefore, there is a clear need for the development of new low-cost biotreatments for the treatment of nitrogen-rich wastewaters.

The ANAMMOX process is a promising new way of removing nitrogen from wastewater. In this process, energy can be conserved from anaerobic ammonium oxidation with nitrite serving as the electron acceptor without addition of external carbon source. The ANAMMOX bacterial activity is 25-fold higher than aerobic nitrifying bacterial oxidation of ammonium under anoxic conditions when using nitrite as the electron acceptor. Anaerobic ammonium oxidation is more than seven times slower than aerobic ammonium oxidation. The main product of anaerobic ammonium oxidation is $N_2$, with approximately 10% of the N-feed (nitrite and ammonium) converted to $NO_3^-$. From this research, the overall nitrogen balance gave a ratio of $NH_4^+$ conversion to $NO_2^-$ conversion of $1:1.31 \pm 0.06$. The ratio of $NO_2^-$ conversion to $NO_3^-$ production was $1:0.22 \pm 0.02$. The ANAMMOX process is combined with a preceding partial nitrification step, therefore, only part of the ammonium needs to be nitrified to nitrite. The ANAMMOX process combines the remaining ammonium with the nitrite to yield dinitrogen gas. This greatly reduces oxygen demand in the nitrification reactor and reduces the operation cost. In addition, the biomass yield is low, and consequently, little sludge is produced. This is another factor that contributes to a substantially lower operation cost of ANAMMOX compared to the conventional denitrification process. However, the low biomass yield also necessitates an efficient system for sludge retention, and long start-up times are required to obtain a sufficient biomass concentration. More importantly, the following advantages should also be recognized for ANAMMOX over nitrification/denitrification: (1) minimal site needed for implementation; (2) amenability of modification of existing construction of current plant operations; (3) operational simplicity; and (4) washout protection for nitrifiers. Operation cost comparison of ANAMMOX and traditional nitrification/denitrification is summarized in Table 4.

### 5.3 MAP Precipitation for Treating Landfill Leachate

Since MAP is a physicochemical process, a substantially smaller reactor volume is needed owing to the short reaction time. In addition, MAP can be purified in order to become a valuable commercial by product that can assist in cost recovery.

Table 4. Comparison of Nitrification/Denitrification with ANAMMOX

| Item | Nitrification/Denitrification | ANAMMOX |
|---|---|---|
| Sludge Production | High | Low |
| Organic Requirement | High | Average |
| Oxygen Requirement | High | Low |
| Operation Cost | High | Average |

However, the removal efficiency of ammonium after sedimentation was not satisfactory, especially for leachate with low initial ammonium content. The low ammonium removal efficiency through MAP precipitation and sedimentation was attributed to the fact that micro-scale MAP cannot settle down efficiently. After filtration, the ammonium removal efficiency reached above 95% for the leachate investigated in this study regardless of the initial ammonium content. The goethite-coated silica sand also had high removal efficiency for phosphorus from the leachate. When introduced in goethite-coated silica sand, more phosphorus was retained in the column. Phosphorus adsorption in goethite-coated silica sand was thought to occur owing to the replacement of $OH^-$ groups with phosphorus on the goethite surface.

Adsorption should play a more important role in phosphorus removal. This research demonstrated that phosphorus can be easily retained through adsorption by goethite. The retention of phosphorus on goethite-coated silica sand is attributed to the fact that adsorption of phosphorus to $Fe(OH)^+$ is the dominating phosphorus removal mechanism.

# 6. Conclusions

Currently, landfills, especially municipal landfills are facing the problem of the disposal of leachate, which is usually considered a complex effluent containing organic compounds, heavy metals, ammonium, chloride and many other soluble compounds. Leachate treatment is complicated and generally requires various processes because of high concentrations of organic compounds and ammonium. Anaerobic biological treatment systems are efficient in treating high concentrations of organic compounds, but they are not efficient in treating high concentrations of ammonium. New methods such as suspended carrier biofilm processes have been studied for leachate nitrogen removal, even at low temperatures (Welander and Henrysson 1998; Welander et al. 1997). These processes are reliable, but they typically have high capital costs. Therefore, there is a need for low-cost and low-maintenance systems to treat landfill leachate with high ammonium content. This research compares the onsite application of ANAMMOX (biological process) and MAP precipitation (physicochemical process) for the treatment of leachate with high organic and ammonium contents.

For ANAMMOX, ammonium was removed and nitrogen was produced. Nitrogen production was a function of both initial ammonium concentration and the solution alkalinity. With the increase of initial ammonium concentration, more nitrogen was produced and the optimal alkalinity corresponding to initial ammonium concentration of 54.1 mg/l, 75.4 mg/l and 90.5 mg/l was 280 mg/l, 378 mg/l and 445 mg/l as calcium carbonate, respectively. Nitrogen production was also a function of dissolved oxygen. The optimal dissolved oxygen corresponding to initial ammonium concentration of 54.1 mg/l, 75.4 mg/l and 90.5 mg/l was 0.12 mg/l, 0.14 mg/l and 0.15 mg/l, respectively.

MAP can efficiently precipitate ammonium and phosphorous from the leachate. Leachate with high ammonium content had much better ammonium removal results. When $Mg^{2+}$, $NH_4^+$ and $PO_4^{3-}$ were provided at 1:1:1 ratio, ammonium removal increased linearly with initial ammonium concentration. After filtration, ammonium removal efficiency reached above 80%. Ammonium removal increased with the increase of pH until pH 10, after which, ammonium removal decreased with the increase of pH.

Both processes are designed to save operation costs. ANAMMOX has advantages over MAP precipitation in that organic decomposition is incorporated with ammonium removal. For MAP precipitation, although energy may be generated from microbial fuel cell applications, their existence complicates the onsite physicochemical treatment process. In addition, the overall organic and ammonium removal rates were low as compared with ANAMMOX, although precipitated MAP can be recovered and commercialized as a fertilizer to offset the operation and chemical cost. As a biological system, ANAMMOX saves operation costs. ANAMMOX was considered superior to MAP precipitation for the treatment of landfill leachate with high ammonium content.

# 7. Future Work

For the continuation of this research, it is recommended that the design and testing of two continuous microbial fuel cell reactors (i.e., an ammonium oxidation/ microbial fuel cell reactor and a microbial fuel cell/ANAMMOX reactor for power generation as well as organic compound decomposition and nitrogen removal from leachate) be conducted. Specifically,

- ➢ Leachate collected from landfills located in Northwest Florida will be treated in a laboratory scale continuous ammonium oxidation/microbial fuel cell reactor, which is composed of an in-line nitrification column and a microbial fuel cell reactor. Impact factors such as the organic load, retention time, pH, and alkalinity as well as nitrification reaction time would be investigated.
- ➢ Leachate collected from landfills located in Northwest Florida will be treated in a laboratory-scale continuous microbial fuel cell/ANAMMOX reactor. Besides factors impacting organic removal such as the organic load and retention time, factors that may impact the nitrite accumulation in the ANAMMOX reaction such as the dissolved oxygen concentration and alkalinity would be evaluated. The microbial fuel cell/ANAMMOX reactor will be compared with the ammonium oxidation/microbial fuel cell reactor in terms of power generation as well as organic compound decomposition and nitrogen removal.

# 8. Student Training

One graduate student, Pawan Subramaniam, was trained as part of this project. So far, he has published three technical journal papers in leading journals based on the work sponsored by the Hinkley Center for Solid and Hazardous Waste Management. He has also presented his research work at four national conferences. He holds a Master of Science degree from the Florida State University and finished his Ph.D. study in the summer of 2011 from the Department of Civil and Environmental Engineering at FAMU-FSU College of Engineering.

Pawan stands by his poster at 96th Annual American Society of Microbiology Southeastern Branch Conference, Montgomery, AL.

# 9. Acknowledgements

This work was supported by the Hinkley Center for Solid and Hazardous Waste Management through Grant No. UF-EIES-0632020-FSU to the Florida State University. We thank Lee Martin, Peter Grasel, Jim Langenbach, Casey Taylor, Michael Watts, and Kamal Tawfiq for serving on the TAG committee. They provided invaluable advice on improving the laboratory design and data analysis.

### 9.1 Peer-reviewed journal paper publication:

1. Subramaniam, P. K., Martin, L., Grasel, P., Tawfiq, K. and Chen, G. (2110) *Treatment of Landfill Leachate with High Ammonium Content through Struvite Precipitation*, Environmental Technology, under review.

### 9.2 Conference presentation:

1. Subramaniam, P. and Chen, G. (Presented on November 6, 2010). *Comparison of Physicochemical and Biological Treatment of Landfill Leachate*. 96th Annual American Society of Microbiology Southeastern Branch Conference, Montgomery, AL: American Society of Microbiology Southeastern Branch.
2. Subramaniam, P. and Chen, G. (To Be Presented on October 21, 2011). *Landfill Leachate Treatment through ANAMMOX*. 97th Annual American Society of Microbiology Southeastern Branch Conference, Gainesville, FL: American Society of Microbiology Southeastern Branch.

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