To my husband, daughters, parents and sister
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<td>ALS</td>
<td>Advanced life support</td>
</tr>
<tr>
<td>ALSB</td>
<td>Air-lift loop sludge blanket</td>
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<td>ANAMMOX</td>
<td>Anaerobic ammonium oxidation</td>
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<tr>
<td>BMP</td>
<td>Biochemical methane potential</td>
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<td>CANON</td>
<td>Completely autotrophic nitrogen removal over nitrite</td>
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<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>ESM</td>
<td>Equivalent system mass</td>
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<tr>
<td>OFMSW</td>
<td>Organic fraction of municipal solid waste</td>
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<td>OLAND</td>
<td>Oxygen-limiting autotrophic nitrification-denitrification</td>
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<td>SEBAC</td>
<td>Sequential batch anaerobic composting</td>
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<tr>
<td>STP</td>
<td>Standard temperature and pressure</td>
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<tr>
<td>TAN</td>
<td>Total ammonia nitrogen</td>
</tr>
<tr>
<td>TKN</td>
<td>Total kjeldahl nitrogen</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>UASB</td>
<td>Upflow anaerobic sludge blanket</td>
</tr>
<tr>
<td>USB</td>
<td>Upflow sludge blanket</td>
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<td>VS</td>
<td>Volatile solids</td>
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This study reports on the development of methods for pretreatment of feedstock and post treatment of residue and leachate to improve the performance of UF-patented anaerobic composting technology. These improvements were needed for application of this technology as a solid waste management subsystem on long-term NASA space missions, as well as in treating industrial organic wastes, such as beet pulp and beet tailings from the beet sugar industry.

Post treatments were needed for the NASA space mission application and beet pulp application, and involved stabilization of anaerobically digested solid residue by a nitrification process, integration of ammonia removal from liquid leachate within the anaerobic digestion process, and incorporation of both treatments into the overall system configuration. Pre-treatment of readily biodegradable feedstock was needed for the sugar beet tailings application, and consisted of adding a pre-wash step to wash and remove the large fraction of soluble organic matter in the sugar beet tailings, and holding this fraction in a separate reservoir for subsequent controlled release into the system.

Results from post treatment of solid residue showed that nitrification initiated within 2 days by continuous flow of air through the residue at a rate of 187 mL/minute/kg wet residue,
and 85% of the initial ammonium-nitrogen nitrified within 16 days at a maximum rate of 0.41 mg/g wet weight/day. Post treatment of liquid leachate showed that the ammonia removal rate was 70 -95 mg/L/day initially, and increased to 200-245 mg/L/day after 8-days of continuous operation, when concentration of total ammonia nitrogen reached 500 mg/L. However, repeated aeration reduced the efficacy of the leachate as an inoculum, suggesting that only part of the leachate should be aerated.

System integration involved a modification in which aeration was carried out by holding air within the reactor at a pressure of ~10 psi over 13 days for stabilization of digested residue, and a similar system and operation for ammonia removal from the liquid leachate.

Results from the pre-treatment of sugar beet tailings revealed that addition of water solubilized a large fraction (0.6 g COD/g VS) of organic matter from the tailings. Methanogenesis could be initiated if the solubilized material was leached out. Most of the methane potential remaining in the solids was generated within a week. The methane yield of tailings was estimated to be 295 L/kg VS, of which 50-60% was from the readily solubilized organic matter. A volume reduction of 70-80% was achieved, and approximately 60% of dry matter and 75% of volatile solids were degraded. These demonstrated performance levels suggested that incorporating these pre and post treatments as a routine part of the system operation will greatly enhance the potential for widespread use of this technology in waste management applications.
1.1 Introduction

1.1.1 Anaerobic Digestion of Solid Waste

The term solid waste would apply to any garbage, refuse, sludge and other discarded material resulting from community activities or commercial or industrial operations. Solid waste management has become a major concern in the world recently due to the huge quantities generated world-wide. Anaerobic digestion of solid waste is becoming a popular method to treat these wastes because it can generate biogas as an energy resource. For example, Canada generates approximately $1.45 \times 10^8$ t of biomass (one type of solid wastes) per year. Anaerobic digestion of these biomass using conventional technologies could generate $1.14 \times 10^{10}$ m$^3$/year of CH$_4$ with a heating value of $4.56 \times 10^8$ GJ, which is equivalent to about 4.4 % of Canada's current annual energy use (Levin et al, 2007). At the same time, the digested residue from anaerobic digestion could serve as fertilizer for plant growth (Svensson et al., 2004). Moreover, anaerobic digestion has limited impact for our environment (Mata-Alvarez et al., 2000). There are several ongoing studies on modeling and kinetics of the process for anaerobic digestion of solid waste (Borja et al., 2003; Borja et al., 2006); as well as enhancement of digester performance, overcoming ammonia inhibition and digester design improvement (Mata-Alvarez et al., 2000).

Anaerobic digestion occurs primarily in two steps: acid formation and methane formation. These processes are mediated by different groups of microorganisms, which require different nutritional compounds and environmental conditions. This could lead to some problems of stability and control if the whole process occurs in one reactor (Demirel and Yenigun, 2002; Pohland and Ghosh, 1971). Therefore, at present more researchers put their efforts into a two-
phase anaerobic digestion process, which means a physical separation of acid-formers and methane-formers in two separate reactors. In this case, optimum environmental conditions for each group of microorganisms could be provided separately to improve the whole process (Demirel and Yenigun, 2002). Ghosh et al (2000) showed that given the same operating conditions, the two-phase anaerobic digestion of municipal solid wastes exhibited 18% higher methane yield, 22% higher methane production rate and 13% higher methane concentration than the corresponding performance parameters for one-stage operation. However, others (Weiland et al., 1990) believed that it was unnecessary to treat all kinds of solid wastes in two separate reactors; it depends on the physical and chemical properties of biodegradable wastes. They recommended that one-stage operation could be used to treat solid waste with low protein content such as beet pulp (Weiland et al, 1990).

Anaerobic digestion may be operated in psychrophilic (12-16 °C), mesophilic (35-37 °C) or thermophilic conditions (55-60 °C). At thermophilic temperatures, the rates of degradation and biogasification are faster, and have greater potential to destroy weed seeds and plant pathogens, which is especially beneficial for reapplying the digested residue with little post treatment back on to the fields to recycle nutrients (Koppar and Pullammanappallil, 2007). Thermophilic operations were found to provide better results than mesophilic conditions in most cases. For example, in Mace et al’s study (2003) biodegradability of municipal solid waste could be enhanced by thermophilic operation and the corresponding ultimate methane yield was about 10% higher. On the other hand, disadvantages of thermophilic anaerobic digestion are the reduced processs stability and reduced dewatering properties of the fermented sludge and the requirement for large amounts of energy for heating (Gallert and Winter, 1997). The greater
energy demand for thermophilic temperature is approximately the same as the excess energy produced in the process in many cases. Therefore, the choice of optimal temperature depends on the type of substrate and the type of system used and levels of pathogen or weed seed control required in the digested residue (Mata-Alvarez et al., 2000).

1.1.2 High-Solids Anaerobic Digestion

Studies have been devoted to the anaerobic digestion of high-solids organic wastes for solid waste management because of the problems associated with reduction in process water and reactor size requirement. Some efforts focus on the environmental factors affecting the efficiency of high-solids waste digestion, including chemical nature of feedstock, moisture content, pH, ammonia concentration and nutrient requirements (Lay et al, 1997, 1998; Kayhanian and Rich, 1995). It was reported that the methanogenic activity decreased with the decrease in moisture content. At optimum pH, the methanogenic activity in high-solids digestion dropped from 100% to 53% when the moisture content decreased from 96% to 90%. However, for some feedstocks such as carrot and cabbage, the methanogenic activity was inhibited by the high level of organic acids instead of moisture content (Lay et al, 1997; Lay et al, 1998). Methanogenic bacteria have a variety of mineral nutrient requirements for robust growth. The addition of wastewater treatment plant sludges and dairy manure as a nutrient supplement may increase the gas production rate by 30% and improve the digestion stability (Kayhanian and Rich, 1995).

Several other studies focused on the improvement of process and reactor design. The leachbed design is one of the options most applicable to high solids operation. The leachbed design uses recycle of leachate between new and mature reactors to inoculate, wet and provide nutrients for rapid startup of new cells. Organic acids produced during startup are conveyed via leachate to the mature reactor for conversion (Chynoweth et al, 1991, 1992). This design does
not require mixing and was developed and patented as the Sequential Batch Anaerobic Composting (SEBAC) process at the University of Florida.

1.1.3 Sequential Batch Anaerobic Composting (SEBAC)

The SEBAC process is a patented high-solids, batch, leach-bed process that uses a combination of solid state fermentation and leachate recycle to provide a simple, reliable process that inoculates new batches of waste, removes volatile organic acids and concentrates nutrient and buffer (Chugh et al., 1999; Chynoweth and Legrand, 1993; Chynoweth et al., 1992). This process has already been commercialized (Teixeira et al, 2003). It also has been considered by NASA as one option for the principal solid waste management component in Advanced Life Support (ALS) systems for long-range space missions (Xu et al, 2002).

The whole system requires a minimum of 3 bioreactors linked through a leachate handling, piping and pumping system. As illustrated in Figure 1-1, coarsely shredded feedstock is placed into a bioreactor that is ready for a new cycle of anaerobic digestion (stage 1). Leachate from a bioreactor containing residue that has been digested (Stage 3) is recycled between that reactor and the newly loaded bioreactor (Stage 1) to provide moisture, inoculum, nutrients, and buffer necessary for start-up. Volatile organic acids formed in the newly loaded bioreactor during start-up are removed via leachate recycle to the active mature bioreactor for conversion to methane and carbon dioxide. After start-up, the newly loaded bioreactor becomes sustainable methanogenic and is maintained by recycling leachate upon itself (Stage 2) until it graduates to Stage 3 as a fully mature reactor. The waste from the mature reactor (Stage 3) is unloaded and taken away as a by-product of the process. A fresh supply of waste is now loaded into the reactor which becomes a new Stage 1. Near the end of the process, which may take approximately three weeks depending on feedstock characteristics, leachate from the now mature reactor is used for the start-up of a new reactor that is once again ready to begin a new cycle. Biomass is not moved
during the process; it passes through different stages over time in the same reactor vessel. After completion, the biomass is dewatered to supply water, and conserve nutrients and buffer for a new run. For some feedstocks, additional make-up water is required (Teixeira et al., 2003; Chynoweth et al., 2002; Xu et al., 2002).

The biogas produced from the SEBAC process can be used like “natural” gas. The average composition for the biogas is 60% methane, 40% carbon dioxide and includes traces of hydrogen sulfide, hydrogen, nitrogen and carbon monoxide. The SEBAC biogas can be used readily in all applications designed for natural gas such as direct combustion, fueling engines and fuel cells for production of mechanical work and electricity (Teixeira et al, 2003). The SEBAC process also produces solid and liquid by-products. The amount, quality and nature of these products depends on the quality of the feedstock, and the extent of the post-treatment refining process (Teixeira et al, 2003). The solid anaerobically digested residue called digestate can be used as a fertilizer or soil amendment.

The SEBAC process has been tested on organic fraction of municipal solid waste (OFMSW), woody biomass, yard wastes and mixtures of yard wastes and biosolids (Chynoweth and Legrand, 1993; Chynoweth et al., 1992). The performance of the SEBAC pilot system on several feedstocks is listed in Table 1-1 (Teixeira et al, 2003).

Compared with other traditional anaerobic digestion technologies, the SEBAC process design offers greater stability. The design allows for easy removal of inhibitory products, which may lead to imbalance. The leachbed design not only facilitates rinsing of toxic substances, such as metals, from the final product, but also eliminates the need for solids movement and mixing (Teixeira et al, 2003).
In the original SEBAC system, gravity was relied upon to bring cascading liquid leachate in contact with the organic feedstock by pumping leachate into the top of the reactor and allowing it to flow by gravity and collect at the bottom for subsequent recycling. In SEBAC-II the system was modified to move leachate through the bed under flooded operation using forced pumping, and recycling leachate through external gas-liquid separators that could accommodate vortex gas/liquid separation systems (Figure 1-2). This design modification was driven by the NASA requirement for operation in the absence of a gravity field.

1.2 Statement of Problem

Currently there are several applications for SEBAC-II system. Firstly, it can be used as a sub-system of solid waste management in Advanced Life Support (ALS) system for NASA. Secondly, it can also be used for anaerobic digestion of sugar beet pulp and sugar beet tailings. However, there still exist some problems in these applications.

When it is used as a sub-system of solid waste management in ALS system, anaerobically digested residue from this process is desired to serve as fertilizer for plant growth for long term space missions. Digested residue directly obtained from SEBAC-II system has high moisture content. So it is usually dried before application. On the other hand, ammonia released from anaerobic digestion may attach on the digested residue. Drying would lead to loss of ammonia by volatilization. Thus it is necessary for us to have a post-treatment process on the anaerobically digested residue to fix nitrogen.

Sugar beet pulp contains a lot of nitrogen, which may be converted to ammonia during anaerobic digestion. Most of this ammonia appears in the leachate. Leachate is reused in the SEBAC-II system, which may cause ammonia accumulate and inhibit the anaerobic digestion. Ammonia inhibition during reuse of leachate is shown in Figure 1-3. The cumulative and daily
methane yield slightly decreased after the leachate was reused for 3 times and dramatically
decreased after the leachate was reused for 4 times (Koppar and Pullammanappallil, 2007).

Sugar beet tailings have a large fraction of readily soluble organic matter. The
fermentation of soluble organic matter produced a lot of organic acids and lowered the pH. When
sugar beet tailings were anaerobically digested in SEBAC-II system, the daily methane yield
failed to increase even after 30 days (Figure 1-4).

Therefore, further investigation was needed to address the problems discussed above, and
this study was undertaken to carry out such investigation.

1.3 Objectives

The work undertaken in this study was organized into four separate but related
investigations to meet each of the following specific objectives:

- Stabilize anaerobically digested residue by a nitrification process,
- Integrate the need for ammonia removal from leachate to become part of the anaerobic
digestion process,
- Integrate the in situ treatment of anaerobically digested residue and leachate into the
SEBAC-II system configuration, and
- Develop a method for pre-treatment of readily biodegradable feedstock in application to
sugar beet tailings.

Each of these objectives is discussed in further detail in the following subsections, while a
report of the work undertaken (and subsequent results) to meet each objective is presented
respectively in the following four chapters.

1.3.1 In Situ Treatment of Anaerobically Digested Residue and Leachate

1.3.1.1 Stabilization of anaerobically digested residue by nitrification process

The digested residue is usually dried before applications, which may lead to ammonia
volatilization. So it is necessary to have a stabilization process to convert ammonia to nitrate by
nitrification. The goal for the first part of this study was to find the feasibility for integration of an in situ stabilization step into the SEBAC-II configuration with the following objectives:

- Confirm the availability of microorganisms required for nitrification in anaerobically digested residue.
- Measure the rate of NH₃ transformations and losses during stabilization process.
- Quantify the rate of NH₃ transformations using a kinetics mathematic model
- Measure the oxygen requirement for the stabilization process.

Ammonia transformation and volatilization during stabilization of anaerobically digested residue were investigated. This work is reported in Chapter 2, and will present results of the preliminary work conducted in the small reactors, including the feasibility of initiating the nitrification process, extent and rate of nitrification and mass balance on nitrogen during aerobic stabilization. Moreover, a method was developed for the determination of total ammonia nitrogen (TAN) in anaerobically digested residue since there was no available method in the literature.

1.3.1.2 Integration of ammonia removal from leachate within anaerobic digestion process

During anaerobic digestion process, organic nitrogen compounds are transformed to ammonia nitrogen and most of them remain in leachate. Leachbed digestion such as the SEBAC-II system, uses recycle of leachate between new and mature reactors to inoculate, wet and provide nutrients for rapid startup of new cells (Ghosh, 1984; Chynoweth et al, 1991, 1992). However, ammonia is accumulated in the system when leachate is reutilized from a mature reactor. Accumulation of ammonia may negatively affect the anaerobic digestion performance (McCarty and McKinney, 1961; Gallert and Winter, 1997).
The goal for the second part of this study is to determine the feasibility of integration of an in situ ammonia removal process within SEBAC-II system. Specific objectives were the following:

- Develop a biological ammonia removal process
- Determine the kinetics of ammonia removal process
- Determine the viability of aerated leachate as inoculum for subsequent leach-bed digestion

This work is reported in Chapter 3, and will present the results of biological ammonia removal from anaerobic leachate using digested residue bed and the feasibility of reuse of the aerated leachate as inoculum for subsequent leach-bed anaerobic digestion.

1.3.1.3 Integration of in situ treatment of anaerobically digested residue and leachate in SEBAC-II configuration

The SEBAC-II system, which is in flood mode operation, recycles the leachate using forced pumping rather than gravity. A bench-scale study was implemented to test the concept of SEBAC-II with external gas/liquid separation (Chynoweth et al, 2002). Several studies were also conducted for design, installation, start-up, preliminary operating performance (Xu et al, 2002; Teixeira et al, 2004) and modifications (Luniya et al., 2005) for a full-scale prototype SEBAC-II configuration for a space mission. However, little work has been devoted to post-treatment of the anaerobic digestion in this system. The objectives for the third part of this study include:

- Design the operation mode for in situ treatment of anaerobically digested residue and leachate in the SEBAC-II configuration
- Measure the nitrification rate in the process of digested residue stabilization
- Determine ammonia removal rate from leachate using digested residue bed during aeration

This work is reported in Chapter 4, and will present the results of the study. The results includes the nitrification rate on anaerobically digested residue and ammonia removal rate from
leachate during aeration, and prediction of the in situ post-treatment processing time in full prototype of SEBAC-II system according to these results.

### 1.3.2 Pre-Treatment of Readily Biodegradable Feedstock

Efforts for enhancement of anaerobic digestion of solid wastes have been dedicated, including different pretreatment methods to improve the digestibility of feedstock (Palmowske and Muller, 2003; Valo et al, 2004; Bougrier et al., 2005, 2006; Li and Noike, 1992; Neyens, E. and Baeyens E.; 2003; Lin et al, 1997; Penaud et al, 1999; Mshandete et al, 2005). However, different feedstock needs different pretreatment method according to its characteristics. Recent work in developing application of SEBAC-II technology for processing sugar beet waste (tailings) in the beet sugar industry is a good example.

Sugar beet tailings contain a large fraction of readily soluble organic matter, which makes too much initial digestibility. In preliminary experiments, sugar beet tailings were anaerobically digested using the SEBAC-II process at 38 °C (Teixeira et al., 2005). Results showed that the rate of methane generation was poor compared to that from digestion of other organic residues. Persistently high volatile organic acid concentrations were measured in digester liquor, and daily methane production rates failed to increase even after 30 days of digestion. This indicated a need for further modifications and improvements to integrate an in situ pre-treatment process to anaerobically digest tailings within the SEBAC-II configuration. The objectives for this fourth part of the study were the following:

- Confirm the availability of microorganisms required for anaerobic digestion of sugar beet tailings
- Develop a pre-treatment method for anaerobic digestion of sugar beet tailings in the SEBAC-II system
- Measure the methane yield and methane production rate in anaerobic digestion of sugar beet tailings after incorporating the new pre-treatments
Chapter 5 will present findings related to these modifications and improvements for anaerobic digestion of sugar beet tailings. These modifications included removal of readily soluble COD, and operation of the digester within a thermophilic temperature range (50 – 57 °C) where the rates of degradation and biogasification are faster.

Figure 1-1. Sequential batch anaerobic composting (SEBAC) process

Stage 1
New

Stage 2
Activated

Stage 3
Mature

Hydrolysis Products and Volatile Acids From Stage 1
Figure 1-2. Sequential batch anaerobic composting II (SEBAC-II) process
Figure 1-3. Ammonia inhibition during reuse of leachate in anaerobic digestion of sugar beet pulp (Koppar and Pullammanappallil, 2007)

Figure 1-4. Anaerobic digestion of sugar beet tailings in SEBAC-II system under mesophilic temperature
Table 1-1. Performance data from the SEBAC process for various feedstocks at 55 °C (Teixeira et al, 2003)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MSW</th>
<th>Yard waste</th>
<th>Brewery chips</th>
<th>Shredded office paper</th>
<th>Space mission wastes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane yield, L/g VS added</td>
<td>0.30</td>
<td>0.07</td>
<td>0.06</td>
<td>0.35</td>
<td>0.3</td>
</tr>
<tr>
<td>VS reduction (%)</td>
<td>57</td>
<td>20</td>
<td>26</td>
<td>96</td>
<td>85</td>
</tr>
<tr>
<td>Volume reduction (%)</td>
<td>65</td>
<td>15</td>
<td>15</td>
<td>94</td>
<td>86</td>
</tr>
<tr>
<td>Solids retention time (days)</td>
<td>30</td>
<td>70</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

* Blend of rice straw, paper and dog food (simulates feces); this run was conducted at 35 °C
CHAPTER 2
STABILIZATION OF ANAEROBICALLY DIGESTED RESIDUE BY NITRIFICATION PROCESS

2.1 Introduction

The process of anaerobic digestion is an important component in sustainable waste management. Anaerobic digestion not only generates biogas which is an energy source but also produces a stable digested residue. Depending on the feedstock, the amount of residue produced in this process varies, which is related to the degradable fraction of the wastes. Dry matter reduction varies from 35% to 90% depending on the feedstock. In anaerobic digestion, dry matter reduction of municipal solid waste (MSW), milled wheat stems and sugar beet tailings were 35 – 60% (Chugh et al, 1999), 70-77% (Chynoweth et al, 2002) and 82-90% (Polematidis, 2007) respectively, which means MSW produces more anaerobically digested residue (dry weight) than wheat stems and sugar beet tailings. The digested residue may be used as a soil-quality enhancer (Svensson et al., 2004; Rivard et al., 1995) and microorganism carrier in biofilter for air revitalization (Xu et al, 2002). Moreover, addition of anaerobic residue from co-composting was shown to improve humic acid formation that led to the production of high quality composts used for plant growth (Meissal et al, 2007)

The SEBAC process is a patented anaerobic digestion technology for odorless bioconversion of organic solid waste to methane and solid residue. The process uses a combination of solid-phase fermentation and leachate recycle between new and old reactors to provide a simple, reliable process that inoculates new batches, removes volatile organic acids, and concentrates nutrients and buffer. The process doesn’t require high temperature and pressure while producing methane, carbon dioxide, nutrients and digested residue as valuable products. Comparing with other traditional anaerobic digestion technologies, the SEBAC process is more stable. The design allows for easy removal of inhibitory products, which may lead to imbalance.
The leachbed design not only facilitates rinsing of toxic substances, such as metals, from the final product, but also eliminates the need for solids movement and mixing (Teixeira et al., 2003). The modified SEBAC-II process means the system which was modified to recycle leachate under flooded operation using forced pumping instead of gravity, and recycling leachate through external gas-liquid separators.

When organic compounds containing nitrogen are anaerobically digested, they are hydrolyzed and the nitrogen is converted to ammonia. Most of this ammonia appears in the leachate. Dissolved ammonia exists in equilibrium with the ammonium ion (the ratio of the concentration of ammonia to ammonium ion being dictated by the pH) and it also partitions between the liquid and gas phase. The digested residue also contains some ammonia. Thus, during anaerobic digestion nitrogen from the solid waste is not removed, and most of it remains in the residue/leachate. This feature may be a drawback from a waste treatment perspective, but can be turned into an advantage if nitrogen in the residue/leachate could be utilized for plant growth purposes.

The digested residue is usually dried before application because it is expensive to transport, handle and spread the residue when it has a high moisture content (Svensson et al., 2004). However, drying would lead to loss of ammonia (Svensson et al., 2004; Rivard et al., 1995), which decreases its effect as a fertilizer because of nitrogen loss. So a stabilization process for the digested residue is necessary. In this study, a stabilization process means an aeration stage after anaerobic digestion. This process not only removes any residual volatile organic acids that formed during anaerobic digestion, but could also nitrify ammonia to nitrate. Nitrate is odorless and non-volatile thereby minimizing any loss of nitrogen in a subsequent drying process.
A lack of literature on stabilization of anaerobically digested residue in terms of the extent and rate of nitrification indicated that not much attention has been devoted to this process during aerobic curing stage. In this chapter the feasibility of initiating nitrification process, the kinetics, extent of nitrification, mass balance for nitrogen transformations and oxygen consumption during stabilization are presented. A method for determination of total ammonia nitrogen (TAN) in anaerobic residue was also developed and tested for its accuracy and recoverability.

2.2 Methods

2.2.1 Experimental Apparatus

Experiments were carried out in a set of 1 L glass jars. Anaerobically digested residue was loaded in the glass jar, the mouth of which was closed with a rubber stopper. Two glass tubes were inserted through the rubber stopper for gas inlet and outlet. The glass tube for inlet was inserted deep into the bottom of the jar. Aeration was carried out from a high purity gas cylinder containing 20 % oxygen and 80% helium (Compressed air was used in the experiment 2.2.3.1 to measure the effect of air flow rate on transformation of nitrogen in anaerobically digested residue). Gas vented from the jar was purged through a dilute H₂SO₄ (concentration 0.04N) solution to absorb any volatilized ammonia. A schematic diagram of this reactor is shown in Figure 2-1.

2.2.2 Anaerobically Digested Residue

The residue used in these studies was an anaerobically digested mixture of wheat straw (55% of wet weight), shredded paper (37% of wet weight) and commercial dog food (7.6% of wet weight). This mixture in terms of its composition simulated the proportions of crop residue, paper wastes and human feces, respectively expected in long-term space missions (Chynoweth et al, 2002). Anaerobic digestion of this synthetic waste was carried out in a SEBAC II system as described in Luniya et al (2005). Basic characteristics of the resulting residue are listed in Table
2.1. After digestion, the residue was stored in a refrigerator for about six months before it was
used in experiments reported here.

2.2.3 Experiments

2.2.3.1 Effect of air flow rate on transformation of nitrogen in anaerobically digested
residue

The glass jar was loaded with 80 g of wet residue. The jar was purged by compressed air
and the inlet air flow rate was 24 mL/min. Aeration was terminated after 8 days. Upon
termination of aeration the residue was taken out and analyzed for TAN, nitrite- and nitrate-
nitrogen. The TAN in dilute H₂SO₄ solution, which was used to absorb any volatilized ammonia,
was also determined. The experiment was carried out in triplicate.

After completion of aeration by 24 mL/min air flow rate, the air flow rates of 15 mL/min
and 6 mL/min were also tested. The air flow rate which yielded better values for nitrification was
then used to carry out the following experiments.

2.2.3.2 Transformation of nitrogen in anaerobically digested residue

Six glass jars were loaded with 80 g of wet residue each. The seventh jar was used as a
control to quantify nitrogen ingress into the system from outside via diffusion and was treated
identically to the other jars but with no residue in it. The same gas cylinder was used for aeration
of all jars through manifold tubing. The inlet flow rate of gas was set at 15 mL/min (which
yielded better values for nitrification). Outlet gas was analyzed for N₂ and O₂ content every day.
Aeration was terminated after 1, 2, 5, 8, 10 and 16 days respectively in each glass jar. Upon
termination of aeration the residue was taken out and analyzed for pH, total Kjeldahl nitrogen
(TKN), TAN, and nitrite- and nitrate-nitrogen. If pH of the residue was below ~7.5 then sodium
bicarbonate was added to bring pH up to ~7.5. The amount of sodium bicarbonate required was
noted and the same amount was then added to the rest of the jars. Experiments were carried out twice, the second after completion of the first run.

2.2.4 Analysis

2.2.4.1 Measurement of TAN

Since a method was not available in the literature to determine TAN in anaerobically digested residue an assay was developed and tested for its accuracy and recoverability. A sample of 10 g wet residue was placed in a flask, to which 10 mL NH₄Cl solution (Concentration: 1 g NH₄Cl/L) was added, and the solution allowed to be absorbed completely by the residue. Into another flask containing 10 g of wet residue 10 mL of distilled water was added. This served as the control. Then 100 mL of 2M KCl was added to each flask as the extraction solution and the flasks were closed by a rubber stopper to avoid ammonia volatilization. Then the flasks were placed on a shaker for 10 minutes. The TAN concentration in the extraction solution was measured by ammonia electrode (Accumet Cat# 13-620-508). This was followed by adding another 100 mL KCl solution and the extraction procedure was repeated. At the end of each extraction TAN was determined.

In another set of experiments, first NaOH was used to increase the pH of KCl solution to 11. Then the extraction procedure as described previously was repeated with KCl. TAN was measured in the extracted solutions. Extractions were repeated twice.

In third set of experiments, distilled water rather than KCl solution was used as extraction solutions. In order to increase pH above 11, NaOH was also added to the distilled water. The TAN was measured in the extracted solutions. Extractions were repeated twice. The method which yielded better values for TAN was then used to measure TAN in the digested residue.
For the measurement of TAN in extraction solutions, ammonia-selective electrode method was used. Firstly, a standard curve was calculated. Standard solutions of 0.1, 1.0, 10, 100, and 1000 mg/L TAN were prepared using NH₄Cl. The electrode was immersed into 100 mL standard solution and a magnetic stirrer was used to mix. A sufficient volume of 10N NaOH solution was added to raise pH above 11. The electrode was kept in solution until a stable millivolt reading was obtained. The relationship of log (Concentration) and millivolt should be linear. A representative standard curve is shown in Figure 2-2. The same procedure was used to measure extraction solutions, and the TAN concentration was read from the standard curve. Ammonia in the diluted sulfuric acid trap was also measured using ammonia electrode.

2.2.4.2 Measurement of nitrite

A sample of 10 g wet residue was placed in a flask, to which 100 mL of 2 M KCl solution was added as the extraction solution, and the flask was closed with a rubber stopper. Then the flask was placed on a shaker for 10 minutes.

A colorimetric method was used to measure nitrite nitrogen in the extraction solutions. Diazotizing reagent was prepared by dissolving 0.5g of sulfanilamide in 100 mL of 2.4 M HCl. Also, 0.3g of N-(1-naphtyl)-ethylenediamine dihydrochloride was dissolved in 100 mL of 0.12 M HCl to make a coupling reagent. A sample of 2 ml of the extract was pipetted into a 50 mL volumetric flask, and deionized water was added to make the total volume about 45 ml. Then 1 ml of the diazotizing reagent was added. After 5 min, 1 ml of the coupling reagent was added. The solution was mixed and allowed to stand for 20 min. After that the solution was made to volume, mixed thoroughly, and color intensity was measured at 540 nm against a reagent blank solution. Absorbance measurements were calculated by analysis of standards whose concentrations were 0, 0.1, 0.2, 0.3 mg/L of NO₂⁻-N. The absorbances of standards were measured for analysis of the extract. A representative standard curve is shown in Figure 2-3. The
\[ \text{NO}_2^- \] concentration of the sample was determined using the equation obtained by linear regression of the concentration of the standards against the corresponding absorbance measurements.

### 2.2.4.3 Measurement of nitrate

A sample of 10 g wet residue was placed in a flask. 100 mL of distilled water was added to the flask as the extraction solution and the flask was closed with a rubber stopper. Then the flask was placed on a shaker for 10 minutes. A nitrate electrode was used to measure nitrate nitrogen in the extraction solutions.

Firstly, a standard curve was calculated. Standard solutions of 1.4, 14, 140, 1400 mg/L \( \text{NO}_3^- \) - N were prepared using \( \text{NaNO}_3 \). A sample of 2 ml of ionic strength adjuster (2 mol/L \((\text{NH}_4)_2\text{SO}_4\)) was added to each 100 ml of standard. The nitrate electrode was used to get potential of each solution. Potential measurements against log (NO\(_3\)-N concentration) were plotted on graph paper and the standard curve was obtained. A representative standard curve is shown in Figure 2-4. The same procedure was used to measure extraction solutions and read nitrate nitrogen concentration from the standard curve. These measurement methods are summarized in Table 2-2.

### 2.2.4.4 Gas samples

Gas samples from outlets of jars were analyzed every day for nitrogen and oxygen by a gas chromatograph equipped with a thermal conductivity detector (Fisher Gas Partitioner, Model 1200). The gas chromatograph was calibrated with an external standard containing N\(_2\): O\(_2\): CH\(_4\): CO\(_2\) in volume ration 20:5:45:30. Gas chromatograms were processed and recoded using an integrator (SP 4200 Integrator, Spectra Physics, Inc.).
2.2.4.5 Stabilization performance

The performance of stabilization was evaluated by fitting the cumulative nitrified nitrogen data to the modified Gompertz equation (Zwietering et al, 1990). The assumption of this model is that the cumulative amount of nitrified nitrogen into batch reactor is a function of bacterial growth. The modified Gompertz equation could be expressed below:

\[
y = A \exp\left\{-\exp\left[\frac{\mu_m e^{\lambda}}{A} (\lambda - t) + 1\right]\right\}
\]

where \(y\) is the cumulative nitrified nitrogen (mg/g wet residue) at any time \(t\), \(A\) is the nitrified nitrogen potential (mg/g wet residue), \(\mu_m\) is the maximum nitrification rate (mg/g wet residue/day), \(\lambda\) is the duration of lag time (day), and \(t\) is the time at which cumulative nitrified nitrogen \(y\) is calculated (day). The parameters \(P, \lambda, \mu_m\) were estimated by using the “Solver” feature in MS-Excel. The value of parameters which minimized the sum of the square of errors between fit and experimental data were determined.

2.3 Results and Discussions

2.3.1 Determination of TAN in Anaerobically Digested Residue

An extraction efficiency of TAN from the digested residue was determined using the following formula:

\[
\text{Extraction efficiency} (\%) = \frac{\text{TAN in treatment} - \text{TAN in control}}{\text{TAN added into treatment}}
\]

For extractions in which pH was not increased, the efficiency was 51.4%. However, for those extractions which were carried out after increasing the pH to 11, the efficiency was 107%. The measurements were carried out in triplicate and the recoverability was within 2%. The pH of digested residue as measured after adding the extraction solution was 6. For this situation the
TAN measured was approximately half of that expected. However, if the pH of extraction solution was increased to above 11 by NaOH addition, almost all the TAN was detected. The value was over 100% because the TAN distribution in the digested residue may not be uniform. The average TAN content in the residue was used as the value of the control in the above expression. It was possible that ammonium ion (the dominant form of ammonia at pH 6) was adsorbed strongly on to the surfaces of the residue, which made it difficult to extract. However, when pH was increased the dominant form shifts to dissolved ammonia which was easily extracted. So pH adjustment during extraction is important to recover and measure TAN in digested residue.

Another set of experiments was carried out by using distilled water (pH>11) instead of KCl solution as the extraction solution. After 3 times of extraction, the efficiency was only 50.8%. The extraction using KCl obtained better efficiency because KCl served as a cation exchanger in the extraction process. The 2 M KCl extraction procedure was adopted because use of 2 M KCl instead of 1 M KCl reduces the size of the aliquot of extract, and some analyses showed that the results obtained using 2 M KCl for extraction had slightly higher precision than those obtained using 3 M KCl or 4 M KCl (Bremner and Keeney, 1966). Subsequently TAN was determined after adjusting the pH to 11 and extracted with an aqueous KCl solution.

2.3.2 Effect of Air Flow Rate on Transformation of Nitrogen in Anaerobic Residue

Three different air flow rates were conducted, which were 6 mL/min, 15 mL/min and 24 mL/min. Figure 2-5 shows the effect of air flow rates on transformations of nitrogen in anaerobically digested residue. The results were reported as percentage (%) of total nitrogen in anaerobically digested residue before and after 8-day stabilization process. The experiments were carried out in triplicates. The results shown in Figure 2-5 are the average of these three measurements.
When air flow rate was 24 mL/min, the result showed that no nitrification happened even though TAN content in the residue dropped from 86% to 32%. The results showed that 48.4% of nitrogen in the residue was lost by volatilization and this may be the reason for the decrease of TAN in the digested residue. Therefore, in order to minimize ammonia volatilization, it was necessary to decrease the air flow rate. When the air flow rate dropped to 6 mL/min, volatilized ammonia loss was only 6.5%. However, the total nitrified nitrogen, i.e. the sum of nitrite nitrogen and nitrate nitrogen, was only 20%. There was 40% of total nitrogen that was unknown in this 8-day stabilization process according to the mass balance for nitrogen. In the nitrification process, oxygen was consumed to oxidize ammonia nitrogen to nitrite nitrogen and then to nitrate nitrogen by nitrifying bacteria as followings:

\[ \text{NH}_4^+ + 1.5 \text{ O}_2 \rightarrow 2 \text{ H}^+ + \text{H}_2\text{O} + \text{NO}_2^- \]

\[ \text{NO}_2^- + 0.5 \text{ O}_2 \rightarrow \text{NO}_3^- \]

When the air flow rate was low, limited oxygen was available for the bacteria, so the denitrification process may happen. In this case, nitrate may have been converted to nitrogen gas according to the following equation and exhausted from system.

\[ \text{NO}_3^- + 6 \text{ H}^+ + 5 \text{ e}^- \rightarrow 0.5 \text{ N}_2 + 3 \text{ H}_2\text{O} \]

So, it was possible that the oxygen supply by 6 mL/min of air flow was not enough for the nitrification process and lead to nitrogen loss from the residue by denitrification.

When the air flow rate was 15 mL/min, the nitrite and nitrate nitrogen reached 7.2% and 63.3% of total nitrogen respectively. At the same time, loss of ammonia nitrogen due to volatilization was 11.7% and unknown nitrogen (maybe lost by denitrification) was 4.8%. So apparently a flow rate of 15 mL/min maximized nitrification and minimized volatilized ammonia
losses through stripping with the apparatus used here. Therefore the following experiments were
carried out at a flow rate of 15 mL/min, which was equal to 187 mL/kg wet residue/min.

2.3.3 Effect of Aeration on Transformation of Nitrogen in Anaerobically Digested Residue

A gas tank containing a helium-oxygen mixture (80:20 by volume) instead of air was used
in order to measure nitrogen gas production from the denitrification process. Figure 2-6 depicts
the nitrogen fractions in the form of ammonium, nitrite, nitrate, nitrogen gas and volatilized
ammonia over a 16-day aeration process. All forms of nitrogen are reported as a percentage (%)
of the total nitrogen at the beginning of the experiment (i.e. day 0).

At the beginning, the total initial nitrogen content was 414 mg, 72% of which was TAN
and 28% of which was nitrate-nitrogen. Nitrate could have been formed during storage due to the
presence of oxygen in the headspace. Nitrogen gas production within a time period was
estimated as the product of nitrogen concentration in the outlet of the jar and the gas flow rate
over that time period. Nitrogen measured from the control jar was subtracted. As no N₂ was
detected in the inlet of all jars, nitrogen measured at the outlet of control jar must be due to
diffusion from outside.

About 85% of ammonium-nitrogen was nitrified during the 16-day aeration. It was seen
that the amounts of nitrogen i.e sum of ammonium, nitrite, nitrate, nitrogen gas and ammonia
losses, were in the range of 390 to 479 mg. The differences were around 5~15% of the initial N
content of 414 mg in the residue. From Figure 2-6 it can be seen that the nitrification process
was activated within two days without any inoculum addition, which meant appropriate
microbial populations required for nitrification were naturally available in the anaerobically
digested residue. The presence of nitrate in the residue used for the experiments was due to
formation of nitrate during storage, which also indicated presence of nitrifiers in the residue. In
the nitrification process, ammonium nitrogen is first converted to nitrite-nitrogen by
Nitrosomonas or other nitrifying bacteria, then nitrite is oxidized to nitrate-nitrogen. Nitrite content was very low in the solid residue during the whole aeration period. Low contents of nitrite-nitrogen indicated that most nitrite was consumed as soon as it was generated.

Ammonia lost by volatilization and stripping was less than 6% after 16 days of aeration. An aeration rate of 187 mL/kg wet residue/min was able to minimize volatilization. The TKN measurements showed that organic nitrogen in the residue was maintained between 55 ~ 65 mg N during the 16-day aeration period. This appeared to indicate that any ammonia released by heterotrophic aerobic respiration was matched by assimilation of nitrogen into microbial biomass.

The errors in the results for all the measurements (except N₂ gas) were between 5-15%. However, errors for N₂ gas production varied between 15-30%. This could have been due to two reasons. Firstly, N₂ gas production was estimated based on a N₂ concentration measurement taken daily. Secondly, air flow patterns within the residue could have varied between jars leading to different N₂ production rates. The results shown in Figure 2-6 are the average of two measurements.

2.3.4 Nitrification Kinetics

Figure 2-7 depicts the amounts of nitrogen nitrified and denitrified during the aerobic stabilization process. Total nitrification represents the cumulative production of nitrite-nitrogen, nitrate-nitrogen and nitrogen gas. The curve from Gompertz fit is the result of fitting the cumulative nitrified nitrogen data to the modified Gompertz equation. The parameters P, λ and μₘ estimated by the Gompertz equation are listed in Table 2-3. Denitrification bars show the cumulative generation of nitrogen gas. Figure 2-8 shows the nitrification and denitrification rate during this stabilization process. The rates are expressed in units of mg N/g wet residue/day.
Nitrate production was initiated two days after aeration, and was sustained until completion of the experiment on day 16. The initial nitrification rate was 0.13 mg/g wet residue/day. After that, the nitrification continued to increase and the maximum rate was 0.41 mg/g wet residue/day, which was obtained on the 8th day. The experiment was carried out for 16 days, by which time 85% of original ammonium was removed and the nitrification rate dropped to 0.04 mg/g wet residue/day. Substrate available for microorganism growth, such as inorganic carbon source and ammonia, could have become limited after several days reducing the nitrification activity. The Gompertz model fitted the nitrification data very well. The lag phase for nitrification was 3.7 days in the model, which was a little longer than that in the experiment. The maximum nitrification rate was 0.49 mg/g wet residue/day.

From Figure 2-7, it can be seen that denitrification was initiated soon after beginning nitrification process. Nitrite and nitrate-nitrogen were produced from the nitrification process, providing substrate for denitrification. The rate of denitrification process continued to increase until day 10 after which it dropped. On the 10th day, the rate reached a peak value of 0.36 mg/g wet residue/day. The rate dropped to 0.004 mg/g wet residue/day on the 16th day. The denitrified nitrogen fraction reached ~50% of the nitrified fraction in this 16-day experiment. Denitrification occurs in the absence of molecular oxygen in anoxic zones. It is evident that even though continuous air flow was maintained, anoxic zones may have developed within the residue promoting denitrification. Thus, nitrogen was lost from the residue due to denitrification.

**2.3.5 Oxygen Consumption**

Oxygen consumption based on wet weight of anaerobic digestate is listed in Table 2-4. The cumulative nitrified nitrogen, cumulative oxygen consumption and theoretical oxygen consumption are shown in units of mg per g wet residue. Theoretical oxygen consumption was calculated based on the stoichiometry for nitrification:
\[
\text{NH}_3 + 2 \text{ O}_2 \rightarrow \text{NO}_3^- + \text{H}^+ + \text{H}_2\text{O}
\]

Within the 16-day aeration period, 8.05 mg oxygen was consumed per mg nitrate-N produced, while the theoretical requirement based upon stoichiometry is only 4.57 mg oxygen/mg nitrified nitrogen. This meant that while 57% of oxygen was consumed to oxidize ammonium to generate nitrate, 43% of oxygen was consumed by residual volatile organic acids, sulfide or composting process.

2.4 Conclusion

A method was developed for the measurement of TAN in the digested residue as a method was not available in the literature. The 2 M of KCl was used as an extraction solution, and pH of the extraction solution was increased to above 11 by NaOH addition. The results showed that the extraction efficiency was above 98% and the recoverability was within 2%.

A method for nitrification on the solid digested residue was also developed. As the microorganisms required for nitrification process naturally existed in the anaerobically digested residue, it was possible to stabilize the nitrogen by simply aerating it. Nitrification was accomplished without any inoculum addition.

By continuously blowing air through the residue at 187 mL/kg wet residue/min, the nitrification process could be initiated within two days. Approximately 85% of ammonium-nitrogen was nitrified during a 16-day aeration period and the maximum rate was 0.41 mg/g wet weight/day. The denitrification process occurred soon after nitrification and its fraction reached ~50% of the nitrification. The modified Gompertz model was used to quantify the rate of NH₃ transformations, and the results showed that it fitted the nitrification data very well.

The oxygen consumption during this stabilization process was determined. The result showed that the oxygen consumption was 8.62 mg oxygen per mg nitrified nitrogen even though theoretical requirement based upon stoichiometry was 4.57 mg oxygen /mg nitrified nitrogen.
Figure 2-1. Glass reactor connected to an ammonia trap

Figure 2-2. Representative standard curve of TAN by ammonia-selective electrode method
Figure 2-3. Representative standard curve of nitrite-nitrogen by colorimetric method

Figure 2-4. Representative standard curve of nitrate-nitrogen by nitrate electrode method
Figure 2-5. Effect of air flow rate on nitrogen transformations in anaerobically digested residue during the 8-day stabilization process when the initial total inorganic nitrogen was 300 mg N

Figure 2-6. Fractions of ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, nitrogen gas and volatilized ammonia in the 16-day stabilization process when the gas flow rate was 187 mL/kg wet residue/min (room temperature) and initial total inorganic nitrogen was 414 mg N
Figure 2-7. Cumulative amount of nitrified- and denitrified nitrogen in 16-day stabilization process in the 1 L glass reactor when the gas flow rate was 187 mL/kg wet residue/min (room temperature)
Figure 2-8. Nitrification and denitrification rate in 16-day stabilization process in 1 L glass reactor when the gas flow rate was 187 mL/kg wet residue/min (room temperature)
Table 2-1. Basic characteristics of anaerobically digestated residue

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>81 - 86</td>
</tr>
<tr>
<td>Total solids (TS) (%)</td>
<td>14 - 19</td>
</tr>
<tr>
<td>Volatile solids (VS) (%)</td>
<td>91 - 98</td>
</tr>
<tr>
<td>pH</td>
<td>7 – 8.8</td>
</tr>
<tr>
<td>NH$_4^+$ - N (mg/g wet weight of residue)</td>
<td>3.5 – 5.2</td>
</tr>
<tr>
<td>Organic N (mg/g wet weight of residue)</td>
<td>4.3 – 7.9</td>
</tr>
</tbody>
</table>

Table 2-2. Measurement of NH$_4^+$, NO$_2^-$ and NO$_3^-$ in digestated residue (10 g wet weight)

<table>
<thead>
<tr>
<th></th>
<th>NH$_4^+$ - N</th>
<th>NO$_2^-$ - N</th>
<th>NO$_3^-$ - N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement method</td>
<td>Ammonia electrode</td>
<td>Colorimetric method</td>
<td>Nitrate electrode</td>
</tr>
<tr>
<td>Extraction reagent</td>
<td>100 ml 2M KCl (pH&gt;11)</td>
<td>100 ml 2M KCl</td>
<td>100 ml Distilled Water</td>
</tr>
<tr>
<td>Extraction time</td>
<td>Shake for 10 minutes</td>
<td>Shake for 10 minutes</td>
<td>Shake for 10 minutes</td>
</tr>
<tr>
<td>Extraction efficiency</td>
<td>98% after 3 times extraction</td>
<td>Above 95 %</td>
<td>Above 90 %</td>
</tr>
</tbody>
</table>

Table 2-3. Experimental and Gompertz values of nitrification in 16-day stabilization process (the gas flow rate was 187 mL/kg wet residue/min)

<table>
<thead>
<tr>
<th></th>
<th>Experimental value</th>
<th>Gompertz equation value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrified nitrogen A (mg/g wet residue)</td>
<td>4.39</td>
<td>4.48</td>
</tr>
<tr>
<td>Maximum nitrification rate $\mu_m$ (mg/g wet residue/day)</td>
<td>0.41</td>
<td>0.496</td>
</tr>
<tr>
<td>duration of lag time $\lambda$, day</td>
<td>2</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Table 2-4. Oxygen consumption during 16-day stabilization process (the gas flow rate was 187 mL/kg wet residue/min)

<table>
<thead>
<tr>
<th>Elapsed time (days)</th>
<th>Nitrification (mg N)</th>
<th>$O_2$ consumption (mg O)</th>
<th>Theoretical required $O_2$ consumption (mg O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.22</td>
<td>1.73</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.56</td>
<td>4.19</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>2.11</td>
<td>12.39</td>
<td>3.13</td>
</tr>
<tr>
<td>8</td>
<td>3.35</td>
<td>24.42</td>
<td>8.79</td>
</tr>
<tr>
<td>10</td>
<td>4.15</td>
<td>25.16</td>
<td>12.48</td>
</tr>
<tr>
<td>16</td>
<td>4.39</td>
<td>25.54</td>
<td>13.54</td>
</tr>
</tbody>
</table>
3.1 Introduction

When organic compounds containing nitrogen are anaerobically digested they are hydrolyzed and the nitrogen is converted to ammonia. Most of this ammonia appears in leachate. The leachbed digestion uses recycle of leachate between new and mature reactors to inoculate, wet and provide nutrients for rapid startup of new cells. Organic acids produced during startup are conveyed via leachate to the mature reactor for conversion (Ghosh, 1984; Chynoweth et al, 1991, 1992). However, ammonia is accumulated in the system when leachate is reutilized from a mature reactor. Accumulation of ammonia may negatively affect the anaerobic digestion performance. Many studies reported ammonia inhibition or toxicity in anaerobic digestion processes (McCarty and McKinney, 1961; Gallert and Winter, 1997). Dissolved ammonia exists in equilibrium with the ammonium ion and the ratio of the concentration of ammonia to ammonium ion is dictated by the pH. It has been demonstrated that a free ammonia concentration of 150 mg/L inhibited around 50% of the anaerobic digestion performance (McCarty and McKinney, 1961). Koppar and Pullammanappallil (2007) reported the effect of ammonia accumulation in serially operated, single-stage, batch, leach-bed, thermophilic anaerobic digestion of spent sugar beet pulp. In this study, each subsequent run used the leachate from a previous run (e.g. Run 2 used the leachate at the end of Run 1, Run 3 used the leachate at the end of Run 2 and so on). The free ammonia concentration reached 149 mg/L at the end of Run 3. The maximum methane production rate was 0.086 m$^3$/day after Run 3, dropped sharply to 0.047 m$^3$/day after Run 4 and dropped further to 0.017 m$^3$/day at the end of Run 5. Similarly, the time required to achieve 95% of ultimate methane potential for Run 3 was 5.71 days, increased to 10.92 days by the end of Run 4 and further rose sharply to 21 days. The results and analysis
showed that the inhibition observed in Run 4 and 5 may be due to toxicity from free ammonia accumulation. Lay et al (1997) also showed that in a well-acclimatized bacterial system, the methanogenic activity dropped 10% when the NH$_4^+$-N concentration was 1670-3720 mg/L, 50% when 4090-5550 mg/L and dropped to zero when 5880-6600 mg/L. The pH in these studies ranged from 6.5 to 8.5. It was also shown that the lag phase duration was dependent on the NH$_3$ level instead of NH$_4^+$ (Lay et al, 1997).

Nitrogen in ammonia form can be removed from the leachate by several different processes, including biological nitrification-denitrification (Aspe et al, 2005; Dong and Tollner, 2003; Wang et al, 2003), stripping (Zeng et al, 2006; Bonmati and Flotats, 2003), ion exchange (Sanchez et al, 1995; Milan et al, 1997) and struvite precipitation (Uludag et al, 2005).

Ammonia removal by biological nitrification-denitrification is a popular method due to its high efficiency and low cost. In this process, ammonia is firstly converted to nitrite and then to nitrate under aerobic conditions by nitrifying organisms, after which nitrate is reduced to nitrogen gas under anaerobic conditions by denitrifying organisms. Recently several varieties of the above scheme have been developed, including anaerobic ammonium oxidation (Anammox), completely autotrophic nitrogen removal over nitrite (Canon) (Sliekers et al, 2003) and oxygen-limited autotrophic nitrification-denitrification (Oland) process (Kuai and Verstraete, 1998; Verstraete and Philips, 1998; Peng and Zhu, 2006).

The concept of biological nitrification and denitrification has been employed to remove ammonia from wastewater or leachate. The kinetics of an in situ ammonia removal in both acclimated and unacclimated wastes was evaluated and the ammonia removal efficiencies reached above 97% when the initial ammonia concentration was 500 mg N/L. All rate data fit well to Monod kinetics, with specific rates of removal of 0.196 and 0.117 mg N/day/g dry waste
for acclimated and unacclimated wastes, respectively (Berge et al, 2006). The impact of temperature and gas-phase oxygen on the kinetics was also evaluated, showing that most rate data fit well to an empirically based multiplicative Monod equation with terms describing the impact of oxygen, pH, temperature and ammonia concentration (Berge et al, 2007). The fate of nitrogen in leachate from bioreactor landfills was summarized (Berge et al, 2005). Also, Jun et al (2004) test an upflow sludge blanket (USB) reactor combined by aerobic biofiltration system had been tested with real sewage. About 95% of ammonia was nitrified in the aerobic filter and the denitrification efficiency was in the range of 72-85% in the anoxic filter. Total ammonia nitrogen removal efficiency reached 70% in this process. Anaerobic digestion may be improved by integrating this biological ammonia removal step into the anaerobic digester system. Wang et al (2003) tried to integrate an aerated submerged biofilter into a two-phase anaerobic digestion process for food waste. Ammonia accumulated in the original system due to recycling of the leachate from the methanogenic reactor. In their study, the leachate from a methanogenic reactor was treated by passing the submerged biofilter for ammonia removal under aerobic conditions. Then the leachate was divided into two streams. The flow rate ratio of the stream recycled into the acidogenic reactor to the stream used for dilution of acidogenic leachate was 1:4. The result showed that ammonia removal efficiency was above 90% and methane production in the enhanced system increased by 26% compared to the original system without the aerated biofilter.

The studies presented in Chapter 2 showed that nitrifying activity can be initiated and sustained in anaerobically digested residue. During post aerobic processing of anaerobically digested residue a major fraction of ammonia was nitrified. Therefore, it is possible that the solid residue itself could serve as a biological filter for nitrification when leachate containing ammonia is flushed through it, i.e. leachate nitrification may be combined with post aerobic processing of
digested residue. The nitrified leachate upon exposure to anaerobic conditions would denitrify, provided sufficient carbon source is available. Another issue in this application, unlike in studies presented in the literature (Berge et al, 2006, 2007; Jun et al, 2004), is the viability of the leachate processed in this manner to initiate digestion of subsequent batches of wastes. The objectives of the work presented in this chapter are to determine:

- Feasibility of biological ammonia removal from leachate during aerobic processing of anaerobically digested residue
- Kinetics of ammonia removal
- Viability of aerated leachate as inoculum for subsequent leach-bed digestion.

3.2 Methods

3.2.1 Experimental Apparatuses

3.2.1.1 Anaerobic digester

A digester was constructed by modifying a Pyrex glass jar. The volume of the digester was 5 liters. The digester was sealed with a top lid, using an O-ring fitted for gas and liquid tightness and clamped with a stainless steel clamp. Three ports were provided at the top of the lid, one for gas outlet, and others for sample withdrawal. The digester was also equipped with an outlet at the bottom from which liquid samples were collected. No additional mixing device was applied. The digester was placed in an incubator where the temperature was maintained at 55 °C. The digester set-up is shown in Figure 3-1.

3.2.1.2 Ammonia removal reactor

Another 5-liter glass bottle half filled with pumice stones was used for ammonia removal (Figure 3-2). The mouth was sealed by a top lid with 4 ports. Two ports were used for air supply, one for leachate circulation and the last one for gas exhaust. Two spargers were connected with the air supply lines and inserted to the surface of pumice stones, so that the solid digested residue
on the pumice stones was in the aerobic zone while the leachate with pumice stones was in the anaerobic zone. The glass bottle was also equipped with an outlet at bottom from which leachate was pumped to the top of the reactor. Connected to the port for leachate circulation, a shower head was used inside the reactor in order to equally distribute the leachate across the top of the solid residue. Gas vented from the lip of bottle was purged through a dilute H$_2$SO$_4$ (concentration 0.04N) solution to absorb any volatilized ammonia.

3.2.2 Feedstock for Anaerobic Digestion

Rice straw was used as feedstock and was provided by Earth Saver Company, California. The rice straw was stored at room temperature. Some basic characteristics of rice straw are listed in Table 3-1.

3.2.3 Experiments

3.2.3.1 Anaerobic digestion of rice straw

Eight experiments using inoculum processed in different ways were carried out (Figure 3-3). In each experiment, the digester was loaded with 100 g of rice straw as received, i.e the straw was not subjected to any size reduction. It was then filled with 4.2 liters of liquid, mixture of tap water and inoculum. The make up of this liquid varied in each experiment. The digester was placed in the incubator whose temperature was controlled at 55 °C.

In Experiment 1, 1.4 liters of tap water and 2.8 liters of inoculum were used after loading the rice straw. The inoculum was taken from a thermophilic digester that had been digesting sugar beet tailings for over two years. Once anaerobic digestion was completed in Experiment 1, the digester was opened, the solid residue was taken out and liquid (digested leachate) was drained out from the digester. Then the leachate was processed by aeration for ammonia removal until the TAN concentration was lower than 10 mg NH$_4^+$ -N/L. Experiments 2 and 3 were repetitions of Experiment 1.
In Experiment 4, 2.8 liters of processed leachate (from Experiment 1) and 1.4 liters of tap water were used. In Experiment 5, 2.8 liters of processed leachate (from Experiment 2) and 1.4 liters of tap water were used as in Experiment 4. But in addition, 100 mg/L of ammonia nitrogen was added into the digester.

Experiment 6 was conducted using 1.4 liters of tap water and 2.8 liters of processed leachate from Experiment 4. This time, the leachate (inoculum) had been processed twice.

Experiment 7 was carried out using 2.8 liters of tap water and 1.4 liters of inoculum directly taken from a thermophilic digester that had been actively digesting sugar beet tailings for over two years (i.e., the same inoculum as Experiment 1-3). The dilution factor of the inoculum in Experiment 7 was the same as that in Experiment 6 except that the microorganisms here were not subjected to any aerobic processing, whereas in Experiment 6 they were subjected to processing twice. The unprocessed leachate from Experiment 7 was used to flood rice straw to carry out next Experiment 8 without subjecting it to any aeration.

3.2.3.2 Aerobic processing of leachate for ammonia removal

After the completion of anaerobic digestion in Experiment 1, the solid residue was taken out and the leachate was drained out from the thermophilic digester. Then the solid residue mixed with 30 g (wet weight) commercial Black Kow compost was placed on the surface top of pumice stones in the ammonia removal reactor and the leachate was also loaded. The quantity of pumice stones was such that the residue was not immersed into the leachate. The leachate was recirculated continuously over the solid bed using a pump. The leachate flow rate was 30-45 mL/minute and air flow rate was 300 mL/kg wet residue/min. In this first processing experiment, the initial concentration of ammonia nitrogen was 500 mg/L. After the ammonia concentration decreased to less than 10 mg NH$_4^+$-N /L, the leachate was reused for subsequent anaerobic digestion, i.e. Experiment 4. In the 2nd aerated processing experiment, the fresh residue after
completion of anaerobic digestion was taken out from the digester and loaded on the surface top of old residue from the 1st aerated processing. The leachate was drained out from the digester (Experiment 4) and loaded to the ammonia removal reactor. Additional NH$_4$Cl was added to make initial ammonia concentration up to 500 mg NH$_4$$^+$-N /L. Upon completion of aerated processing, the processed leachate was reused for subsequent anaerobic digestion (Experiment 6). In the 3rd aerated processing experiment, leachate from Experiment 2 was aerated and the processed leachate was used as inoculum for anaerobic digestion in Experiment 5.

3.2.4 Analysis

Gas production was monitored daily. The biogas was metered using a positive displacement gas meter (Figure 3-1). The device consisted of a clear PVC U-tube filled with anti-freeze fluid (ethylene glycol), a 3-way solenoid valve (Fabco Air), a float switch (Grainger), an electromechanical counter (Redington Inc.) and a time delay relay (Dayton OFF Delay Model 6X153E). The U-tube gas meter was calibrated in-line to determine volume of biogas per count. A count was considered as that amount of gas read on a syringe (in mL) for which the gas meter completes one whole number count (e.g. one count=0.045 L, then two counts = 0.09 L and continued on.).

Gas composition (CH$_4$, CO$_2$) was measured using a gas chromatograph equipped with a thermal conductivity detector (Fisher Gas Partitioner, Model 1200). Methane volume was reported at standard temperature and pressure (STP) conditions.

Leachate samples were collected periodically and analyzed for pH, chemical oxygen demand (COD), TAN and nitrate-nitrogen. The COD of leachate was measured by colorimeter (HACH DR/890 colorimeter). The TAN and nitrate nitrogen were determined by ammonia electrode (Accumet Cat # 13-620-508) and nitrate electrode (Accumet, Cat # 13-620-535), respectively. The TAN on the digested residue was extracted by 2 M KCl solutions from the
residue and also measured by ammonia electrode. The details of measurement methods are described in Chapter 2.

The performance of the anaerobic digestion processes was evaluated by fitting the cumulative methane production data to the modified Gompertz equation (Lay et al., 1998). The Gompertz equation describes cumulative methane production from batch digesters assuming that methane production is a function of bacterial growth. The modified Gompertz equation is presented below:

$$M = P \times \exp \left\{ -\exp \left( \frac{Rm \times e^{(\lambda - t)}}{P} + 1 \right) \right\}$$

Where $M$ is the cumulative methane production, m$^3$/kg VS at any time, $t$, $P$ is the methane yield potential, m$^3$/kg VS, $Rm$ is the maximum methane production rate, m$^3$/kg VS/day, $\lambda$ is the duration of lag phase, day, and $t$ is the time at which cumulative methane production $M$ is calculated, day. The parameters $P$, $\lambda$ and $Rm$ were estimated for each of the data sets by using the “solver” feature in MS-Excel. The value of parameters which minimized the sum of the square of errors between fit and experimental data were determined.

3.3 Results and Discussions

3.3.1 Anaerobic Digestion of Rice Straw

Figure 3-5 depicts the cumulative biogas and methane yield, daily methane yield and methane fractions in biogas from Experiment 1-3. In these experiments, 1.4 liters of tap water and 2.8 liters of inoculum taken from a thermophilic digester that had been actively digesting sugar beet tailings for over two years were added to flood the bed of rice straw. The microorganisms recovered and methane production was initiated after one day. The methane production rate continued to increase until day 4 after which it dropped. The experiments were
carried out for 15 days, by which time about 0.222 m³ CH₄ STP/kg VS, 0.203 CH₄ STP/kg VS and 0.214 m³ CH₄ STP/kg VS of methane were produced respectively from Experiment 1-3 (Figure 3-5). To analytically quantify parameters for the batch growth curve, a modified Gompertz equation was fit to the cumulative methane production data from these experiments and the results are listed in Table 3-3. From Gompertz equation, the maximum methane production rate was 0.027±0.003 m³/kg VS/day and the methane yield of rice straw was in the range of 0.205 to 0.219 m³ CH₄ STP/kg VS loaded. This yield was obtained without any pretreatment of rice straw, which was better than those reported in the literatures. It was reported that the methane production of rice straw without pretreatment was 0.170 m³ CH₄ STP/kg VS at mesophilic temperature. The methane yield may be improved to 0.217 m³ CH₄ STP/kg VS when the rice straw was ground to 25 mm length and heated in a pressure cooker for 2 hours at 110 °C as pretreatment (Zhang and Zhang, 1999). Therefore, the methane yield from anaerobic digestion of rice straw in Experiment 1-3 was good enough to be a basis for the conclusion from subsequent results.

3.3.2 Biological Ammonia Removal from Leachate

Three aerated processing experiments were carried out. Each processing experiment included two batches and the 2nd batch was immediately after 1st batch. The leachate from anaerobic digestion of Experiment 1 was aerated by 1st processing experiment, after which the leachate was used to inoculate the anaerobic digestion of Experiment 4. After the completion of 1st processing experiment, the ammonia removal reactor was ceased and stayed for two weeks until the 2nd processing experiment was set up. The leachate from Experiment 4 was treated by aeration and the processed leachate was introduced to the anaerobic digester of Experiment 6. After it was completed, the 3rd aerated processing experiment was started immediately. In this
experiment, leachate from Experiment 2 was aerated and the processed leachate was used as inoculum for anaerobic digestion of Experiment 5.

The TAN concentration in the leachate from anaerobic digestion of rice straw in Experiment 1-3 was around 500 mg NH$_3$-N/L. Because of low nitrogen content in rice straw, it was expected that TAN of leachate remaining at the end of digestion would be low, equal to value in Experiment 4 (around 50 mg NH$_3$-N/L). Therefore higher values of TAN measured in Experiment 1-3 mainly came from the inoculum taken from the thermophilic digester that was digesting sugar beet tailings. For better measurement and observation of ammonia removal, additional NH$_4^+$-N was added to the leachate to make the TAN concentration up to 500 NH$_3$-N/L at the beginning of the 2$^{nd}$ and 3$^{rd}$ processing experiment.

The TAN concentrations and processing time in these experiments are listed in Table 3-2. Ammonia removal rate was reported as amount of removed ammonia per liter of liquid volume per day. Appropriate microorganisms can attach and grow on the digested residue from anaerobic digestion due to its large surface area. In the 1$^{st}$ processing experiment, the residue was seeded by 10 % (wet weight) of commercial Black Kow compost. Ammonia oxidization was initiated within 3 days by purging air into the reactor. Daily leachate analysis showed that no ammonia was removed during the first 2 days. The TAN concentration decreased from 505 mg/L to 3.17 mg/L in 7 days in the 1$^{st}$ batch processing. The ammonia removal rate was higher in the 2$^{nd}$ batch processing, in which TAN concentration dropped from 496 mg/L to 0.34 mg/L within 3 days. After completion, the ammonia removal reactor was ceased and stayed inoperable during the anaerobic digestion of Experiment 4. In the 2$^{nd}$ aerated processing experiment, the fresh residue from anaerobic digestion of Experiment 4 was loaded on the surface top of old residue containing ammonia oxidizing bacteria. The measured initial ammonia removal rate was 95.4 mg
/L/day, which indicated the microorganisms need some time to recover and spread out to the fresh residue. The rate increased to 165 mg/L/day in the 2nd batch processing. Upon completion of the 2nd processing experiment, the leachate from anaerobic digestion of Experiment 2 was processed by aeration immediately (termed the 3rd processing experiment). The TAN concentration dropped from 500 mg/L to 4 mg/L in the 1st batch and from 500 to 23.7 mg/L in the 2nd batch within 2 days. So the average ammonia removal rate was 200-245 mg/L/day in this experiment.

The performance of this ammonia removal process was evaluated by fitting the TAN concentration data to the 1st order equation as follows:

\[ \ln \left( \frac{C}{C_0} \right) = -k (t - t_0) \]

where \( C \) = TAN concentration in leachate at elapsed time \( t \), mg/L

\( C_0 \) = Initial TAN concentration in leachate, mg/L

\( k \) = rate constant, day\(^{-1}\)

\( t \) = Elapsed time \( t \), day

\( t_0 \) = lag time, day

The experimental data and the modeling fit in 1st, 2nd and 3rd processing are described in Figure 3-4. Concentrations of TAN, processing time and model parameters are listed in Table 3-2.

In the 1st processing experiment, the residue was seeded by 10 % (wet weight) of commercial Black Kow compost. Then the ammonia oxidization was initiated within 3 days by purging the air into reactor because daily leachate analysis showed that little ammonia was removed during the first 2 days. The lag time was 2.97 days from the model, which showed consistent with the experimental value. The rate constant was only 0.82 day\(^{-1}\), which increased to
2.52 day\(^{-1}\) in 2\(^{nd}\) batch. In 2\(^{nd}\) processing experiments, the rate constant dropped to 0.96 day\(^{-1}\) in 1\(^{st}\) batch, which indicated the microorganisms need some time to recover after 2 weeks without feeding. After that, it increased to 1.3 in 2\(^{nd}\) batch, then to 1.7 - 2.3 day\(^{-1}\) in 3\(^{rd}\) processing experiment. At the same time, the lag time \(t_0\) was in the range of 0-0.2 days. The results demonstrated that the ammonia removal process could be initiated immediately after set up and the removal rate may not increase dramatically any more after 2\(^{nd}\) processing experiment, i. e. after 8-day continuous operation. The experimental ammonia removal rate was 200-245 mg/L at that time. This result, quick startup of nitrification, was better than those in some literatures. Ahn et al (2007) reported 60 mg/L/day of ammonia removal rate within 10 days, 135 mg/L/day after 50 days and 235 mg/L/day after 80 – 90 days in continuous operation in combined anaerobic upflow bed filter and aerobic membrane bioreactor. In an air-lift loop sludge blanket (ALSB) treatment and a sequential upflow anaerobic sludge blanket (UASB) treatment, ammonia removal rate was 170-180 mg/L/day and 35-40 mg/L/day respectively after continuous operation of 50 days (He et al, 2007). In a rotating biological contactor, the nitrogen removal rate reached as high as 858 mg/L/day, but that rate was only obtained after 450 days in continuous operation (Wyffels et al, 2003).

The air flow rate for purging the reactor was 300 mL/minute/kg wet residue. Low concentration of nitrate-nitrogen, about 50 mg/L, was detected at the end of treatments. At the same time, only 5% and less than 1% of initial TAN was in the form of nitrite-nitrogen and volatilized ammonia-nitrogen. These results indicated that more than 85% of TAN may be removed in the form of nitrogen gas by denitrification due to oxygen limitation. The pH increased from 7.46 to 7.72 in the 1\(^{st}\) processing experiment and from 7.86 to 8.90 in the 2\(^{nd}\)
processing experiment. The increase of pH also may be the result of denitrification process by the explanation of proton consumption in the nitrification-denitrification reaction as following:

Nitrification: \( \text{NH}_4^+ + 2 \text{O}_2 \rightarrow \text{NO}_3^- + 2 \text{H}^+ + \text{H}_2\text{O} \)

Denitrification: \( \text{NO}_3^- + 6 \text{H}^+ + 5 \text{e}^- \rightarrow 0.5 \text{N}_2 + 3 \text{H}_2\text{O} \)

Overall: \( \text{NH}_4^+ + 2 \text{O}_2 + 4 \text{H}^+ + 5 \text{e}^- \rightarrow 0.5 \text{N}_2 + 4 \text{H}_2\text{O} \)

The ammonia wasn’t absorbed on the digested residue either. The result showed that the TAN attached on the digested residue could be removed at the same time. In the 2\textsuperscript{nd} processing experiment, the ammonia content decreased from 0.134 mg/g residue (dry weight) to 0.035 mg/g residue (dry weight).

### 3.3.3 Viability of Aerated Leachate as Inoculum for Subsequent Leach-Bed Anaerobic Digestion

Leach-bed anaerobic digestion uses recycle of leachate from mature reactors to inoculate new reactors for rapid startup. Microorganisms used for inoculation are anaerobes, which may not sustain activity under aerobic conditions. So leachate may loose bacterial activity after aerated processing and cannot serve as inoculum for subsequent anaerobic digestion. Therefore, it is necessary to test viability of aerated leachate as inoculum for subsequent anaerobic digestion.

Five experiments were conducted to test the sustained bacterial activity in processed leachate. Experiment 4 was carried out using the inoculum which was processed once and Experiment 6 was implemented using the inoculum which was processed twice. There are another three possible reasons to affect the digestion performance besides aeration. (1) Little ammonia is available in processed inoculum, which may be turned into a limit factor for anaerobic digestion because ammonia nitrogen is a nutrient for bacteria growth. The implementation of Experiment 5 is to test the effect of TAN concentration in leachate. (2)
Inoculum was diluted due to the addition of makeup water. Experiment 7 was carried out to test the effect of inoculum dilution. The dilution factor of inoculum in Experiment 7 was the same as that in Experiment 6. (3) Microorganisms may sustain in leachate and accumulate during reutilization of leachate. It was also possible that microorganisms attached on the digested residue and their population decreased when leachate was reutilized. The objective of Experiment 8 is to test the effect of reutilization of leachate.

Experiment 4 and 5 were conducted using the processed leachate from Experiment 1 and 2, respectively. In these two experiments same amount of rice straw was flooded with 1.4 liters of tap water and 2.8 liters of inoculum after ammonia removal process. In Experiment 5, 100 mg/L of ammonia nitrogen was added into the digester while this was not done in Experiment 4. The results, including cumulative and daily methane yield, COD, pH and TAN in leachate, are described in Figure 3-6 and 3-7. The methane production was initiated within 2 days and the experiment was carried out for 22 days. The total methane yield was 0.211 m³ STP/kg VS and 0.214 m³ STP/kg VS in experiment 4 and 5 respectively. The pH of the leachate was maintained in the range of 7 to 8. For TAN in the leachate in experiment 4, it increased to 22 mg/L on day 4, after which it drop to 15 mg/L. Then it increased again and the final TAN was 50.64 mg/L. In experiment 5, it increased to 133 mg/L on day 2 and dropped to 91 mg/L on day 3. After that it slowly increased to above 140 mg/L.

In Experiment 6, same amount of rice straw was flooded with 1.4 liters of tap water and 2.8 liters of inoculum after ammonia removal process. At this time, the inoculum used had been aerated for ammonia removal twice. Figure 3-8 depicts the cumulative and daily methane yield, COD, pH and total ammonia nitrogen (TAN) in leachate during the anaerobic digestion process. The microorganisms still need two days to recover and produce methane. The peak methane
production rate showed up on day 4, which was same as that in Experiment 1-3. On day 15, the cumulative methane yield was 0.173 m$^3$ STP/kg VS. The value was 18.8% and 18.0% lower than those from Experiment 1-3 and 4-5 respectively. The initial pH in this process was 8.9. On day 3, it decreased to 7.3 due to the presence of organic volatile acids. After that, it increased gradually until 8.5 at the completion of anaerobic digestion. The TAN in leachate increased to 83 mg/L after 1 day and decreased to 16 mg/L on day 5. The final TAN concentration was 48 mg/L.

In Experiment 7, 2.8 liters of tap water and 1.2 liters of unprocessed inoculum directly from thermophilic sugar beet tailings digester were used to flood the same amount of rice straw. The dilution factor of inoculum was the same as that in Experiment 6. The performance of the digester was described in Figure 3-9. Methane production was activated on day 1 and the maximum methane production rate was obtained on day 5, which was 0.042 m$^3$ CH$_4$/kg VS/day. After 15 days, the cumulative methane yield was 0.191 m$^3$ STP/kg VS.

The unprocessed leachate from Experiment 7 was used to flood rice straw to carry out Experiment 8. The performance of the digester was described in Figure 3-9. The methane production was activated immediately after setup and the maximum methane production rate was obtained on day 3, which was 0.037 m$^3$ CH$_4$/kg VS/day. After 15 days, the cumulative methane yield was 0.196 m$^3$ STP/kg VS.

To analytically quantify parameters of batch growth curve, a modified Gompertz equation was fit to cumulative methane production data from all experiments and the results are listed in Table 3-4.

### 3.3.3.1 Effect of TAN and nitrate in leachate

Figure 3-6, 3-7 and 3-8 depicts TAN concentration in leachate from Experiment 4, 5 and 6. In all cases, TAN concentration increased first before dropping. In Figure 3-6 (Experiment 4) and 3-8 (Experiment 6), the lowest concentrations of TAN were around 16 mg/L. After that the
concentration began to climb to about 50 mg/L until the completion of digestion. There may be two reasons for the increase of TAN during the first several days. The first one is the result of hydrolysis of organic matter containing nitrogen. The second possible reason is transformation of nitrate-nitrogen (50-60 mg/L in processed leachate) because of dissimilatory nitrate reduction. Rivard et al (1988) demonstrated that in thermophilic anaerobic digesters, nitrate was reduced to nitrite and finally to ammonia. Microorganisms need ammonia nitrogen as a nutrient for growth, so TAN concentration dropped due to the consumption by microorganisms. The results showed that the ammonia was produced soon after the digester was set up and the amount of ammonia was enough for the microorganism growth. The lowest concentration of TAN was 15.3 mg/L and 16.7 mg/L in Experiment 4 and 6 respectively, which were extra ammonia nitrogen left after the consumption by microorganisms. After the completion of anaerobic digestion, the TAN concentration was around 50 mg/L. Given that there was ammonia nitrogen available during the whole anaerobic digestion, the limitation of TAN in leachate may not be a reason of lower methane yield in Experiment 6.

This was confirmed by results from Experiment 5, which also showed that the concentration of TAN in leachate was not a limited factor. In Experiment 5, 100 mg/L of additional ammonia nitrogen was added into the system. In Figure 3-7, the concentration of TAN increased to 133 mg/L on day 2 and dropped to 91 mg/L on day 3. After that it slowly increased to above 140 mg/L. The final experimental methane yield was 0.214 m³ STP/kg VS, which was close to that in Experiment 4 (0.211 m³ STP/kg VS).

Both reduction of nitrate to ammonia and methane formation from carbon dioxide consume electrons:

\[
\text{NO}_3^- + 10 \text{H}^+ + 8 \text{e}^- \rightarrow \text{NH}_4^+ + 3 \text{H}_2\text{O}
\]
\[ \text{CO}_2 + 8 \text{H}^+ + 8 \text{e}^- \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \]

The competition for electrons may result in the decrease in gas production because methane formation from carbon dioxide only happens after all available nitrate has been reduced (Rivard et al, 1988). The concentration of nitrate in processed leachate was around 50-60 mg/L. According to electron consumption from above two stoichiometric equations, the decrease of methane production is approximately 5% due to the electron competition by nitrate.

From Table 3-4, experimental values and Gompertz fit shows that methane yields from Experiment 4 and 5 were the same as those from Experiment 1-3 (within error bar) and the lag time \( \lambda \) were also similar, which indicates TAN and nitrate-nitrogen in leachate as well as processed inoculum (processed once) didn’t inhibit the performance of anaerobic digester in Experiment 4 and 5.

### 3.3.3.2 Effect of inoculum dilution

Inoculum was diluted due to the addition of makeup water. Experiment 7 was carried out to test the effect of inoculum dilution. The dilution factor of inoculum in Experiment 7 was the same as that in Experiment 6, which was double than that in Experiment 1-3.

From Table 3-4, Gompertz fit shows that the lag time, \( \lambda \), was 1.091 days in Experiment 1-3. It kept similar in Experiment 4 and 5 while increased to 1.744 - 1.844 days in Experiment 6 and 7. This may indicate that the microorganisms need more time to recover when dilution factor of inoculum was higher (The ratio of inoculum and tap water was 1:2 in Experiment 6 and 7).

The maximum methane production rate (\( R_m \)) from Gompertz model was 0.027 m\(^3\) CH\(_4\)/kg VS/day in Experiment 1-3 (mean value). In Experiment 7, it was 0.032 m\(^3\) CH\(_4\)/kg VS/day. Even though the bacteria need more time to recover, the maximum methane production rate didn’t drop due to the inoculum dilution once the digestion was activated. However, the
cumulative methane yield in Experiment 7 was slightly lower than that from Experiment 1-3, which indicated that the dilution of inoculum may be one of the reasons leading to the decrease of methane yield in Experiment 6.

3.3.3.3 Effect of reutilization of leachate

Leachate was reutilized in these experiments. Experiment 8 was carried out to test the effect of reutilization of leachate with no dilution and aerating process. Figure 3-9 depicts the performance of anaerobic digestion in Experiment 7 and 8. The inoculum used in Experiment 8 was unprocessed, undiluted leachate from Experiment 7. The final methane yields were 0.191 m³/kg VS and 0.196 m³/kg VS respectively.

For the parameters from a modified Gompertz equation, the lag time of t₀ was 0 in Experiment 8 indicated that microorganisms in leachate were active to inoculate a new run immediately if the leachate hadn’t been diluted and processed by aeration. The cumulative methane yield (P) was 0.196 m³ CH₄/kg VS in Experiment 8, which was higher than that in Experiment 7 (0.187 m³ CH₄/kg VS) even though the maximum methane production rate was lower. Therefore the reutilization of leachate may not decrease methane yield from anaerobic digestion.

3.3.3.4 Effect of pH in leachate

The initial pH in Experiment 6 was 8.9 and the pH of leachate maintained in the range of 7.5 to 8.5, which was higher than those in Experiment 1-5. However, despite the high pH values there was no effect on the rate of methanogenesis in Experiment 6. The maximum methane production rate was 0.025 m³ CH₄/kg VS/day in Experiment 6, which was in the range of 0.027±0.003 m³ CH₄/kg VS/day from Experiment 1-3 and even higher than those from
Experiment 4-5. Methanogenic rate kept the same indicted higher pH didn’t inhibit the digestion and lead to the lower methane yield (Koppar and Pullamanappallil, 2007)

3.3.3.5 Effect of aerating process

The cumulative methane yield from Experiment 6 was 0.173 m$^3$ CH$_4$/kg VS and methane potential from Gompertz model was 0.171 m$^3$ CH$_4$/kg VS. This value was lower than those from Experiment 1-3, which served as basis for comparison. Based on measurements and operations during these experiments, the lower methane yield in Experiment 6 was initially attributed to five reasons: (1) limited TAN and exist of nitrate in leachate; (2) dilution of inoculum; (3) effect of reutilization of leachate; (4) high pH and (5) aeration process. According to previous discussions, the reasons from TAN, nitrate-nitrogen, reutilization and high pH were discounted. The possible reasons for lower methane yield from Experiment 6 included dilution of inoculum and aeration process. However, from the result of Experiment 7, dilution of inoculum may not be the only reason that leads to methane yield was as low as 0.173 m$^3$ CH$_4$/kg VS in Experiment 6.

Aeration process provides oxygen. Microorganisms required for anaerobic digestion was anaerobes, which may loose activity under aerobic conditions. So the bacteria activity from processed inoculum was lower than that from unprocessed inoculum and resulted in lower methane production in Experiment 6. Another possibility was that aeration process removal other nutrients for bacterial growth, such as phosphorus and trace metal. However, Experiment 4 and 5 also used processed inoculum to activate the anaerobic digestion. The inoculum used was only processed once by aeration and the methane yields were 0.211 m$^3$ CH$_4$/kg VS and 0.214 m$^3$ CH$_4$/kg VS in Experiment 4 and 5 respectively. These values of methane yield were the same as those in Experiment 1-3. But the maximum methane production rate, $R_m$, in Experiment 4 and 5 were slightly lower than those in Experiment 1-3. These results showed that when the inoculum
was processed by aeration once, methane yield didn’t decrease whereas maximum methane production rate did. The methane yield became lower after the inoculum was processed twice.

It would be possible that not only ammonia inhibition due to leachate reutilization could be avoided but also bacterial activity in leachate could be remained if only part of leachate was processed by aeration. For example, TAN concentration in leachate was 500 mg/L. Free ammonia concentration would be around 140 mg/L at pH 8 according to formula as following (Hansen et al, 1998):

\[
\frac{[NH_3]}{[TNH_3]} = (1 + \frac{10^{-pH}}{0.09018 + \frac{2739.92}{T(K)}})^{-1} \quad (1)
\]

where \([NH_3]\) is the concentration of free ammonia, \([TNH_3]\) is the concentration of TAN and \(T(K)\) is the temperature (Kelven). It would be safe to avoid ammonia inhibition if free ammonia concentration was 100 mg/L, i.e. TAN concentration was 350 mg/L. In this case, only 30% of leachate needs to be processed by aeration for ammonia removal.

### 3.4 Conclusion

A post-treatment method, which may be integrated into the SEBAC-II system, was develop to biologically remove ammonia from leachate on the stabilized digested residue by simply aerating the reactor. At the same time, the viability of aerated leachate as inoculum for subsequent anaerobic digestion was also determined as little literature reported the results about the viability of reusing the aerated leachate.

The results showed that when a nitrification-denitrification processing step for ammonia removal from leachate using the stabilized digested residue was integrated into the anaerobic digestion, the ammonia removal rate was 70 -95 mg/L/day initially and increased to 200-245 mg/L/day after 8-day continuous operation. The original concentration of TAN in leachate was
500 mg/L. More than 85% of TAN may be removed as the form of nitrogen gas by
denitrification when the air flow rate was 300 mL/minute/kg wet residue and leachate flow rate
was 30-45 mL/minute.

Viability of aerated leachate as inoculum for subsequent anaerobic digestion was also
determined. The results showed that after the inoculum was processed by aeration for one time,
the cumulative methane yield of the anaerobic digestion almost didn’t decrease. However, after
the inoculum was processed for two times, the cumulative methane yield of the anaerobic
digestion decreased comparing with those using unprocessed inoculum. Therefore, it would be
better to only process part of the leachate instead of the total leachate as it was not necessary to
remove all TAN in leachate to avoid ammonia inhibition. The fraction of leachate needs to be
processed can be calculated according to original and objective TAN concentration in leachate
and formula (1).
Figure 3-1. Digester setup for anaerobic digestion of rice straw

Figure 3-2. Ammonia removal reactor
Figure 3-3. Experiment operation for anaerobic digestion of rice straw using inoculum processed in different ways
Figure 3-4. Experimental data and first order fit of TAN removal from leachate by aeration in the 1st, 2nd and 3rd processing.
Figure 3-5. Cumulative biogas and methane yield, daily methane yield and methane fractions from Experiment 1-3.
Figure 3-6. Cumulative methane production, COD, pH and TAN in leachate during anaerobic digestion of rice straw from Experiment 4
Figure 3-7. Cumulative methane production, pH and TAN in leachate during anaerobic digestion of rice straw from Experiment 5
Figure 3-8. Cumulative methane production, COD, pH and TAN in leachate during anaerobic digestion of rice straw from Experiment 6
Figure 3-9. Cumulative methane production and daily methane yield from Experiment 7 and 8
Table 3-1. Characteristics of rice straw

<table>
<thead>
<tr>
<th>Rice straw characteristics</th>
<th>value</th>
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</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8 - 13</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>87-92</td>
</tr>
<tr>
<td>Volatile solids (% of TS)</td>
<td>83 - 88</td>
</tr>
<tr>
<td>Bulk density (kg/m³)</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 3-2. Concentrations of ammonia-nitrogen, nitrate-nitrogen and ammonia removal rate in biological ammonia removal process

<table>
<thead>
<tr>
<th></th>
<th>1st processing experiment</th>
<th>2nd processing Experiment</th>
<th>3rd processing experiment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1st batch</td>
<td>2nd batch</td>
<td>1st batch</td>
</tr>
<tr>
<td>NH₃-N (mg/L)</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td></td>
<td>505</td>
<td>3.17</td>
<td>496</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.5</td>
<td>500</td>
</tr>
<tr>
<td>Processing Time (days)</td>
<td>7</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Average removal rate (mg/L/day)</td>
<td>70.54</td>
<td>163.58</td>
<td>95.41</td>
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<tr>
<td>k (day⁻¹)</td>
<td>0.82</td>
<td>2.52</td>
<td>0.96</td>
</tr>
<tr>
<td>1st order fit t₀ (day)</td>
<td>2.97</td>
<td>0.39</td>
<td>0.74</td>
</tr>
<tr>
<td>R²</td>
<td>0.65</td>
<td>0.91</td>
<td>0.69</td>
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Table 3-3. Performance of anaerobic digestion of rice straw from Experiment 1-3

<table>
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<tr>
<th>No.</th>
<th>Temperature (°C)</th>
<th>Final methane yield (experimental) (m³ CH₄ STP /kg VS)</th>
<th>Gompertz parameters (model)</th>
<th>Duration to produce 95% methane yield potential (days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P (m³ CH₄/kg VS)</td>
<td>Rm (m³ CH₄/kg VS/day)</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>0.222</td>
<td>0.221</td>
<td>0.029</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>0.203</td>
<td>0.207</td>
<td>0.024</td>
</tr>
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<td>3</td>
<td>55</td>
<td>0.214</td>
<td>0.210</td>
<td>0.027</td>
</tr>
<tr>
<td>Mean values</td>
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<td>0.212</td>
<td>0.027</td>
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<tr>
<td>Standard deviation</td>
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<td>0.010</td>
<td>0.007</td>
<td>0.003</td>
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<tr>
<td>Final range</td>
<td></td>
<td>0.213±0.010</td>
<td>0.212±0.007</td>
<td>0.027±0.003</td>
</tr>
<tr>
<td>No.</td>
<td>Temperature (°C)</td>
<td>Final methane yield (experimental) (m³ CH₄ STP /kg VS)</td>
<td>Gompertz parameters (model)</td>
<td>Duration to produce 95% methane yield potential (days)</td>
</tr>
<tr>
<td>-----</td>
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<td>-----------------------------------------------------</td>
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<tr>
<td></td>
<td></td>
<td>P (m³ CH₄/kg VS)</td>
<td>Rm (m³ CH₄/kg VS/day)</td>
<td></td>
</tr>
<tr>
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<td>0.222</td>
<td>0.221</td>
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<tr>
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<td>0.020</td>
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<tr>
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<td>6</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
<td>55</td>
<td>0.196</td>
<td>0.196</td>
<td>0.017</td>
</tr>
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</table>
4.1 Introduction

Among the various technologies that are available for anaerobic digestion of solid wastes, the high-solids, batch process offers several advantages. The process does not require fine shredding of waste, does not require mixing or agitation of digester contents, does not require bulky, expensive, high-pressure vessels as it can be carried at low (ambient) pressures and can be operated stably at both mesophilic and thermophilic temperatures (Pullammanappallil et al., 2005). Sequential Batch Anaerobic Composting (SEBAC) is one such process that uses a combination of solid state fermentation and leachate recycle to provide a simple, reliable process that inoculates new batches of waste, removes volatile organic acids and concentrates nutrient and buffer. Comparing with other traditional anaerobic digestion technologies, the SEBAC process design offers greater stability. The design allows for easy removal of inhibitory products, which may lead to imbalance. The leachbed design not only facilitates rinsing of toxic substances, such as metals, from the final product, but also eliminates the need for solids movement and mixing (Teixeira et al, 2003). The process has been tested on organic fraction of municipal solid waste (OFMSW), woody biomass, yard wastes and mixtures of yard wastes and biosolids (Chugh et al., 1999; Chynoweth and Legrand, 1993; Chynoweth et al., 1992).

In the original SEBAC system, gravity was relied upon to bring cascading liquid leachate in contact with the organic feedstock by pumping leachate into the top of the reactor and allowing it to flow by gravity and collect at the bottom for subsequent recycling. In SEBAC-II the system was modified to recycle leachate under flooded operation using forced pumping, and recycling leachate through external gas-liquid separators that could accommodate vortex gas/liquid separation systems. Since leachate flow rate is not dependant on gravity in SEBAC-II,
higher solid waste bulk density in the leachbed can be used to increase the loading rate and reduce the reactor size and system footprint (Teixeira et al, 2004).

A bench-scale study (SEBAC-II model) was implemented to test the concept of SEBAC-II using the simulated space waste and the results were promising as degradation kinetics, in flood mode operation, were substantially higher than expected (Chynoweth et al, 2002). Besides terrestrial operation, SEABC-II configuration is also suitable for the operation under micro-gravity, so several studies were devoted to its application in space. A preliminary design, installation, start-up and preliminary operating performance for a full-scale prototype SEBAC-II system for space mission were presented (Xu et al, 2002; Teixeira et al, 2004). Recently, it was modified to improve kinetics and reduce solids processing time by incorporating flooded operation and periodic reversal of direction of leachate flow (Luniya et al., 2005). However, little work has been devoted to post-treatment of the anaerobic digestion, including stabilization of anaerobically digested residue and in situ ammonia removal from anaerobic leachate using digested residue bed. Preliminary studies in this field have been presented in Chapter 2 and 3.

The objectives of this chapter included:

- Design the operation mode for in situ treatment of anaerobically digested residue and leachate in SEBAC-II configuration
- Measure the nitrification rate in the process of digested residue stabilization
- Determine ammonia removal rate from leachate using digested residue bed during aeration

4.2 Methods

4.2.1 Experimental Apparatuses

The apparatus (termed SEBAC-II model, Figure 4-1) consisted of a bench-scale reactor made from a PVC tube, which was enclosed by caps glued at either end. The inner diameter of this reactor was 4 inches, and the total volume was 6 liters. The reactor was held upright by a
stand. Plastic ports were drilled into each cap. This system was originally used for anaerobic digestion of the simulated space waste. Air was supplied from a compressor and was introduced from the bottom and vented from top using solenoid valves automatically operated by a CR10 control system. The CR10 (from Campbell Scientific Inc.) control system is a fully programmable data logger / controller with several digital and analog inputs, and digital outputs. It was used to open and close the solenoid valves for aeration of reactor at pre-specified time intervals. A basket was used to hold the residue in the reactor. Loss of ammonia by volatilization was tracked by bubbling the gas vented from the reactor through a bottle of diluted \( \text{H}_2\text{SO}_4 \) (0.04N) before purging.

During the aeration for stabilization of anaerobically digested residue, the inlet valve was opened for 30 seconds to introduce the air while the outlet valve remained closed. The reactor was pressurized at ~ 10 psi. After 20 minutes (this time interval was chosen to ensure that oxygen concentration remained above 20%), the outlet valve was opened for one minute to vent air from the system. The aeration cycle was then repeated (Figure 4-2).

For ammonia removal from leachate, a pump which was also controlled by the CR10 system was used to pump the leachate from the bottom to the top (Figure 4-3). During the aeration, the inlet valve was opened for 15 seconds to introduce the air while the outlet valve remained closed. The reactor was pressurized at ~ 10 psi. After 18.5 minutes (this time interval was chosen to ensure that oxygen concentration remained above 20%), the outlet valve was opened for 30 seconds to vent air from the system. After the outlet valve was closed, the pump was turned on to pump the leachate for 1 minute. The aeration cycle was then repeated (Figure 4-4).
4.2.2 Anaerobically Digested Residue

The residue used in the studies was the same as that used in Chapter 2. It was an anaerobically digested mixture of wheat straw (55% of wet weight), shredded paper (37% of wet weight) and commercial dog food (7.6% of wet weight). Anaerobic digestion of this synthetic waste was carried out in a SEBAC-II system as described in Luniya et al (2005). After digestion, the residue was stored in a refrigerator for about six months before it was used in experiments reported here.

4.2.3 Experiments

4.2.3.1 Stabilization of anaerobically digested residue

The reactor was loaded with 1.5 kg of wet residue. Initially 100 mL of a 1000 mg NH$_4^+$-N/L NH$_4$Cl solution was added from the top. Additional ammonium chloride solution was added to the reactor every day and the amounts depended on the nitrification performance. The TAN, nitrite, nitrate-nitrogen content and pH in the residue were measured every week. The bench-scale reactor was unloaded periodically and the wet residue was mixed to take a representative sample. Sub samples were collected from different locations within the residue. These sub samples were mixed to make the sample for analysis. After this run, the reactor was loaded again with 1.5 kg of wet residue for the 2$^{nd}$ run. This time sodium bicarbonate was used to keep pH at ~ 7.5.

4.2.3.2 Ammonia removal from leachate

A sample of 1.2 kg wet residue was loaded into the reactor and 1.5 liters of NH$_4$Cl solution at the concentration of 35, 75, 150, 400, 650 and 1000 mg/L were added to the system respectively. Leachate sample was collected daily for the measurement of TAN. The ammonia removal rate is reported in the unit of mg removed N per liquid volume per day.
4.2.4 Analysis

The TAN, nitrite- and nitrate-nitrogen in anaerobically digested residue and leachate were measured periodically by the methods described in Chapter 2 and 3.

4.3 Results and Discussions

4.3.1 Stabilization of Anaerobic Residue

The stabilization process in a bench-scale reactor (SEBAC-II model) was carried out. In the experiment, 100 mg NH₄⁺-N was added on the 2nd and 4th day respectively. On the 5th day, the results showed that the nitrification was already initiated. Initially in this experiment no attempt was made to control pH. Nitrification reactions produce H⁺ causing pH to drop. Figure 4-5 shows the changes in pH and its effect on the nitrification rate. On day 5, the specific nitrification rate was 0.068mg/g wet weight/day. Then the rate dropped to 0.025 mg/g wet weight/day when pH was as low as 4.7 on day 15. On day 23, sodium hydroxide was added to increase pH above 8 and sodium bicarbonate was also added to serve as a buffer. The specific nitrification rate recovered a little on day 24. On day 36, the specific nitrification rate increased to 0.049 mg/g wet weight/day, and the pH was maintained above 8. Specific nitrification rate was affected by pH significantly. The reason is that nitrifiers prefer neutral pH conditions. Besides, nitrifiers use ammonia (NH₃) as substrate. In the system as ammonia is equilibrium with ammonium (NH₄⁺), pH affects the ratio of ammonia and ammonium. At high pH, the ratio is high, that is to say, ammonia is the major fraction. However, ammonium dominates at low pH. Therefore, there would be little substrate available for nitrifiers as pH dropped, even though the total ammonium content is high.

Another run was carried out in this SEBAC-II model. In this experiment, 4 g NaHCO₃ was added as a pH buffer. After one day, the result showed that the nitrification was already initiated
and the specific nitrification rate was 0.26 mg/g wet weight/day, which was much higher than the first run without pH control.

For the stabilization process in 1 L glass reactors described in Chapter 2, the initial specific nitrification rate was 0.13 mg/g wet weight/day on day 2. But in this SEBAC-II model, the nitrification could be activated in shorter time and the reaction rate was higher. The reason is that higher pressure in this bench-scale reactor may have improved the contact between digested residue and oxygen by overcoming the preferential channel flow of air when it was simply blown continuously through the residue. In the SEBAC-II system, a similar procedure could be used for stabilization.

A stabilization process can be easily incorporated into the SEBAC-II system and a schematic diagram of the SEBAC-II process incorporating stabilization is shown in Figure 4-6. The vessel would be aerated following the aeration procedure used in the SEBAC-II model described here. For the digested residue used in this study, assume the TAN content in residue is around 4.0 mg/g wet residue. Based on the assumption that the initial nitrification rate would be 0.26 mg/g wet weight/day (i.e. specific nitrification rate measured using SEBAC-II model) and the nitrification rate would fit the Gompertz equation, it was estimated that more than 90% of TAN in the anaerobically digested residue could be nitrified after 13-day aeration.

4.3.2 Ammonia Removal from Leachate

The efficiency for the ammonia removal from leachate was above 95% in this SEBAC-II model. The relationship of initial ammonia removal rate and original TAN concentration in leachate may be described as linear in SEBAC-II model (Figure 4-7). When the TAN concentration in leachate was as low as 35 mg/L, the initial ammonia removal rate was around 22.75 mg/L/day. The initial ammonia removal rate increased with the TAN concentration in leachate. According to this linear relationship, when the concentration was 500 mg/L, the initial
removal rate should be around 85 mg/L/day. This result was consistent with the one obtained in previous experiment using apparatus in Figure 3-2, which was described in Chapter 3.

A treatment of leachate for ammonia removal can be incorporated into SEBAC-II and a schematic diagram of SEBAC-II process incorporating stabilization of residue and treatment of leachate is shown in Figure 4-8. The vessel would be aerated following aeration procedure used in the SEBAC-II Model described here. The air enters into the reactor from the bottom and vents from the top. The Pump E is used to pump the leachate from the reservoir to the top of the aerobic reactor for ammonia removal. The leachate will flow down to the bottom of the reactor through the solid digested residue containing microorganisms required for nitrification. The leachate accumulated at the bottom of the aerobic reactor is pumped back to the reservoir by the Pump D. From the results in Chapter 3, aeration process may slightly lower the microbial activity in leachate. So it would be better to only process part of leachate to avoid ammonia inhibition. The fraction of leachate needs to be processed can be determined from the TAN concentration in leachate and implemented using flow rate of leachate controlled by pump.

**4.4 Conclusion**

A new operation mode was developed for in situ treatment of anaerobically digested residue and leachate in the SEBAC-II model. Instead of supplying a continuous air flow, air was held under pressure at ~ 10psi for 20 minutes before venting and filling again.

For the stabilization of solid digested residue, initial specific nitrification rates were higher at 0.26 mg /g wet weight /day showing that this method of aeration was more efficient since higher pressure in this bench-scale reactor may have improved the contact between digested residue and oxygen by overcoming the preferential channel flow of air when it was simply blown continuously through the residue. Based upon the specific nitrification rate obtained in this SEBAC-II model and modified Gompertz equation, more than 90% of TAN in the anaerobically
digested residue could be nitrified after 13-day aeration in the SEBAC-II system. It was also seen that decreases in pH caused by nitrification reactions can inhibit nitrification activity. So it is essential to incorporate pH monitoring and control during the stabilization process.

In situ ammonia removal from leachate using a digested residue bed rather than a dedicated reactor was also studied in this SEBAC-II model. The similar operation was utilized. The ammonia removal efficiency was above 95%. Initial ammonia removal rate had linear relationship with original TAN concentration in leachate. According to results from Chapter 3, it would be better to only process part of the leachate instead of the total leachate as it was not necessary to remove all TAN in leachate to avoid ammonia inhibition. The fraction of leachate needs to be processed can be calculated according to original and objective TAN concentration in leachate, and implemented using flow rate of leachate controlled by pump.
Figure 4-1. Bench-scale reactor operated by an automatic CR10 control connected to an ammonia trap

Figure 4-2. Operation of inlet and outlet valves by automatic CR10 control
Figure 4-3. Bench-scale reactor (SEBAC-II Model) for biological removal process

Figure 4-4. Operation of pump, inlet and outlet valves by automatic CR10 control
Figure 4-5. Specific nitrification rate in a SEBAC-II model and simultaneous pH change during stabilization process

Figure 4-6. Integration of stabilization process into SEBAC-II system
Figure 4-7. Relationship of initial ammonia removal rate and original TAN concentration in bench scale reactor

Figure 4-8. Integration of step for stabilization of residue and treatment of leachate into SEBAC-II system
CHAPTER 5
THERMOPHILIC ANAEROBIC DIGESTION OF SUGARBEET TAILINGS USING COD REMOVAL AS PRE-TREATMENT

5.1 Introduction

Nearly 40% of all refined sugar consumed in the USA is made from sugar beets grown in the north central and north western regions of the United States. Beet sugar processing generates significant quantities of both solid and liquid wastes. Raw sugar beets when brought into the processing plant from storage in outdoor stockpiles, are first washed and separated from “tailings” which mainly consist of sugar beet chips (10-30%), weeds, sugar beet tops, debris and soils held by sugar beets when harvested (Kumar et al., 2002). The washed beets proceed for further processing and juice extraction generating another solid waste stream namely spent beet pulp. This waste stream is a valuable by-product and can be used as cattle feed, for fertilizer production (Zhang and Shi, 2000), for pectin production (Karpovich et al., 1989) or as an ethanol feedstock source (Doran and Foster, 2000).

A lack of literature on the disposal of tailings, the first solid waste stream, indicated that not much attention has been devoted towards processing this waste stream for value addition. Usually, tailings are stockpiled outside the factory and hauled away for disposal onto landfills or applied on nearby farmland at a significant cost to the factory. For example, American Crystal Sugar Company spends close to $1 million per year disposing 400 tons of tailings that are generated daily at its East Grand Forks plant (Teixeira et al., 2005).

Anaerobic digestion of tailings would not only generate biogas but also reduce the quantity of waste stream that requires disposal. Biochemical methane potential assays of tailings carried out at a mesophilic temperature of 38 °C yielded 250 L of methane/kg VS (Teixeira et al., 2005). Based on this methane yield, a preliminary economic analysis showed that taking into account the reduced cost of disposal, electricity revenues, and natural gas savings, a conservative
estimate of the net savings from anaerobically digesting 400 tons/day of tailings was $4,873 per day (Teixeira et al., 2005).

In preliminary experiments, sugar beet tailings were anaerobically digested using the SEBAC-II process at 38 °C (Teixeira et al., 2005). It was found that the rates of methane generation were poorer compared to that from digestion of other organic residues, persistently high volatile organic acid concentrations were measured in digester liquor and daily methane production rates failed to increase even after 30 days of digestion. This indicated a need for further modifications and improvements to integrate an in situ pre-treatment process to anaerobically digest tailings within the SEBAC-II configuration. The objectives of the work in this chapter were:

- Confirm the availability of microorganisms required for anaerobic digestion of sugar beet tailings
- Develop a pre-treatment method for anaerobic digestion of sugar beet tailings in the SEBAC-II system
- Measure the methane yield and methane production rate in anaerobic digestion of sugar beet tailings after incorporating the new pre-treatments

This chapter presents findings related to these objectives. The digester was modified to operate within a thermophilic temperature range (50 – 57 °C) where the rates of degradation and biogasification are faster, and has a greater potential to destroy weed seeds and plant pathogens, which is especially beneficial for reapplying the undigested residue with little post treatment on to the fields to recycle nutrients. Since a thermophilic inoculum was not available in our laboratory, a method of quickly culturing such an inoculum was also developed in this study.
5.2 Methods

5.2.1 Anaerobic Digester

A 5-liter glass bottle with a wide mouth was used as the anaerobic digester. The mouth was closed with a rubber stopper through which a glass rod was inserted for venting the biogas from the digester. The digester was also equipped with an outlet at bottom from which liquid samples were collected. A schematic diagram of this apparatus is shown in Figure 5-1. The digester was placed in an incubator where the temperature was maintained at 55 °C. The biogas was metered using a wet tip gas meter which was placed outside the incubator.

5.2.2 Feedstock

Beet tailings were shipped in a frozen state from American Crystal Sugar Company’s East Grand Forks plant in five gallon pails. Upon receipt, the tailings were stored at -20 °C in a cold room. The basic characteristics of beet tailings are listed in Table 5-1 and 5-2.

5.2.3 Experiments

Three experiments were carried out. The digester was loaded with 1.5 kg of tailings as received (i.e. the tailings were not subjected to any size reduction) in all experiments. The tailings were taken out of cold storage and thawed at room temperature before being loaded into the digester. In each experiment, the tailings were initially flooded with 2 liters of tap water and then drain this liquid (henceforth this liquid will be referred to as wash leachate) out. The process would remove readily soluble organic matter, which may be helpful to avoid pH drop and activate methanogenesis. However, the initial fill, leach and drain procedure were different in these three experiments.

In Experiment 1, 2 liters of tap water containing 12 g/L of sodium bicarbonate was initially added to flood the bed of tailings. Next day liquid was drained and fresh water containing 12 g/L of sodium bicarbonate was added which was drained on day 3. After which
water with sodium bicarbonate was again added and anaerobic digestion was started up. The COD of wash leachate was measured. The experiment was carried out for 25 days. At the end of this period, the digested leachate was drained and stored at room temperature. Solid residue from the digester was analyzed for dry matter and volatile solids content.

In Experiment 2, the tailings were initially flooded with 2 liters of tap water containing 12 g/L of sodium bicarbonate. After three days the bed was drained and this wash leachate stored for subsequent use. Then digested leachate from the end of Experiment 1 (which was stored at room temperature for 3 days) was introduced into the bed to start up the anaerobic digestion. From day 15 onwards wash leachate that was drained initially was added to the digester. On days 15 and 16, 50 ml of leachate was added; on day 17, 150 ml; on days 18 and 19, 200 ml; and from day 21 onwards, 300 ml of leachate until all leachate was used up by day 28. After that, the digested leachate was drained and solids analyzed for dry matter and volatile solids content.

In Experiment 3, wash leachate was drained out on day 1 itself. Tap water was added, the bed was drained on day 2 and the procedure repeated on day 3. No sodium bicarbonate was added this time. Digested leachate from the end of experiment 2 (which was stored at room temperature for 3 days) was then added. On day 11 the wash leachate that was drained out of the bed at the start of Experiment 3 was then added. About 200 ml of this leachate was added every day until day 17. The digester was operated for 45 days so as to evaluate ultimate degradability and quality of leachate.

5.2.4 Analysis

Total solids (TS) were determined gravimetrically after drying overnight at 105 °C.

Volatile solids (VS) content was determined by ashing a dried sample at 550°C for 2 hours and determining the ash-free dry weight. Gas production was monitored daily using the gas meter.
described in Chapter 3. Gas composition (CH$_4$, CO$_2$) was measured using a gas chromatograph (Fisher Gas Partitinoer, Model 1200) equipped with a thermal conductivity detector. The gas chromatograph was calibrated with an external standard containing N$_2$: CH$_4$: CO$_2$ in volume ratio 25: 45: 30. Leachate samples were collected daily and analyzed for pH, COD and volatile organic acids. Volatile organic acid concentrations (acetic, propionic, butyric, and valeric acids) were measured using a gas chromatograph equipped with a flame ionization detector. COD of leachate was measured by colorimetric method (Greenberg et al, 1992). Frozen tailings and digested residue samples (50 g each) were stored in air-tight bags, packed in an insulated envelope and shipped to Dairy One, Inc., Ithaca, New York, a commercial forage testing laboratory. These samples were analyzed for crude protein, soluble protein, nonfibrous carbohydrates, lignin, hemicellulose and cellulose.

5.3 Results and Discussions

The performance of experiments 1, 2 and 3 are depicted in Figures 5-2, 5-3 and 5-4 respectively. In these figures, the cumulative biogas and methane production, methane percentage, daily methane production, pH, COD and volatile organic acid concentrations of leachate are shown. Cumulative biogas and methane production values were normalized on the basis of kg VS loaded in each experiment. Daily methane production was reported per L of active (sum of liquid and solids volume) reactor volume. All the reported gas volume was converted to volume at standard temperature and pressure (STP).

In Experiment 1, 2 liters of water containing 12 g/L of sodium bicarbonate was initially added to flood the bed of tailings. Upon addition of water, a large amount of organic matter was solubilized. Fermentation of the soluble COD caused the pH to drop to very low values of 3 or below. Next day liquid was drained (henceforth this liquid will be referred to as wash leachate) and fresh water containing 12 g/L of sodium bicarbonate was added which was drained on day 3.
After which water with sodium bicarbonate was again added. The amount of readily soluble organic matter removed was 95 g COD. Methane production was initiated two days later, which was sustained and continued to increase until day 11 after which it dropped. The maximum methane production rate was approximately 0.4 L/L/d. The experiment was carried out for 25 days, by which time about 64 L CH₄/kg VS loaded was produced. Since the removed readily soluble COD (95 g) represents about 50% of the total methane potential, the final methane yield was lower.

Volatile organic acid profiles showed accumulation and degradation of all acids that were analyzed. Acetic acid accumulated to 1300 mg/l, and propionic and butyric acids to 1250 mg/L. Degradation of these acids caused their concentration to drop after day 9.

In Experiment 2, the tailings were initially flooded with 2 liters of tap water containing 12 g/L of sodium bicarbonate. After three days the bed was drained and this wash leachate stored for subsequent use due to its high COD content. Then digested leachate from the end of Experiment 1 was introduced into the bed. Methane production was initiated on day 5, soluble COD of leachate dropped and pH began to climb. By day 13, the pH of leachate was above 8 and continued to fluctuate around this value for rest of the duration of experiment. Methane production rate peaked to 0.79 L/L/d on day 13. Volatile organic acid concentrations were higher in this experiment compared to Experiment 1. On day 11, the concentration of butyric acid was still 4700 mg/L and that of propionic acid was almost 1900 mg/L. This may be because all soluble COD may not have been completely washed out during the fill, leach and drain step. Soluble COD was 20 g/L on day 6 compared to values less than 8 g/L from day 4 onwards in Experiment 1.
From day 15 onwards wash leachate that was drained initially was added to the digester. On days 15 and 16, 50 mL of leachate was added; on day 17, 150 mL; on days 18 and 19, 200 mL; and from day 21 onwards, 300 mL of leachate until all leachate was used up by day 28. Upon addition of this leachate, methane production continued from the digester showing that the readily soluble organic matter in the wash leachate can be anaerobically digested. The addition and digestion of wash leachate caused the fluctuations of pH and COD values.

In Experiment 3, the initial fill, leach and drain procedure was modified because it seemed that all soluble COD might not be completely washed out in experiment 2. This time wash leachate was drained out on day 1 itself. Fresh water was added, the bed was drained on day 2 and the procedure repeated on day 3. Digested leachate from the end of experiment 2 was then added. It can be noted that at this stage the soluble COD was less than 7 g/L. Methane production was initiated as soon as the digested leachate was added on day 3 and peaked at a value of 1.01L/L/d by day 9 and began to drop soon after. Individual volatile organic acids accumulated initially but dropped to below 500 mg/L by day 11. On day 11 the wash leachate that was drained out of the bed at the start of Experiment 3 was then added. About 200 mL of this leachate was added every day until day 17. The digester was operated for 45 days so as to evaluate ultimate degradability and quality of leachate. By day 45 the soluble COD was 1,900 mg L⁻¹. Among the volatile organic acids only propionic and valeric acids were detected on day 45 and its concentrations were 430 mg L⁻¹ and 270 mg L⁻¹ respectively.

5.3.1 Characteristics of Beet Tailings and Residue

Tailings as received in our laboratories had high moisture content. The average moisture content was 88-89%. The volatile solids content of the tailings was about 90% of dry matter. Therefore, upon loading 1.5 kg of tailings in the anaerobic digester in each experiment, only about 156 g of solids (which are the volatile solids) were available for degradation based on
11.5% dry matter. A large fraction of the tailings is made up of readily soluble organic content. This was measured as COD in wash leachate; which was very high. About 80 to 95 g COD of soluble organic matter was removed during the fill, leach and drain steps. The readily soluble organic fraction in tailings was about 0.5 - 0.6 g COD /g VS. Chemical analysis of tailings for animal feed constituents is listed in Table 4-2. Carbohydrates (including non-fibrous carbohydrates, hemicellulose and cellulose) are the primary constituents.

After anaerobic digestion, the average moisture content of the residue upon draining the leachate was 80 - 88% with volatile solids ~ 55% of dry matter. The volume of the bed decreased 70-80%. Dry matter and volatile solids reduction was measured to be ~60% and ~75% respectively. Chemical analysis of digested residue for animal feed constituents is listed in Table 5-2. Since all constituents are present in the residue, none of them are completely degraded during the digestion process. The fractions of protein, lignin and cellulose are higher in the residue than in the tailings. The extent of degradation is of individual constituents is also listed in Table 5-2. More than 50% of the protein and cellulose was degraded, whereas about 80% of the nonfibrous carbohydrates and hemicellulose was degraded. In the case of lignin it is likely that 25.5% of the lignin was solubilized and released into solution rather than being degraded, as digestibility to methane was considered limited (Polematidis, 2007). Since cellulose was bound to be present in the tailings within a ligno-cellulose matrix and that this matrix is resistant to biodegradation, the extent of cellulose degradation was only 61.4%.

5.3.2 Biochemical Methane Potential (BMP)

Based on methane production from a complete digestion run with a batch of tailings and the readily solubilized organic matter, the biochemical methane potential of the tailings is approximately 295 L at STP/kg VS. Of this about 160 – 180 L (50 – 60%) is produced from the readily soluble component of beet tailings. This indicated that even after the readily solubilized
matter is removed, significant methane production potential exists within the tailings (40-50%). The BMP estimated in these experiments is more than that determined from biochemical methane potential assays carried out at mesophilic temperatures (Teixiera et al., 2005). It should be noted that these mesophilic BMP assays were carried out using sample sizes of 2 g and may not be representative of the feedstock. Moreover, the inoculum used for the BMP assays came from a slurry digester that was fed a synthetic feed solution made from dog-food. On the contrary, by Experiment 3 the inoculum used in experiments here would have been acclimatized to degrading beet tailings, hence able to break down more of the feedstock. It can be seen from Figure 5-5 that over a duration of 35 days, Experiment 3 yielded more methane than Experiment 1 or 2 (after discounting methane yield from addition of high COD leachate from day 11 in Experiment 3 and day 15 in Experiment 2) which indicated that there was more degradation of the solids as the inoculum became progressively adapted. From the amount of constituents listed in Table 5-2 that were degraded during digestion, the theoretical methane yield was calculated to be ~278 L CH4 at STP/ kg VS, assuming an average COD of 1.1 g/g for the constituents. This value is in close agreement to the experimentally measured value of 295 L CH4 at STP/ kg VS.

5.3.3 Microbial Populations

Methane production was initiated in Experiment 1 by simply flooding the bed with water containing only buffer. Interestingly, appropriate microbial populations required for anaerobic digestion of the feedstock was naturally present within the tailings. Mineralization of organic matter to methane and carbon dioxide in an anaerobic digester requires the concerted action of several populations of microorganisms. Of these populations, those involved in acidogenesis (conversion to volatile organic acids), acetogenesis (conversion of higher chain volatile organic acids like propionic and butyric acids to acetic acid) and methanogenesis (formation of methane from acetic acid and methane production from hydrogen and carbon dioxide) are of primary
importance. It is well known that acidogenic organisms readily establish within anaerobic environments where in they cause rapid fermentation of organic matter due to their high growth rates. This was also confirmed here as addition of water and establishment of anaerobic environment quickly caused volatile organic acids to build up and pH to drop to below in 3 in Experiment 1. On the contrary, acetogenic and methanogenic populations (especially aceticlastic methane populations) are slow growing organisms. Highly imbalanced anaerobic digestion process, like sludge digesters with high concentrations of propionic and butyric acids, have been known to take long periods of time to recover requiring elaborate operational protocols with close monitoring to nurse them back to balanced conditions. However, it was seen here that even though higher chain volatile organic acids like propionic and butyric acid accumulated to levels around 1,680 mg/L (by day 7 in Experiment 1) these were degraded to below 750 mg/l within ten days. This degradation was mediated by microbial populations naturally occurring within the waste bed as an external inoculum was not added to the digester in Experiment 1. Valeric acid accumulated to 1000 mg/L initially; but it was reduced to 400 mg/L within couple of days. Initiation and sustenance of methane production from uninoculated tailings also indicated the presence of adequate number of methanogenic populations within the tailings. Methane production rate increased quickly from 0.05 L/L/d to 0.4 L/L/d within 4 days.

5.3.4 High Solids Anaerobic Digestion of Beet Tailings in an Unmixed Digester

Previous investigations into use of the flooded SEBAC process for anaerobic digestion of beet tailings (Texeira et al., 2005) showed poor performance because of the process’ inability to deal with the high amount of readily soluble organic compounds. Previously, the SEBAC process has been shown to initiate methanogenesis rapidly in the feedstocks like organic fraction of municipal solid waste, yard waste, mixtures of biosolids and yard waste, simulated municipal solid waste etc. The readily soluble organic matter in these feedstocks is lower than that
generated by beet tailings. For example, soluble COD of leachate from a flooded vegetable waste bed was around 8 g/L (Hegde and Pullammanappallil, 2007) and that from organic fraction of municipal solid waste was 12 g/L (Lai, 2001) compared to up to 47.5 g/L from beet tailings. In a SEBAC process, the leachate recirculation strategy ensures that the readily soluble COD generated in a fresh waste bed is completely converted to methane in a stabilized waste bed. The leachate that is returned to the waste bed from the stabilized waste bed is low in soluble COD. But in the case of beet tailings, the COD that was loaded into the stabilized waste bed with the leachate exceeded its assimilation capacity. This caused the leachate that was returned to the fresh waste bed to contain residual undegraded COD, low alkalinity and low pH. These conditions were unfavorable for initiation of methanogenesis in the fresh waste bed.

Therefore, an anaerobic digestion process for beet tailings should include a step in which the readily soluble organic matter is leached and removed from the waste bed. Since, this readily soluble COD represents about 50% of the total methane potential, the leachate may be anaerobically digested in a high rate anaerobic treatment system. It was shown that the organic matter that is leached out initially can indeed be anaerobically digested.

Digested leachate from Experiment 1 was used to start up the subsequent experiment (i.e. Experiment 2) and digested leachate from Experiment 2 was used to start up Experiment 3. This process ensured adaptation and enrichment of the inoculum. Figure 4-6 compares the cumulative methane production from the three experiments. It can be seen that the rate of methane production (or initial slopes of the cumulative methane production plot) increased with each subsequent experiment. The rate was highest in Experiment 3 and cumulative methane production began to level-off by day 10. Moreover, methane production was initiated as soon as digested leachate from Experiment 2 was introduced into the digester on day 3. As explained in
Results section, continued methane production after day 10 was due to addition of high COD wash leachate. Also, the microbial populations in the leachate continued to be able to rapidly degrade higher chain volatile organic acids. Individual volatile organic acid concentrations dropped below 550 mg/L by day 11 in Experiment 3.

5.3.5 Integration of Removal of Soluble COD as a Pre-treatment Step into SEBAC-II Process

The tailings contain a large fraction (0.6 g COD/g VS) of readily soluble organic matter, which lead to high soluble COD during the anaerobic digestion. This high COD that was loaded into the stabilized waste bed with leachate exceeded the assimilation capacity of the mature reactor in the SEBAC-II system. One solution was to increase the volume of the mature reactor, but in this case it would be failed to implement the sequence of anaerobic digestion in the SEBAC-II. In the two-phase digestion developed by Barry (1983), leachate containing the soluble organic matter was withdrawn from an acidification reactor and replaced by fresh effluent from a methanogenesis reactor to ensure optimal nutrient levels in the acidification reactor. The rate for leachate withdrawn and replacement was dependent on the concentration of soluble organic matter. The acidification reactor would be disconnected to methanogenesis reactor to function independently as a single-phase system as soon as the rate of acidification decreases if the feedstock exhibited an initial rapid conversion of the easily biodegradable components followed by a slower rate of hydrolysis and fermentation of the more recalcitrant components (Rijkens 1981; Rijkens and Voetberg, 1982). However, these solutions were based on the intermittent or continuous operation. The SEBAC-II technology is used in the batch digestion.

So when the SEBAC-II technology is used for sugar beet tailings, the first step was to wash and remove the soluble organic matter in the tailings. A whole SEBAC-II system is
described in Figure 2-7 if a filling reactor for sugar beet tailings and a reservoir for washing water storage are added. The beet tailings are loaded into the filling reactor for pre-treatment. Tap water is added to flood the tailings bed and solubilize the organic matter. After a while the washing water containing high soluble COD is pumped to the reservoir and the tailings are ready for anaerobic digestion. The washing water in the reservoir is pumped into the activated reactor (Stage 2) which is in the process of anaerobic digestion for conversion to methane. As leachate is recirculated upon itself in this activated reactor, the COD that is loaded into the activated waste bed with the washing water may be controlled according to its assimilation capacity, and is successfully converted to methane.

5.4 Conclusions

A pre-treatment process, which may be incorporated into SEBAC-II system, was developed to accommodate anaerobic digestion of sugar beet tailings. The pre-treatment step was to wash and remove the large fraction of soluble organic matter in sugar beet tailings. After this, the methane production was initiated as soon as digested leachate was introduced into the digester. At the same time, the washing water containing high COD content was added to the activated reactor for bioconversion to methane. Critical findings on the modifications of the SEBAC-II system to accommodate sugar beet tailings were:

- The tailings contained naturally occurring microbial communities to carry out anaerobic digestion. A method of culturing a thermophilic inoculum was developed by simply flooding a bed of tailings with pH buffer solution and incubating this bed at thermophilic temperatures. Inoculum thus cultured was shown to robustly and stably initiate methanogenesis in subsequent batches of tailings.

- As the tailings contain a large fraction (0.6 g COD/g VS) of readily soluble organic matter, the first step was to wash and remove the soluble organic matter. After this, methanogenesis was initiated immediately upon flooding the bed with digested leachate containing adapted inoculum. Most of the methane potential of the solids was recovered within a week.
• A methane yield of 295 L at STP/kg VS was determined for the tailings, of which 50-60% was contributed by the solubilized organic matter. After anaerobic digestion, a volume reduction of 70-80% was achieved and approximately 60% of dry matter and 75% of volatile solids in tailings were degraded.

• The methane production rate, consequently the kinetics of the process, could be improved by flooding tailings using digested leachate from the end of a previous experiment.
Figure 5-1. Anaerobic digester filled with tailings and leachate
Figure 5-2. Cumulative biogas and methane production, methane percentage, daily methane, pH, COD and volatile organic acid concentrations from Experiment 1
Figure 5-3. Cumulative biogas and methane production, methane percentage, daily methane, pH, COD and volatile organic acid concentrations from Experiment 2
Figure 5-4. Cumulative biogas and methane production, methane percentage, daily methane, pH, COD and volatile organic acid concentrations from Experiment 3
Figure 5-5. Comparison of cumulative methane production from Experiments 1, 2 and 3
Figure 5-6. Incorporation of removal of soluble COD as pre-treatment step into SEBAC-II process
Table 5-1. Sugar beet tailings characteristics

<table>
<thead>
<tr>
<th>Tailings characteristics</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>88 - 90</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>13 - 17</td>
</tr>
<tr>
<td>Volatile solids (%)</td>
<td>80 - 92</td>
</tr>
<tr>
<td>Bulk density (kg/m³)</td>
<td>333</td>
</tr>
</tbody>
</table>

Table 5-2. Chemical characteristics of tailings and digested residue, and extent of degradation of the individual constituents during anaerobic digestion

<table>
<thead>
<tr>
<th>Component</th>
<th>Tailings (% VS)</th>
<th>Digested Residue (% VS)</th>
<th>Extent of degradation or solubilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>7.5</td>
<td>15.2</td>
<td>49.3</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>1.95</td>
<td>3.7</td>
<td>52.5</td>
</tr>
<tr>
<td>NFC (non fibrous carbohydrates)</td>
<td>44.9</td>
<td>22.9</td>
<td>87.2</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.7</td>
<td>14</td>
<td>25.5</td>
</tr>
<tr>
<td>Hemi cellulose</td>
<td>17.8</td>
<td>14.7</td>
<td>79.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>18.1</td>
<td>27.9</td>
<td>61.4</td>
</tr>
</tbody>
</table>
CHAPTER 6
CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

This research work contained four different parts, including preliminary studies of post-treatment of anaerobically digestated residue, leachate, their bench-scale (SEBAC-II model) studies and pre-treatment of feedstock. The results of the study suggested that incorporating these pre and post treatments as a routine part of SEBAC-II operation will greatly enhance the potential for wide-spread use of SEBAC-II technology in waste management applications. The critical findings in the study are as follows:

6.1.1 Stabilization of Anaerobically Digested Residue by Nitrification Process

A method was developed for the measurement of TAN in the digested residue as a method was not available in the literature. The 2 M of KCl was used as an extraction solution, and pH of the extraction solution was increased to above 11 by NaOH addition. The results showed that the extraction efficiency was above 98% and the recoverability was within 2%.

A method for nitrification on the solid digested residue was also developed. As the microorganisms required for nitrification process naturally existed in the anaerobically digested residue, it was possible to stabilize the nitrogen by simply aerating it. Nitrification was accomplished without any inoculum addition.

By continuously blowing air through the residue at 187 mL/kg wet residue/min, the nitrification process could be initiated within two days. Approximately 85% of ammonium-nitrogen was nitrified during a 16-day aeration period and the maximum rate was 0.41 mg/g wet weight/day. The denitrification process occurred soon after nitrification and its fraction reached ~50% of the nitrification. The modified Gompertz model was used to quantify the rate of NH₃ transformations, and the results showed that it fitted the nitrification data very well.
The oxygen consumption during this stabilization process was determined. The result showed that the oxygen consumption was 8.62 mg oxygen per mg nitrified nitrogen even though theoretical requirement based upon stoichiometry was 4.57 mg oxygen /mg nitrified nitrogen.

6.1.2 Integration of Ammonia Removal from Leachate within Anaerobic Digestion Process

A post-treatment method, which may be integrated into the SEBAC-II system, was develop to biologically remove ammonia from leachate on the stabilized digested residue by simply aerating the reactor. At the same time, the viability of aerated leachate as inoculum for subsequent anaerobic digestion was also determined as little literature reported the results about the viability of reusing the aerated leachate.

The results showed that when a nitrification-denitrification processing step for ammonia removal from leachate using the stabilized digested residue was integrated into the anaerobic digestion, the ammonia removal rate was 70 -95 mg/L/day initially and increased to 200-245 mg/L/day after 8-day continuous operation. The original concentration of TAN in leachate was 500 mg/L. More than 85% of TAN may be removed as the form of nitrogen gas by denitrification when the air flow rate was 300 mL/minute/kg wet residue and leachate flow rate was 30-45 mL/minute.

Viability of aerated leachate as inoculum for subsequent anaerobic digestion was also determined. The results showed that after the inoculum was processed by aeration for one time, the cumulative methane yield of the anaerobic digestion almost didn’t decrease. However, after the inoculum was processed for two times, the cumulative methane yield of the anaerobic digestion decreased comparing with those using unprocessed inoculum. Therefore, it would be better to only process part of the leachate instead of the total leachate as it was not necessary to remove all TAN in leachate to avoid ammonia inhibition. The fraction of leachate needs to be processed can be calculated according to original and objective TAN concentration in leachate.
6.1.3 Integration of In Situ Treatment of Anaerobically Digestated Residue and Leachate in SEBAC-II Configuration

A new operation mode was developed for in situ treatment of anaerobically digested residue and leachate in the SEBAC-II model. Instead of supplying a continuous air flow, air was held under pressure at ~ 10psi for 20 minutes before venting and filling again.

For the stabilization of solid digested residue, initial specific nitrification rates were higher at 0.26 mg /g wet weight /day showing that this method of aeration was more efficient since higher pressure in this bench-scale reactor may have improved the contact between digested residue and oxygen by overcoming the preferential channel flow of air when it was simply blown continuously through the residue. Based upon the specific nitrification rate obtained in this SEBAC-II model and modified Gompertz equation, more than 90% of TAN in the anaerobically digested residue could be nitrified after 13-day aeration in the SEBAC-II system. It was also seen that decreases in pH caused by nitrification reactions can inhibit nitrification activity. So it is essential to incorporate pH monitoring and control during the stabilization process.

In situ ammonia removal from leachate using a digested residue bed rather than a dedicated reactor was also studied in this SEBAC-II model. The similar operation was utilized. The ammonia removal efficiency was above 95%. Initial ammonia removal rate had linear relationship with original TAN concentration in leachate. According to results from Chapter 3, it would be better to only process part of the leachate instead of the total leachate as it was not necessary to remove all TAN in leachate to avoid ammonia inhibition. The fraction of leachate needs to be processed can be calculated according to original and objective TAN concentration in leachate, and implemented using flow rate of leachate controlled by pump.
6.1.4 Pre-treatment of Readily Biodegradable Feedstock to Improve Digestibility

A pre-treatment process, which may be incorporated into SEBAC-II system, was developed to accommodate anaerobic digestion of sugar beet tailings. The pre-treatment step was to wash and remove the large fraction of soluble organic matter in sugar beet tailings. After this, the methane production was initiated as soon as digested leachate was introduced into the digester. At the same time, the washing water containing high COD content was added to the activated reactor for bioconversion to methane. Critical findings on the modifications of the SEBAC-II system to accommodate sugar beet tailings were:

- The tailings contained naturally occurring microbial communities to carry out anaerobic digestion. A method of culturing a thermophilic inoculum was developed by simply flooding a bed of tailings with pH buffer solution and incubating this bed at thermophilic temperatures. Inoculum thus cultured was shown to robustly and stably initiate methanogenesis in subsequent batches of tailings.
- As the tailings contain a large fraction (0.6 g COD/g VS) of readily soluble organic matter, the first step was to wash and remove the soluble organic matter. After this, methanogenesis was initiated immediately upon flooding the bed with digested leachate containing adapted inoculum. Most of the methane potential of the solids was recovered within a week.
- A methane yield of 295 L at STP/kg VS was determined for the tailings, of which 50-60% was contributed by the solubilized organic matter. After anaerobic digestion, a volume reduction of 70-80% was achieved and approximately 60% of dry matter and 75% of volatile solids in tailings were degraded. The methane production rate, consequently the kinetics of the process, could be improved by flooding tailings using digested leachate from the end of a previous experiment.

6.2 Future Work

The SEBAC-II system could be enhanced by integration of an in situ post-treatment of anaerobically digestated residue (stabilization) and leachate (ammonia removal) and pre-treatment of feedstock. Preliminary and bench-scale studies of post-treatment have been carried out and some data in full scale SEBAC-II system has been predicted according to the results
from preliminary and bench-scale studies. Conduct these post-treatment processes in full scale SEBAC-II system could be part of future work:

- Modify SEBAC-II system to integrate the steps of stabilization of digestate and treatment of leachate

- Measure the extent and rate of nitrification, oxygen requirement in stabilization process in full scale SEBAC-II system and hence determine the purging time and frequency which is controlled by CR10 automatic system

- Conduct mass balance of nitrogen during simultaneous stabilization of digestate and treatment of leachate in full scale SEBAC-II system

- Modify the SEBAC-II system to treat only half of leachate for ammonia removal if necessary, because full treatment of aeration may lower the bacteria activity

For pre-treatment of feedstock to improve anaerobic digestion, only readily biodegradable feedstock has been studied. The interest in pre-treatment of non-readily biodegradable feedstock can be addressed:

- Develop an in-situ pre-treatment method of non-readily biodegradable feedstock, such as wheat straw, to enhance anaerobic digestion

- Measure the methane yield and methane production rate of the feedstock in anaerobic digestion

- Modify the SEBAC-II system to integrate the steps of pre-treatment of feedstock
APPENDIX A
PROGRAM CODE OF CR10X FOR STABILIZATION PROCESS

01: 1200 Execution Interval (minutes)
; run this program every 20 minutes

1: Do (P86)
   1: 42 Set Port 2 High
   ; open the vent/exhaust

2: Excitation with Delay (P22)
   1: 1 Ex Channel
   2: 6000 Delay W/Ex (units = 0.01 sec)
   3: 0000 Delay After Ex (units = 0.01 sec)
   4: 0000 mV Excitation
   ; keep the vent open for 60 seconds

3: Do (P86)
   1: 52 Set Port 2 Low
   ; close the vent valve

4: Do (P86)
   1: 41 Set Port 1 High
   ; open the gas supply valve

5: Excitation with Delay (P22)
   1: 1 Ex Channel
   2: 3000 Delay W/Ex (units = 0.01 sec)
   3: 0000 Delay After Ex (units = 0.01 sec)
   4: 0000 mV Excitation
   ; keep the supply open for 30 seconds

6: Do (P86)
   1: 51 Set Port 1 Low
   ; close the supply valve and go back to the top of the program
APPENDIX B
PROGRAM CODE OF CR10X FOR AMMONIA REMOVAL PROCESS

01: 1200 Execution Interval (minutes)
; run this program every 20 minutes

1: Do (P86)
  1: 42 Set Port 2 High
  ; open the vent/exhaust

2: Excitation with Delay (P22)
  1: 1 Ex Channel
  2: 3000 Delay W/Ex (units = 0.01 sec)
  3: 0000 Delay After Ex (units = 0.01 sec)
  4: 0000 mV Excitation
  ; keep the vent open for 30 seconds

3: Do (P86)
  1: 52 Set Port 2 Low
  ; close the vent valve

4: Do (P86)
  1: 43 Set Port 3 High
  ; open the pump

5: Excitation with Delay (P22)
  1: 1 Ex Channel
  2: 6000 Delay W/Ex (units = 0.01 sec)
  3: 0000 Delay After Ex (units = 0.01 sec)
  4: 0000 mV Excitation
  ; keep the pump open for 60 seconds

6: Do (P86)
  1: 53 Set Port 3 Low
  ; close the pump

7: Do (P86)
  1: 41 Set Port 1 High
  ; open the gas supply valve

8: Excitation with Delay (P22)
  1: 1 Ex Channel
  2: 1500 Delay W/Ex (units = 0.01 sec)
  3: 0000 Delay After Ex (units = 0.01 sec)
  4: 0000 mV Excitation
  ; keep the supply open for 15 seconds
9: Do (P86)
1: 51 Set Port 1 Low
    ; close the supply valve and go back to the top of the program
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Wei Liu was born in China. She received her Bachelor of Science in biochemical engineering from Beijing Technology and Business University, China in 2000 and Master of Science in organic chemistry from Chinese Academy of Sciences in 2003. In Aug. 2003, she came to University of Florida to pursue her Ph.D. degree in Agricultural and Biological Engineering Department under the supervision of Dr. Pullammanappallil and Dr. Teixeira.