The Effects of Ammonia on Anaerobic Digestion of the Organic Fraction of Municipal Solid Wastes

By

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Abstract

The effect of ammonia on anaerobic digestion of the organic fraction of municipal solid wastes (OFMSW) was investigated in this study. This study involved two sets of experiments. First set involved the investigation of ammonia toxicity on AD of synthetic OFMSW only (SW), at three different phases and pH 7.5, 8.0 and 8.5.

Phase 1 was the Batch Methane Potential (BMP) phase. BMP tests were conducted under ammonia concentration of 2500 mg/L, 5000 mg/L, 7500 mg/L and 10,000 mg/L and at pH 7.5, 8.0, and 8.5, using 500 mL Kimax® glass bottles. The total working volume of the mixture was 300 mL comprising 120 mL of mesophilic anaerobically digested inoculums, 30 g of OFMSW, various TAN concentrations ranging from 2,500, 5,000, 7,500 to 10,000 mg/L, and equal portions of buffer in form of $NaHCO_3$ and $KHCO_3$.

The second phase of the experiment examined whether the tolerance of the bacteria to high ammonia concentration would improve by acclimating the microbes to high ammonia concentrations, through gradual TAN loading. TAN concentration was increased gradually at pH 7.5, 8.0 and 8.5 weekly.

The third phase of the experiment was Semi-continuous batch phase. This phase examined the possibility of reducing the inhibitory effect of ammonia on AD, batch reactors at pH values of 8.0 and 8.5 containing initial TAN concentrations of 7500 mg/L and 10,000 mg/L. 3 g of the digestate containing high ammonia concentration(s) was replaced with fresh substrate at every 4 days, 7 days and 15 days.

The second set of experiment involved study of the effects of ammonia on anaerobic digestion of OFMSW with real landfill leachate (SW+L).

Phase 1 was BMP in which the effect of ammonia was examined at TAN concentrations of 7,500 and 10,000 mg/L.

The phase 2 of the (SW+L) gradual TAN TAN loading. The possibility of adapting mesophilic bacteria to high ammonia concentration was examined.

The results of the study confirmed that ammonia is toxic to AD, at high concentrations. Biogas production reduced with increase in TAN concentration. Reduction in Cumulative

Biogas Production (CBP) compared with control reactors was as much as 43 %, 64 % and 77 % in reactors containing 7500 mg/L TAN at pH 7.5, pH 8.0 and pH 8.5. CBP reduced to 80-85 % in reactors containing 10,000 mg/L TAN across the pH examined. Also, replacing 3g of digestate containing high TAN concentrations of 7500 mg/L and 10,000 mg/L with 3 g fresh substrate improved the activity of the mesophilic bacteria as seen in the surges in biogas production when fresh substrate was injected into the reactors.

Similar results were obtained on effect of ammonia on AD of OFMSW mixed with real landfill leachate to simulate an anaerobic bioreactor landfill. CBP reduced as the TAN concentration increased. Compared with control reactors, reactors containing 7500 mg/L TAN at pH 8.0 and pH 8.5 had 61 % and 80 % reduction in CBP. Likewise, reactors containing 10,000 mg/L TAN at pH 8.0 and pH 8.5 had 68 % and 85 % reduction in CBP, compared with control reactors.

Study confirmed that pH influenced the toxicity and composition of Total Ammonia Nitrogen (TAN). At high pH (i.e. 8.5), FAN component of TAN was about 26 % and was inhibitory to the methanogens. Results also showed that mesophilic bacteria could be adapted to a TAN concentration of about 5000 mg/L at pH 7.5 through gradual TAN loading.

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Dedicated to my Brother and Super Hero, Adeyinka Ayodeji Ojediran

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Special thanks to Bro. Kenny Ojo, my spiritual leader-turned brother. You are very amazing.

And to all my friends who stood by me by encouraging me throughout this thesis, thank you and may God bless you all.

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List of abbreviations

AD: Anaerobic digestion

BANANA: Building Absolutely Nothing Anywhere Near Anybody.

BMP: Biochemical Methane Potential

CBP: Cumulative Biogas Production

CB-reactors: Control BMP reactors.

CH_3COOH: acetic acid

CH4: Methane

CO_2: Carbon-di-oxide

COD: Chemical Oxygen Demand

DBP: Daily Biogas Production

DW: Distilled Water

FAN: Free Ammonia Nitrogen

GHG: Greenhouse Gas

GTL: Gradual TAN Loading

HRT: Hydraulic Retention Time

ISWM: Integrated Solid Waste Management

MRT: Mass Retention Time

MSW: Municipal Solid Waste

NIMBY: Not In My Back Yard.

NIMO: Not In My Office time

OFMSW: Organic Fraction of Municipal Solid Waste TAN

OLR: Organic Loading Rate

SLR: Solids Loading Rate

SRB: Sulfate Reducing Bacteria

SRT: Solids Retention Time

TAN: Total Ammonia Nitrogen

TV: Total Solids

USEPA: United States Environmental Protection Agency

VFAs: Volatile fatty acids

VOCs: Volatile Organic Compounds

VS: Volatile Solids

WMS: Waste Management Strategy

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Chapter 1

1 Introduction

1.1 Background

Municipal Solid Waste (MSW) generation is a global concern. The MSW generation rate increases with continuous increase in world population, yearly. Currently, an estimated 1.3 billion tons of MSW is generated globally every year and this value is projected to increase by 70 % by 2025. In the same vein, the annual global cost of waste disposal is expected to increase from $205 billion to $375 billion (World Bank, 2015). In 2008, Statistics Canada published that over 25 million tonnes of MSW was deposited into landfills across Canada. Out of the 25 million tonnes of waste landfilled, 13 million tonnes were household wastes containing mainly organic waste. The Organic Fraction of MSW (OFMSW) is about 40% of the total MSW generated in Canada (Statistics Canada, 2008; Alqaralleh et al., 2015). Anaerobic digestion (AD) is the most widely used process for the treatment of wastes containing high organic content such as municipal wastewater sludge and OFMSW. The AD of the OFMSW is a better alternative compared to aerobic digestion because of the biogas produced in the process (mainly, carbon dioxide and methane), which can be used for energy recovery. In addition, AD results in significant reduction of waste volume. However, the AD process is greatly inhibited by high ammonia concentration, which accumulates during the AD process. Ammonia is the by-product resulting from the AD of proteins present in MSW (Nair et al., 2014). Several studies have been carried out to examine the inhibitory effects of ammonia on AD of organic matter. However; there is limited information about the inhibitory effects of ammonia on AD of OFMSW (Yenigün and Demirel, 2013).

1.2 Research Objectives

The objectives of this research work are summarized below:

- To examine the possible inhibitory effect(s) of different ammonia concentrations on the mesophilic AD of the OFMSW, under different operating pH levels of 7.5, 8.0 and 8.5, at similar operating temperature of 35 ^{o}C.
- To examine the possibility of reducing the inhibitory effect of ammonia on AD of OFMSW by acclimating the bacteria to high ammonia concentrations, through gradual loading of influent ammonia concentrations.
- To examine the possibility of running reactors containing high TAN concentrations in the semi-continuous mode by replacing the digestate containing high ammonia concentration(s) with fresh substrate.
- To examine the possible inhibitory effect(s) of different ammonia concentrations on AD OFMSW in addition with real landfill leachate to simulate anaerobic bioreactor landfill at pH levels of 7.5, and 8.5, at similar operating temperature of 35 ^{o}C. The effect(s) of gradual TAN loading will also be examined.

1.3 Research Questions

What are the factors responsible for the AD inhibition by ammonia? What effect(s) will gradual ammonia loading have on AD of the OFMSW, rather than abrupt ammonia loading? What effects will AD operating pH and temperature have on ammonia inhibition of the AD of the OFMSW?

1.4 Thesis Layout

This thesis has six chapters and it is the form of technical papers. Chapter 1 is introduction, comprising the background, objectives, and layout of the thesis. In chapter 2, a literature review on anaerobic digestion and the inhibitory effects of ammonia on the AD of MSW is presented. Chapter 3 is the materials and methodology used in the experimental study of this research. In chapter 4, the first technical paper titled "The Toxicity Effects of Ammonia on Anaerobic Digestion of the Organic Fraction of Municipal Solid Waste" is presented. The second paper is presented in Chapter 5, titled "The Effects of Ammonia on the Anaerobic Digestion of Synthetic Organic Fraction of Municipal Solid Waste Mixed with Landfill Leachate". A summary of the conclusions of this thesis and the suggested future work is provided in Chapter 6.

Due to the fact that this thesis is in paper based format, there may be repeated information occurring in different chapters.

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Chapter 2

2 Literature Review

2.1 Municipal Solid Waste (MSW) Generation

MSW comprises the waste generated from residential households and apartment buildings, commercial and institutional establishments, construction and demolition waste, municipal services, and treatment plants (Staley and Barlaz, 2009).In North America, 254 million tons of MSW was generated in the year 2013 in the United States of America (USA) while in Canada over 30 million tons of MSW is generated annually (USEPA, 2012). A recent report by the Canadian Broadcast Commission indicated that the per capita waste generated in Canada is higher than in any other country in the world (Canadian Broadcast Commission, 2013).The components of a typical MSW are food wastes, paper, cardboard, plastics, textiles, rubber, leather, yard wastes, wood etc. while construction and demolition waste is tended to be managed separately in industrial mono-landfills (Tchobanoglous et al., 1993). The percentage composition of a typical MSW is shown in Figure 2.1. The OFMSW which represents the biodegradable portion of the MSW contains carbon compounds derived from animal and plant materials i.e. kitchen waste and yard waste.

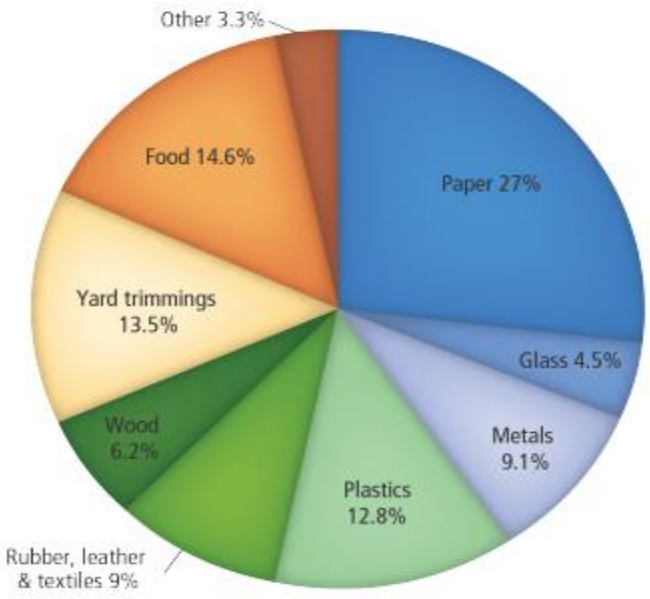

Figure 2.1: MSW Generation in 2013 in the U.S. before recycling (USEPA, 2013)

2.2 Waste Management Strategies

The growing concerns of waste generation and the need to effectively manage the generated waste necessitated a strategic approach for waste management, referred to as the Integrated Solid Waste Management (ISWM). In ISWM, priority is placed in the following order: waste avoidance, waste recycling, waste incineration (energy recovery) and lastly, waste disposal as shown in Figure 2.2.

Figure 2.2: Waste management hierarchy in the ISWM strategy (USEPA, 2012)

2.2.1 Waste Avoidance

Waste avoidance is a waste management strategy (WMS) that is aimed at discouraging industries and individuals from acquiring unnecessary wastes. This WMS is often referred to as waste reduction, aimed at reducing the rate of waste generation from the source. In the developed countries, government policies in terms of MSW management are geared towards waste prevention and reduction. This is achieved through public education and sensitization, and increased waste disposal tariffs. The waste avoidance strategy is also implemented through several programs that encourage individual consumer to reuse used products and buy goods in bulk (USEPA, 2013).

2.2.2 Waste Recycling/Composting

Next on the ISWM is MSW recycling and composting. Through the recycling strategy, items that would otherwise be dumped off as wastes and used items are collected, sorted and processed into raw materials, and remanufactured into new products. MSW recycling and separation is widely practiced in Canada. In the same vein, food scraps, yard

trimmings, and other organic materials are also composted to enhance waste stabilization. Recycling has numerous advantages because it saves energy, enhances the supply of valuable raw materials to the manufacturing industry, creates jobs, stimulates the development of sustainable technology, reduces the need for new landfills and combustors, and prevents the emission of many greenhouse gases and water pollutants (Ara, 2012).

2.2.3 Energy Recovery

In the energy recovery strategy, waste materials are burned to create energy in the form of heat and electricity. The energy recovery process involves thermal, chemical and biological processes through which energy is recovered from waste. Energy recovery is a renewable energy because the garbage which is the source of the fuel is sustainable and is not depleted (Ara, 2012). Energy recovery from waste strategy is implemented in some developed countries such as Japan where up to 70% of its MSW generated is incinerated to reduce epidemics, reduce waste volume and to recover energy from such waste materials (Mastutu, 2014). In addition, the energy recovered from the incineration of MSW provides a better alternative for electricity generation than power plants powered by fossil fuels through which greenhouse gases are emitted in large quantities. The incineration of the OFMSW is neither energy efficient nor cost effective. This is because the OFMSW consists of mostly kitchen food wastes and the incineration of food waste requires lots of energy input while the energy recovered from the process is negligible.

2.2.4 Waste Disposal

Despite the implementation of the ISWM strategies in the order discussed above, considerable amounts of wastes are still being disposed of in landfills (Kheradmand et al., 2010). Landfilling operation is carried out without environmental protection measures in underdeveloped and some developing countries. In developed countries however, the [1]NIMBY, [2]NIMO, and [3]BANANA approaches of individuals towards waste management

and waste disposal into landfills have necessitated the need for the implementation of environmental measures to minimize the environmental risks associated with landfilling, through the use of bioreactor landfills. These measures involve the construction of landfills liners, leachate collect system and biogas (especially, methane gas) collection system for energy recovery (Poulsen, 2014).

2.3 Bioreactor Landfill

A bioreactor landfill is an engineered landfill where the waste microbial degradation process is enhanced to achieve a faster and more extensive stabilization of waste (Malfredi and Thompson, 2009). This is mainly achieved through the recirculation of the collected leachate. Leachate is a liquid which is generated from the landfill when water (i.e. rain or run off) seeps through the solid waste. The schematic diagram of a typical bioreactor landfill is shown in Figure 2.3.The quality of leachate varies from one landfill to another. The recirculation of leachate ensures a continuous supply of nutrients and moisture close to field capacity, which are the two key conditions to promote the degradation reactions. Leachate recirculation may also lead to an increase in waste density, which results in a better utilization of the landfill capacity (Benson et al., 2007).
Leachate quality depends on the age of the landfill, the makeup of the waste and leachate, the operational type of the landfill, leachate recirculation and treatment system, rainfall, runoff design and ambient temperatures (Malfredi and Thompson, 2009).

1. Not In My Back Yard. Public attitude regarding the installation of waste management facilities close a residential area.
2. Not In My Office time. Politicians and administrators try to avoid or postpone decisions in order not to dent their career.
3. Building Absolutely Nothing Anywhere Near Anybody.

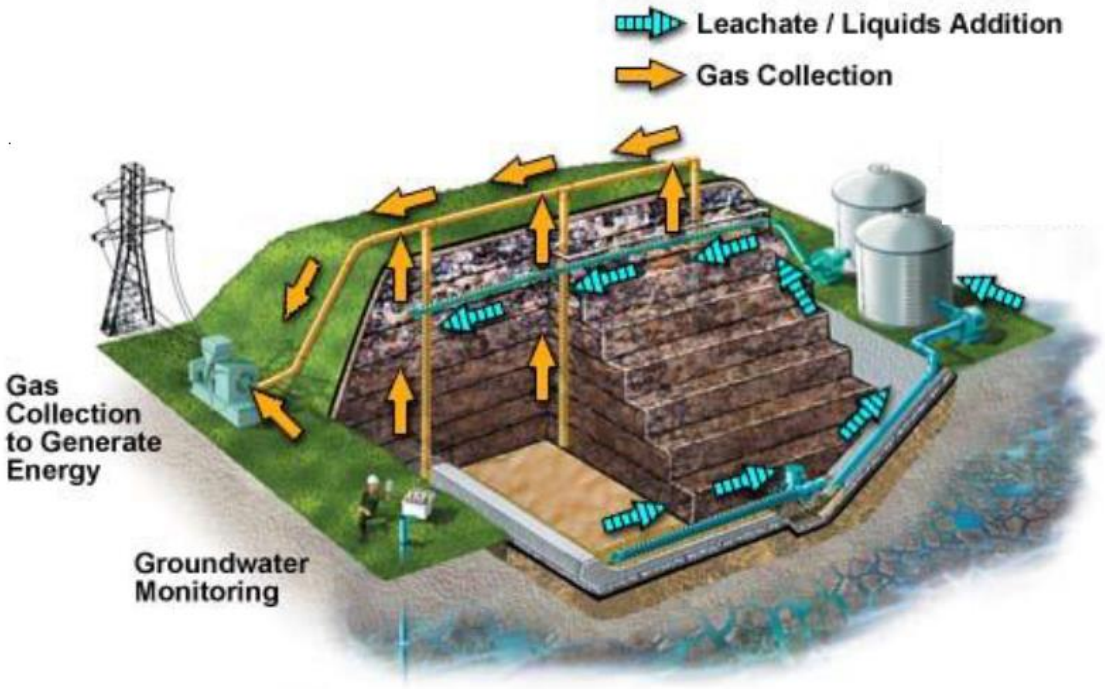

Figure 2.3: Anaerobic Bioreactor Landfill ®Waste Management (USEPA, 2011)

2.3.1 Classifications of Bioreactor Landfills

Based on the processes of operation, bioreactor landfills can be classified into aerobic, anaerobic, hybrid (or facultative) bioreactor, and flushing bioreactor landfills.

Aerobic bioreactors are bioreactors wherein air is injected into the landfill waste mass using blowers, in addition to controlled leachate recirculation, to enhance aerobic activity and accelerate waste stabilization (USEPA, 2011). Due to the bacterial consortium, the rate of waste stabilization in the initial stages of stabilization is relatively quick but the rate of waste stabilization becomes longer towards the latter stages. Aerobic bioreactor landfills also generate landfill gas (poor in methane and rich in carbon dioxide and Nitrogen) and lower leachate production (Malfredi and Thompson, 2009). Aerobic bioreactors are also called aerated bioreactors.

Anaerobic bioreactor landfills, unlike aerobic bioreactor landfills are operated in the absence of air or oxygen. Anaerobic bioreactors require longer time to stabilize waste

when compared to aerobic bioreactors but they are more efficient than the conventional engineered landfills. The entire landfill works as an anaerobic digester and accelerates the waste decomposition by maintaining a healthy anaerobic bacteria consortium. Compared to other classes of bioreactor landfills, anaerobic bioreactor landfills are easier to operate and more cost effective since there is no energy requirement for aeration. One major advantage of using an anaerobic bioreactor landfill for MSW degradation is its ability to trap methane gas (i.e. methane constitutes around 50-60% of the biogas generated from landfills) which can be used for energy recovery.

The hybrid landfill bioreactor combines the processes of anaerobic and aerobic metabolism to stabilize the waste mass (Matsufuji et al., 2005). In the initial stage, the degradation mechanism is anaerobically driven and enhanced by the recirculation of leachate. This first stage (anaerobic stage) continues for 5–10 years, during which the generated methane (CH_4) will be too low for utilization for energy recovery. Then the subsequent aerobic step is initiated by injecting air from the bottom of the landfill. A convective air flow will then proceed within the landfill, due to the temperature gradient between the warm waste (up to 50 – 70 ^oC) and the colder external environment by the so-called ‘‘chimney effect” (Hanashima, 1999). When the natural airflow ceases, anaerobic conditions may naturally re-establish but the waste is already stabilized and therefore the potential for CH_4 generation will be low. The cycling of aerobic and anaerobic conditions also offers possibilities of treatment of some recalcitrant chemicals and chemical by-products, in the same manner as modern wastewater treatment such as nitrification and denitrification of ammonia (Reinhart et. al., 2006).The advantage that hybrid bioreactors have is that they reach the methanogenesis phase faster due to the combined aerobic-anaerobic processes. However, hybrid bioreactors are more difficult to operate and the associated installation and maintenance cost is much more compared to the other classes of bioreactors.

Flushing bioreactor landfills, are a special form of bioreactor landfills where considerable amounts of water and leachate are recirculated in order to flush-out soluble waste, components using a process called ‘‘waste irrigation” or ‘‘waste flushing”. The flushing rate typically ranges from 1 to 5 m^3 of total liquids (leachate and external water) per ton of waste landfill (Hupe et al., 2003; Blakey et al., 1997). Due to the addition of large

volumes of water, flushing bioreactors achieve waste stabilization and contaminant removal quickly (IWMLWG, 1999). However, the cost for the installation of flushing bioreactor may be two to four times higher than the conventional landfill (Karnik and Perry 1997).

2.4 Anaerobic Digestion

AD is a biological process whereby organic and inorganic wastes are decomposed in the absence of oxygen. According to USEPA (2011), AD is defined as a process where microorganisms break down organic materials, such as food scraps, manure and sewage sludge, in the absence of oxygen. The huge energy content in OFMSW can be optimally recovered when anaerobically biodegraded. The AD process may occur naturally in open dump landfills where the bioreactor technology is not applied in portions that are deficient of air, especially in the lower part of the landfills. When this occurs, the biogas (especially methane) produced in this anaerobic part of such landfills is emitted into to atmosphere, resulting in greenhouse gases (GHG) emission.

The ability to recover energy from organics makes AD more attractive than aerobic digestion. It also reduces GHG by using methane as an energy source which would otherwise be emitted from land filling waste (USEPA, 2011). In addition, anaerobic systems produce methane gas, which is unavailable for biomass synthesis, resulting in only 5-20% as much waste biomass, significantly reducing financial and disposal site requirements (Speece, 2008).The application of the AD technology for MSW management enhances the reduction of odours and volatile suspended solids and the destruction of pathogenic organisms. When anaerobically biodegraded, OFMSW generates biogas which is composed of methane (CH_4) and carbon-dioxide (CO_2), in percentages of 55% and 45%, respectively. Methane production is a major consideration in the selection of anaerobic digestion technology for wastewater treatment.

2.4.1 Anaerobic Digestion Process

The AD process is a complex biological process, consisting of four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis as illustrated in Figure 2.4. Each phase has different microbial populations (Gujer and Zehnder, 1983).

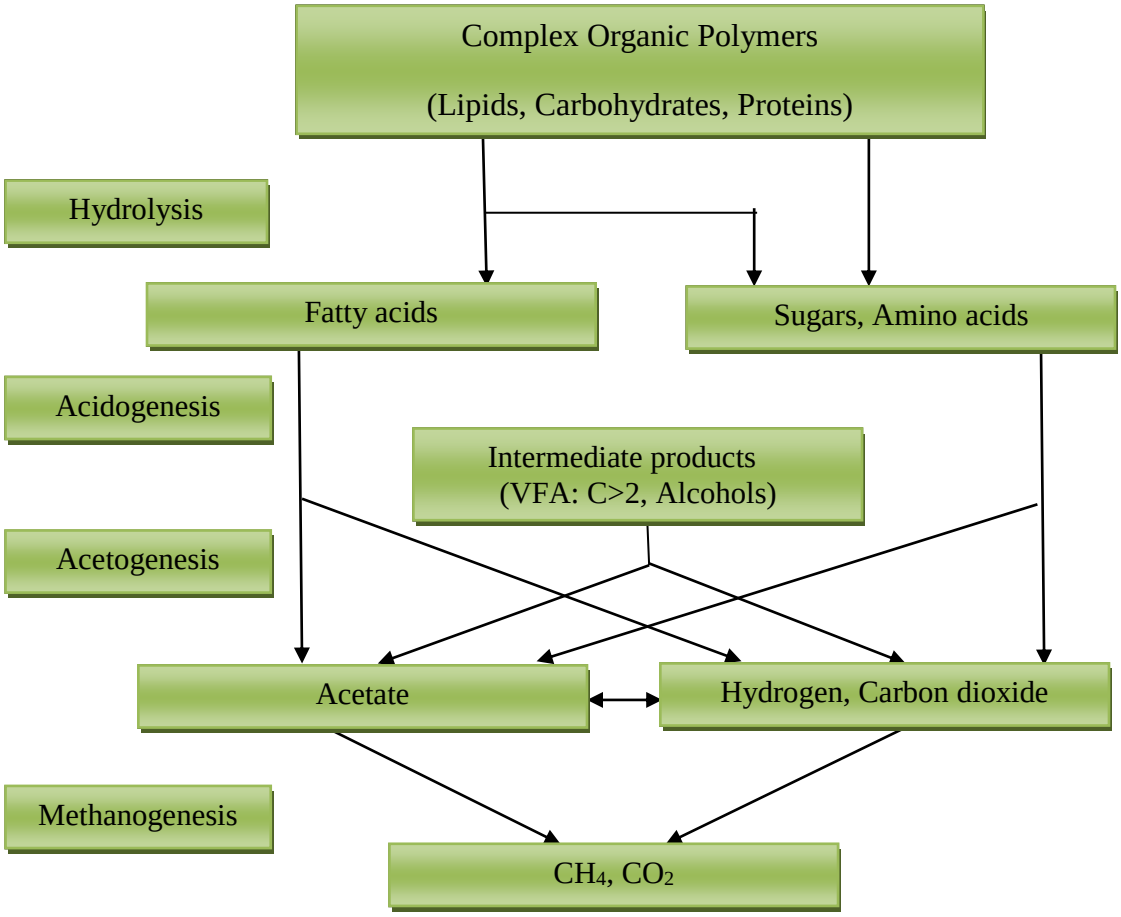

Figure 2.4: Anaerobic digestion process (Gujer and Zehnder, 1983).

2.4.1.1 Hydrolysis

Hydrolysis is the process by which the biopolymer particulate organic compounds and colloidal wastes (proteins, fats, and carbohydrates) are solubilized and degraded to form soluble monomeric or oligomeric organic compounds (Gerardi, 2003). Hydrolysis is catalyzed by hydrolytic enzymes such as proteases, which degrade proteins, lipases,

which degrade fats, and cellulases, which degrade cellulose. The simple organic compounds (amino acids, fatty acids, and sugars) produced during this phase will be utilized as a substrate in the next phase of the AD. Hydrolysis is a very significant process in AD because it that can be a rate limiting step, particularly when anaerobically digesting semi-solid waste (Ferrer et al., 2008).

2.4.1.2 Acidogenesis

During acidogenesis, the products of the hydrolysis phase (amino acids, fatty acids, and sugars) are absorbed, degraded and converted into volatile fatty acids (VFAs), alcohols, carbon dioxide, and hydrogen by different facultative and obligate anaerobic bacteria. By-products, such as ammonia and hydrogen sulfide are also produced during this phase (Strik et al., 2005; Chandra et al., 2012)

2.4.1.3 Acetogenesis

In the acetogenesis phase, the VFAs, alcohols, and carbon dioxide produced in the acidogenesis phase are converted into acetate, hydrogen and carbon dioxide by acetate-forming bacteria. This phase is also characterized by the constant reduction of hydrogen and carbon dioxide to acetate by homoacetogenic microorganisms (Gerardi, 2003; Chandra et al., 2012).

2.4.1.4 Methanogenesis

Methanogenesis occurs in strictly obligate anaerobic conditions. During this phase, methane is produced from the products of acidogenesis/acetogenesis by methanogenic Achaea. The two types of microorganisms responsible for methane formation are acetoclastic methanogens and hydrogen-utilizing methanogens. Acetoclastic methanogens utilize acetate to produce methane and carbon dioxide while the hydrogen-utilizing methanogens use hydrogen and reduce carbon dioxide to form methane (Mara et al., 2003). The methanogenesis phase is critical because methanogens are sensitive to the different conditions such as ammonia, VFAs and pH, and this phase can therefore have a huge impact on AD performance (De Vrieze et al., 2012). About 70% of methane in AD is derived from this pathway (Parawira, 2012).

The AD phases in anaerobic bioreactor landfills are similar to the AD phases in a typical digester, however with some slightly different terminologies.

2.5 Phases of Biodegradation in a Bioreactor Landfill

The analysis of the processes occurring in a typical bioreactor landfill has been summarized into five different phases, as shown in Figure 2.5 (Pohland and Kim, 2003). These phases include Lag or Initial Adjustment Phase, Transition Phase, Acid Formation Phase, Methane Fermentation or Methanogenesis Phase, and Maturation and Stabilization Phase.

2.5.1 Phase I: Lag or Initial Adjustment

Once a waste lift is placed in a landfill and covered with a layer of topsoil, the lag or initial adjustment phase starts. This is followed by aerobic microbial decomposition of the waste due to the presence of moisture and trapped air. The duration of this phase depends on the extent of waste compaction and moisture content.

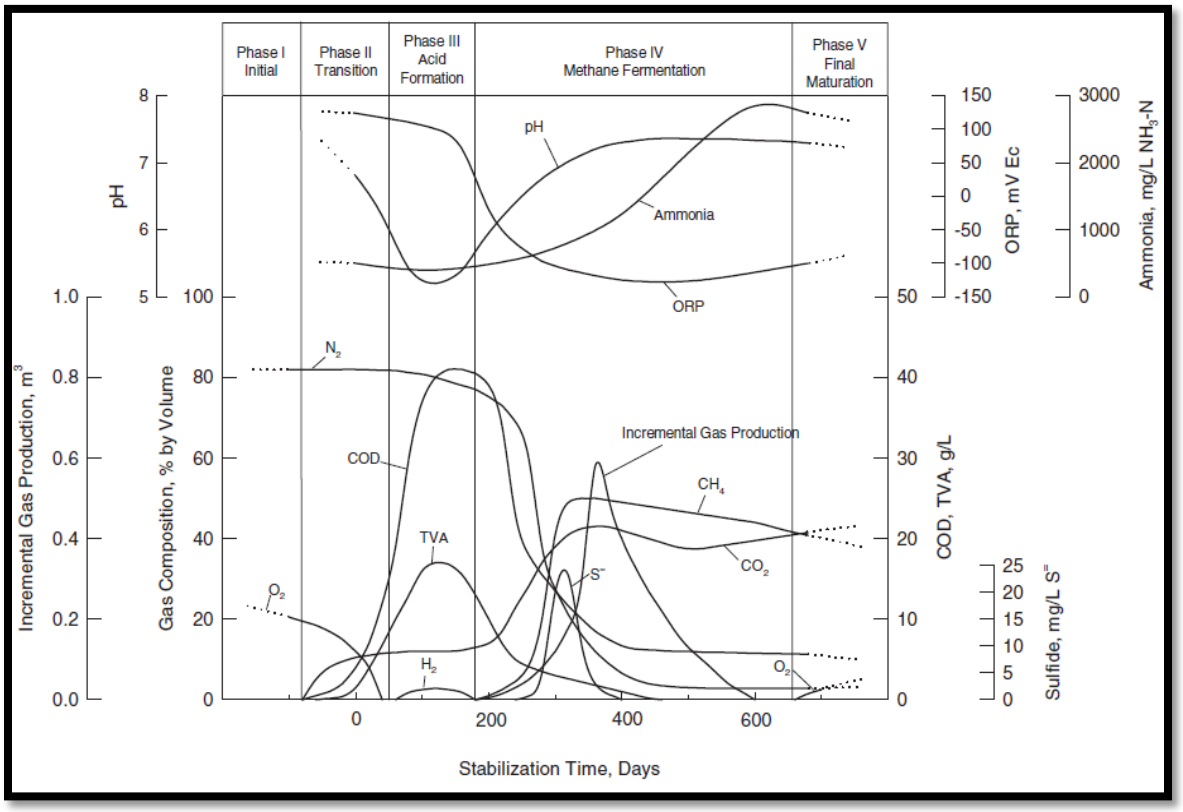

Figure 2.5: Phases of bioreactor landfill stabilization (Pohland and Kim, 2003)

2.5.2 Phase II: Transition

The compaction and aerobic microbial activity in the previous phase result into the depletion of oxygen in the waste as shown in Figure 2.5 and the entire process changes from aerobic to anaerobic, during the transition stage. This switch from aerobic to anaerobic process causes the hydrolysis and breaking down of the hydrocarbons present in the waste. The end of this phase is marked by the significant change in the leachate chemical oxygen demand (COD), and volatile organic acids (Shahriari and Abdallah, 2009).

2.5.3 Phase III: Acid Formation

The acid formation phase commences as the hydrocarbons broken down by hydrolysis become converted into VFAs, composed mainly of acetic acid (CH_3COOH), butyric acid and hydrogen gas. There is a reduction in the pH value at this phase.

2.5.4 Phase IV: Methane Fermentation or Methanogenesis

This phase commences as the intermediate compounds, (i.e. VFAs and hydrogen gas) formed in the acid forming phase, are broken down into methane (CH_4), carbon-di-oxide (CO_2) and water (H_2O) by microbes called methanogens. The consumption of the intermediate compounds causes a rise in pH value to a neutral range (i.e. 7.0). This phase is the most important stage for tapping the methane potential of the OFMSW.

2.5.5 Phase V: Maturation and Stabilization

The last phase of the biodegradation of the OFMSW in bioreactor landfills is called maturation and stabilization. The level of microbial activity at this stage is lower when compared to the previous two stages. This is because the whole process of biodegradation is limited by available carbon substrates and essential nutrients like phosphorous, both of which are essential for cell growth and synthesis. As a result, the methane produced in this stage is low and is associated with the difficulty to biodegrade residual organics in the OFMSW. Waste stabilization is then said to have occurred wherein there is little or negligible methanogenic activity in the landfill (Nair et al., 2014).

2.6 Factors Inhibiting the Performance of the AD Process

There is a wide variation of information regarding the inhibitory effects of identified substances and environmental factors on AD performance. This is largely because of the variation in the properties of different substrates and inoculums used, variation in the method of acclimation adopted, and owing to the fact that the AD process is complex. However, the inhibition of the AD process has been linked to a decrease of the steady-state rate of the biogas produced, the reduction of the methane content of the biogas produced, and accumulation of organic acids (pH decrease) (Kroeker et al., 1979).

2.6.1 Sulfide

One of the constituents found in many industrial wastewaters is sulfate. During AD in digesters, sulfate reducing bacteria (SRB) reduce sulfate to sulfide (Koster et al., 1986; Hilton and Oleszkiewicz, 1988).The reduction of sulfate is accomplished through two main groups of SRB including incomplete and complete oxidizers. Incomplete oxidizers reduce compounds such as lactate to acetate and CO_2, while complete oxidizers completely convert acetate to CO_2 and HCO^-_3. Due to sulfate reduction, there are two noticeable stages of inhibition during AD. The first stage of inhibition is as a result of the competition for available organic and inorganic substrates by SRB, causing a reduction in methane production. The second stage of sulfide inhibition is due to the toxicity of sulfide to different groups of bacteria (Andersonet al., 1982; Colleran et al., 1998).

2.6.2 pH

pH has a significant influence on the growth of microbial population necessary for AD and the accumulation and composition of inhibitory substances such as total ammonia nitrogen (TAN) and VFAs. TAN is made up of free ammonia nitrogen (FAN) and ammonium nitrogen and the percentage composition of these two components largely depends on the operating pH and temperature (Ding and Sartaj, 2015). FAN form of TAN

is more toxic to the AD process than the ammonium form and an increase in pH would result in increased toxicity as will be discussed in more detail later on. It is therefore expedient to keep pH within the growth optimum of microorganisms and to prevent inhibition to the AD process. Braun et al. (1981) observed a VFAs accumulation up to 316 mg/L and an increase in FAN up to 316 mg/L while anaerobically digesting liquid piggery manure, at an operating pH of 8.0. However, the FAN and VFAs concentrations reduced instantly to 86 mg/L and 20 mg/L as the pH was adjusted to 7.4. The reduced pH enhanced the bacteria utilization of the VFAs and the biogas production also increased. Zeeman et al. (1985) observed an increase in methane production during the thermophilic AD of cow manure, when the pH was reduced from 7.5 to 7.0.

Shanmugam and Horan (2009) examined the effect of operating pH on a waste mixture containing a C:N ratio of 15 under various pH values of 4.5, 5.5, 6.5, 7.5 and 8.5. The study showed that cumulative biogas production ranged from 1258 mL at pH 8.5 to 6518 at pH 6.5 as shown in Figure 2.6. Furthermore, a low specific biogas yield of 0.17 mL CH_4/gVS removed and high ammonia production of 3473 mg/L was observed at pH 8.5. However, at pH 6.5, the cumulative biogas production and ammonia accumulation were 6515 mL and 817 mg/L respectively.

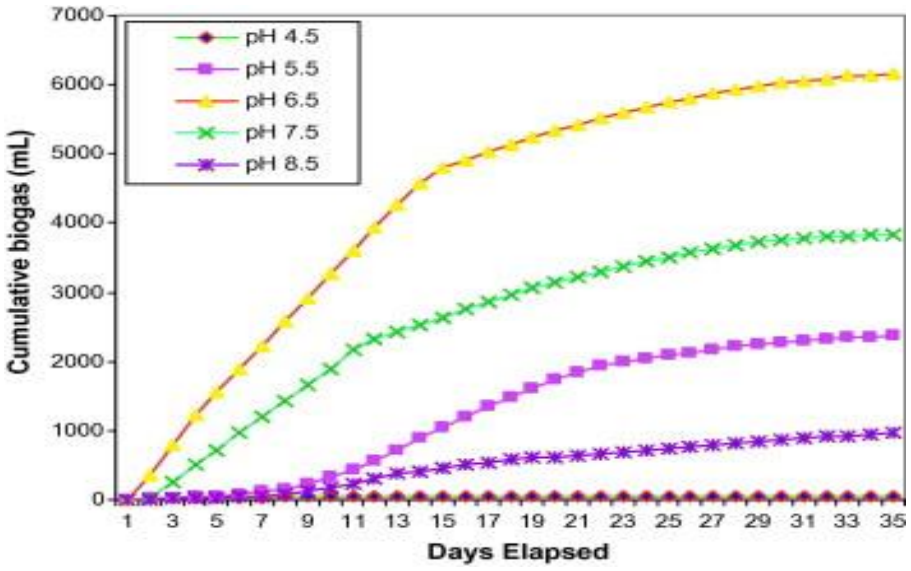

Figure 2.6: Cumulative biogas yield at C:N ratio of 15 at different pH controlled reactors of LF admixed with MSW (Shanmugam and Horan, 2009)

2.6.3 Temperature

The operating temperature of an AD process has a significant effect on FAN accumulation and microbial growth. When the temperature of an AD process is increased the microbial growth rate increases however, this will enhance the accumulation of FAN in high concentrations (Chen et al., 2008).

Research conducted by Braun et al. (1981) showed that operating at the mesophilic temperature range during AD of cow manure containing high FAN concentration enhanced a more stable process and reduced process inhibition, compared to operating at the thermophilic temperature range. Angelidaki and Ahring (1994) and Hansen et al., (1999) observed an increase in biogas production when the operating temperature was dropped from 60 ^{o}C to 37^{o}C during the AD of substrates containing high ammonia concentrations. The increased biogas production was interpreted as an indication of relief from the inhibition caused by the FAN, as the temperature was reduced. However, it may be argued that different bacteria such as mesophilic and thermophilic bacteria dominate depending on the operating temperature thermophilic bacteria.

2.6.4 Ammonia-Nitrogen

The application of anaerobic bioreactor landfills and anaerobic digesters for anaerobically digesting OFMSW and other organic matter, prevents the emission of GHG into the atmosphere, prevents contamination of the groundwater and enhances the optimal collection and utilization of the biogas produced for energy recovery (Ward et al., 2008). However, the lack of degradation pathway for ammonia in anaerobic bioreactor landfills and anaerobic digesters causes the accumulation of ammonia and volatile fatty acids, resulting in reduced biogas production and digester failure. This will be discussed in more details in the latter part of this thesis. High ammonia concentration inhibits methanogenic activities and causes VFA accumulation, causing the failure of AD digesters (Nielsen and Angelidaki., 2008). The inhibitory effect of ammonia on AD is

equally significant in anaerobic bioreactor landfills where leachate is constantly recirculated. Continuous leachate recirculation enhances the persistent increase in the concentration of ammonia in landfills and this a major factor in the treatment cost of leachate and length of post-closure activities for bioreactor landfills (Berge et al., 2005).

2.6.5 Presence of other ions

Research has shown that some ions such as Na^+, Ca^{2+}, and Mg^{2+} have antagonistic effects on ammonia inhibition, a phenomenon whereby the toxicity of one ion is reduced by the presence of other ions (McCarty and McKinney, 1961; Braun et al., 1981; Hendriksen and Ahring, 1991). Research work by Kugelman and McCarty (1964) provided more insights into the mutual antagonism existing between ammonia and sodium. Methane production from acetic acid reduced by 20% as a result 0.15 M ammonia present in the system, and a further 5 % increase in methane production was observed when 0.002–0.05 M Na+ was added to the system, compared to the control. In a related study on the AD of poultry manure by Krylova et al. (1997), biogas generation was stimulated by adding 10% (w/v) phosphorite, regardless of the fact that NH_4Cl was as high as 30 g/L in the system. The stimulating effect of phosphorite was attributed to the immobilization of the biomass on mineral particles, preventing biomass washout from the reactor. Also, biogas stimulation due to phosphorite addition was an indication of the antagonistic effect of phosphorite ore minerals (K^+, Ca^{2+}, Mg^{2+}) on ammonia inhibition. The authors reported however that the toxicity caused by more than 50 g/L of NH_4Cl was irreversible, even with addition of phosphorite.

2.7 Mechanism of Ammonia Inhibition during Anaerobic Digestion

Ammonia is the by-product resulting from the anaerobic digestion of nitrogenous materials available in organic matter, mostly proteins and urea. Other nitrogenous compounds present in wastewaters and OFMSW include phospholipids, nucleic acids and

other nitrogenous lipids. During the hydrolysis and solubilisation of protein, proteolytic bacteria convert protein to alpha-amino acids as shown in Figure 2.7 (Kayhanian, 1999).

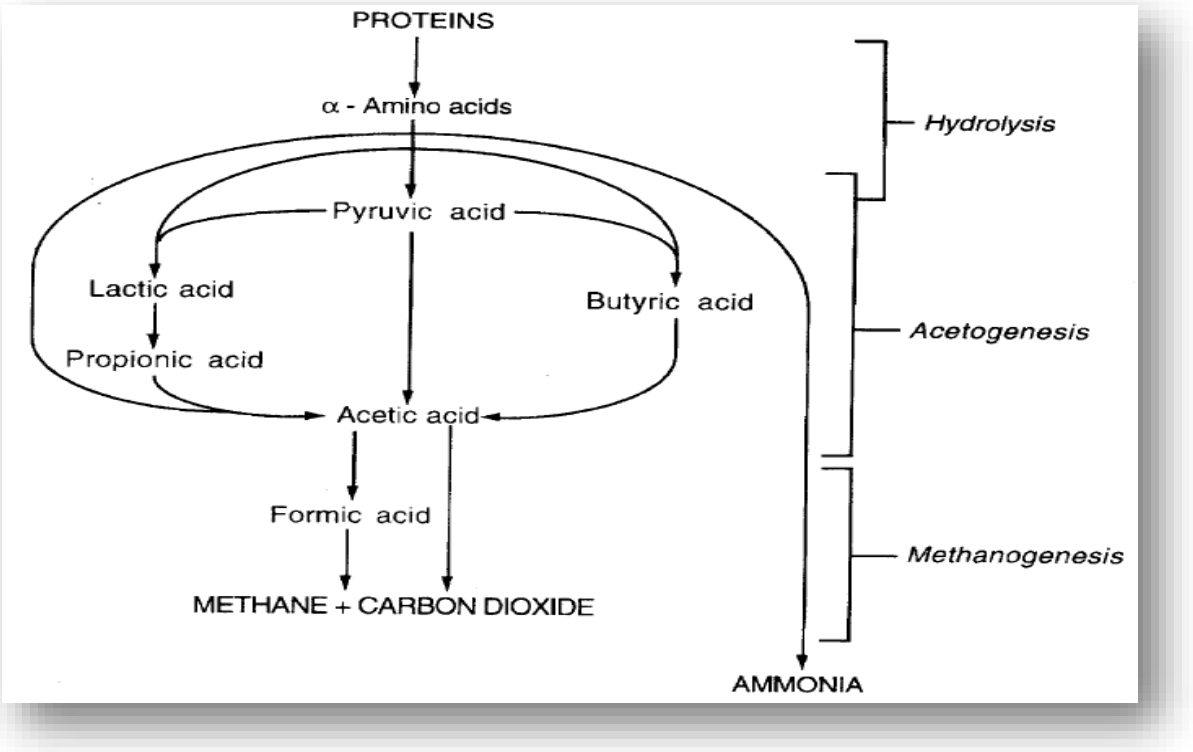

Figure 2.7: steps in the bioconversion of proteins (Blomgren et al., 1990).

As evident in Figure 2.7, ammonia is produced during hydrolysis of protein which is the first stage of bioconversion of protein. Also, hydrolytic bacteria further hydrolyze amino acids to form ammonia, H_2, CO_2 and VFAs. The amount of ammonia that can be produced during the biodegradation of an organic substrate can be quantified using the stoichiometric relationship in Equation (1) given below (Tchobanoglous et al., 1993).

$$C_aH_bO_cN_d + \frac{4a - b - 2c + 3d}{4}H_2O \rightarrow \frac{4a + b - 2c - 3d}{8}CH_4 + \frac{4a - b + 2c + 3d}{8}CO_2 + dNH_3 \quad \ldots . \text{ Eq. (1)}$$

While ammonia is an essential source of nutrient for bacterial growth during AD, its inhibitory effects at high concentrations on methanogenic activities has been widely

reported (VanVelsen, 1979; Kayhanian, 1994; Poggi-Varaldo et al., 1997; Angelidaki and Ahring., 1994; Calli et al., 2005; Duan et al., 2012).The release of ammonia through the hydrolysis of amino acids causes a rise in the alkalinity and pH of the digester liquid (Shanmugam and Horan, 2008). Among the three major anaerobic bacteria (i.e. methanogens, acidogens and acetogens), methanogens have the least tolerance to ammonia and fatty acids overloads. In order to maintain a constant pH and prevent digester failure, there must be a balance between the consumption of fatty acids by methanogens and the production of fatty acids by the acetogens (Kayhanian, 1994).

Oftentimes, ammonia-nitrogen is expressed as total ammonia nitrogen (TAN). TAN is composed of free ammonia-nitrogen (FAN) and ammonium-nitrogen (NH_4^+-N). The FAN content of the TAN is often referred to as the unionized form while the ammonium content is commonly referred to as the ionized form of TAN. The percentages of FAN and NH_4^+-N in TAN depend on pH and temperature as shown in Figure 2.8

The percentage of FAN in TAN can be estimated using Equation (2), as reported by Hansen et al. (1998).

$$NH_3 - N = TAN * \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

where: NH_3-N = Free Ammonia Nitrogen (FAN) mg/L; TAN = Total Ammonia Nitrogen mg/L; T (K) = Temperature (Kelvin).

It is important to provide information on the operating pH and temperature when referring to the TAN concentration of a digester and of any AD system. For instance, at low pH (i.e. 6.0) and temperature (i.e. 35 ^oC), the percentage of FAN in TAN is almost negligible. However, at high pH (i.e. 11) and temperature values, FAN constitutes as much 100% of TAN, as seen in Figure 2.8.

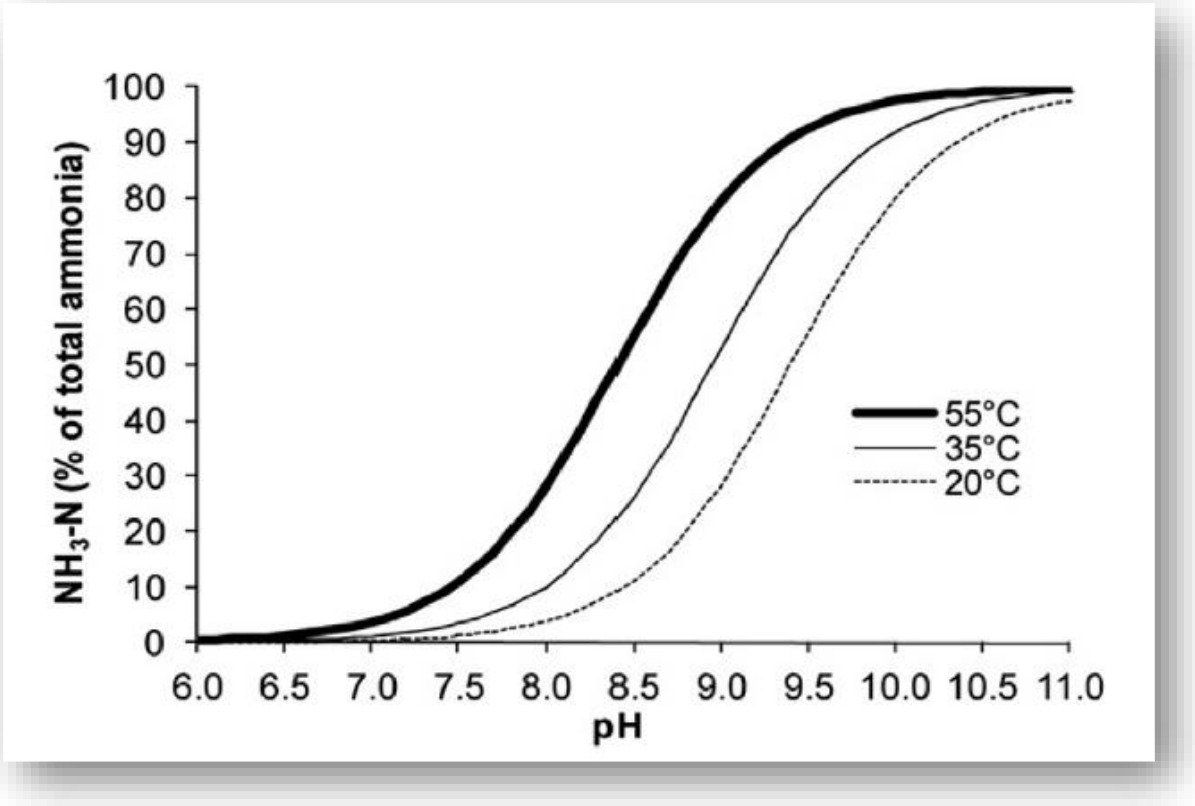

Fig. 2.8: Percentage of free ammonia in solution at temperature of 20, 35 and 55 and varying pH values (Rajagopal et al., 2013).

Sterling et al. (2001), Nielsen and Anglidaki, (2008), and Chen et al. (2008) confirmed that the formation of FAN was more toxic to the AD in a digester was pH dependent.

The FAN component of TAN is more responsible for the inhibition of the AD process than the ionized form of TAN. This is largely due to the fact that FAN is more toxic and capable of penetrating through bacteria cell membrane (Sung & Liu 2003; Muller et al., 2006). FAN toxicity inhibits methanogenic bacteria by disrupting the methane producing enzymes directly and by diffusing passively into bacterial cells, causing proton imbalance and/or potassium deficiency as illustrated in Figure 2.9 (Gallert et al., 1998).

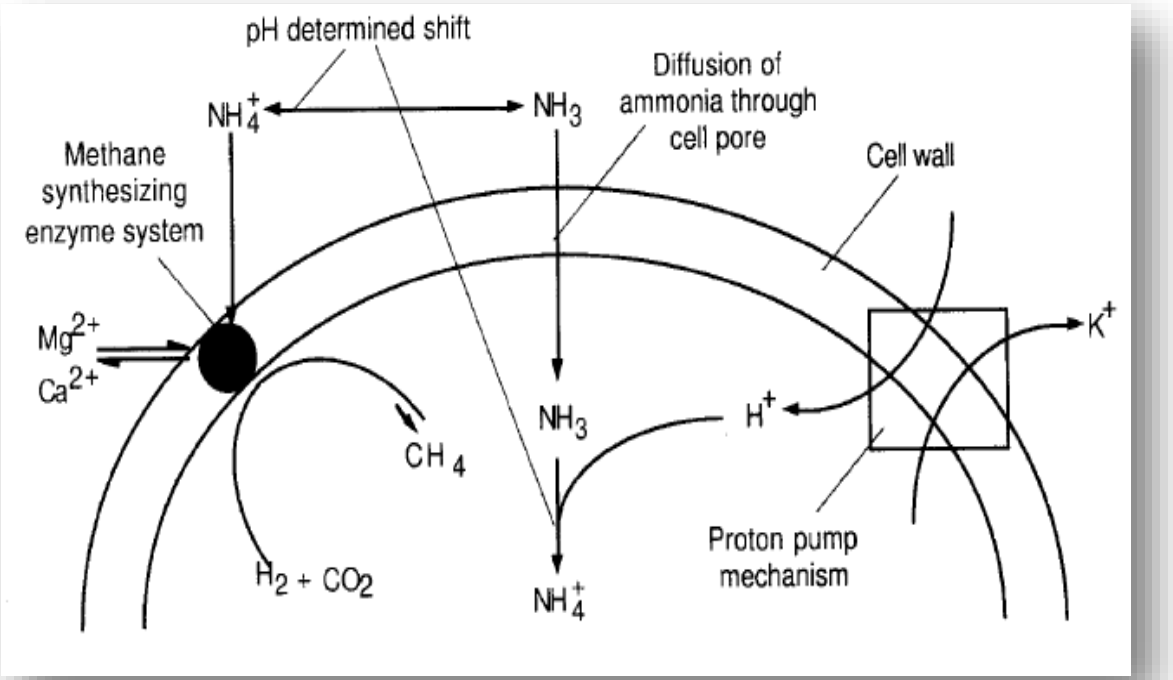

Figure 2.9: Mechanism of ammonia inhibition on methanogenic bacteria (Sprott and Patel, 1986)

As illustrated in Figure 2.9 above, when ammonia diffuses passively into the cells of methanogens, there is a difference in intracellular pH which results into the conversion of some of the ammonia into ammonium ($NH_4^+ - N$), after absorbing protons (H^+). The cells then must dissipate to balance the protons (H^+) deficit, using a potassium (K^+) antiporter (Sprott et al., 1984). In a study conducted by Wiegant and Zeeman (1995), it was observed that high TAN significantly affected the growth rates of hydrogen utilizing methanogens and acetate was quickly produced when the concentration of TAN increased. The authors proposed that intermediate compounds such as hydrogen and/or propionic acids were formed as a result of the inhibition of hydrogen utilizing bacteria, inhibiting the conversion of the acetate formed into methane. Ammonia is also toxic in biological treatment of wastewater. The discharge of effluent of wastewater treatment plant containing high ammonia concentration into water bodies can be toxic to aquatic life (Dong and Sartaj, 2016).

AD process inhibition by ammonia often results in VFAs accumulation. In return, the accumulation of VFAs leads to decrease in pH and a decline in the concentration of FAN. As the FAN, VFAs and pH interact, there is an inhibition to the steady-state of the AD process. Although the process will still be running stably, the biogas and the methane content of the biogas produced will reduce significantly (Angelidaki and Ahring, 1993).

2.8 Studies on Ammonia Inhibition of the AD Process

The inhibitory effect of ammonia on the AD of both high-solids and low-solids under various operating temperatures and pH values have been reported (Pfeiffer, 1974: Braun et al., 1981; Webb, & Hawkes 1985; Hashimoto, 1986; Blomgren et al., 1990; Angelidaki and Ahring, 1993). The impact of high ammonia concentrations on the biodegradation of the OFMSW was studied by Kayhanian (1994), using various ammonia concentrations. An instant inhibition occurred at 1000 mg/L ammonia concentration, followed by a 50% inhibition and a complete bioreactor failure occurring at 1500 and 2500 mg/L ammonia concentrations respectively. The inhibitory effect of ammonia on anaerobic film enriched by methylaminotrophic methane producing Archaea was studied by Sossa et al. (2004), using varying ammonia concentrations of 48.8, 73.8, 98.8, 148.8, 248.8, 448.8, and 848.8 mg/L, respectively. The authors reported that maximum methanogenic activity occurred at 48.8 mg/L ammonia concentration. However, methanogenic activity was significantly inhibited at 848.8 mg/L ammonia concentration. In a study by Koster (2007), 1900 –2000 mg/L ammonia concentration was reported as the cause of inhibition to methane production at pH values above 7.6. When operating in thermophilic temperature range, the inhibitory effect of high ammonia concentrations is more significant than when operating in the mesophilic temperature range. Kayhanian (1994) observed a significant reduction in methanogenic activity and methane gas production at TAN concentration of 500 mg/L, under operating pH of 7.5 and above and thermophilic temperature conditions. Studies by Bhattacharya and Parkin (1998), Angelidaki et al. (1993), Martinelle and Haggstrom (1993) and McCarty and McKinney (1991) showed that FAN was more responsible for AD process inhibition than TAN. Bhattacharya and Parkin (1989)

reported a threshold FAN concentration of 55 ± 11 mg/L – NH_3 while Braun et al., suggested a slightly higher FAN concentration of 80 mg/L – NH_3. In another study by McCarty and McKinney (1994), methanogenic activity was inhibited when FAN concentration reached 150 mg/L – NH_3.

2.9 Measures to reduce the inhibitory effects of Ammonia on AD

Several measures have been employed to reduce the inhibitory effect of ammonia on anaerobic digestion. These include among others pH control, low operating temperature, co-digestion, C:N ratio control, and dilution of digester content with fresh water (distilled water).

2.9.1 C: N Ratio adjustment

Shanmugam and Horan (2009) conducted an experimental study on the AD of leather fleshing waste (LFW) containing high alkaline pH value of 11.4 and a low C:N ratio of 3.2. A low methane yield due to the fact the LFW had excess nitrogen as evident in the C:N ratio and the hydrolysis of the nitrogen, resulting in the formation of high ammonia concentration inhibitory to methanogenesis. The C:N ratio of the LFW was adjusted by blending it with MSW having a high C:N ratio. Various C:N ratios were examined ranging from 3.2 to 30. The study showed that LFW and MSW waste mixtures containing C:N ratios of 15 and 20 gave the highest cumulative biogas production compared with other C:N ratios examined as shown in Figure 2.10. This result conformed with the study conducted by Sievers and Brune (1978) who reported an optimum C:N ratio of 19.9.

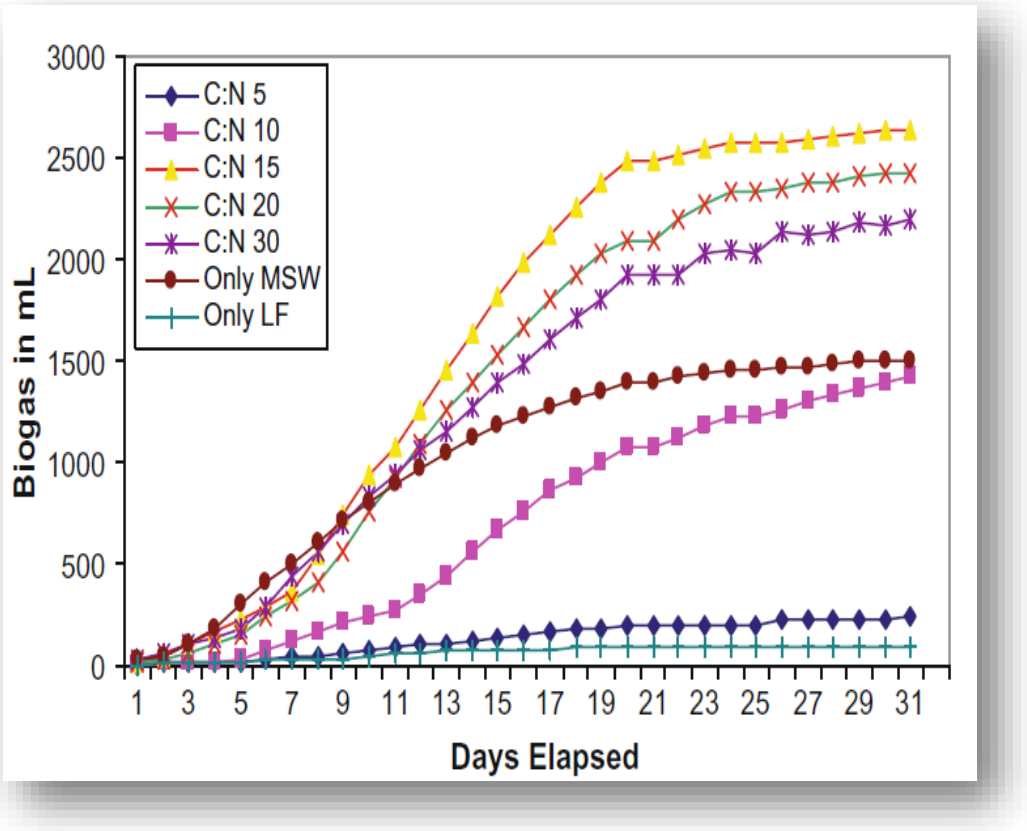

Figure 2.10. Cumulative biogas yield at different C:N ratio of LFW mixed with MSW (Shanmugam and Horan, 2009).

LFW and MSW waste mixtures containing low C:N ratios produced high concentrations of ammonia up to 4289 mg/L as shown in Figure 2.11 and resulted in high alkalinity and pH of 34,020 mg/L and 11.4 respectively. On the other hand, LFW and MSW waste mixtures producing the highest biogas yielded low concentrations of both ammonia and alkalinity 1736 and 8970 mg/L, respectively.

When optimised, the C:N ratio and co-digestion of wastes from different streams can be the least expensive and easiest to implement strategy for reducing the inhibitory effect of ammonia in AD (Shanmugam and Horan, 2009).

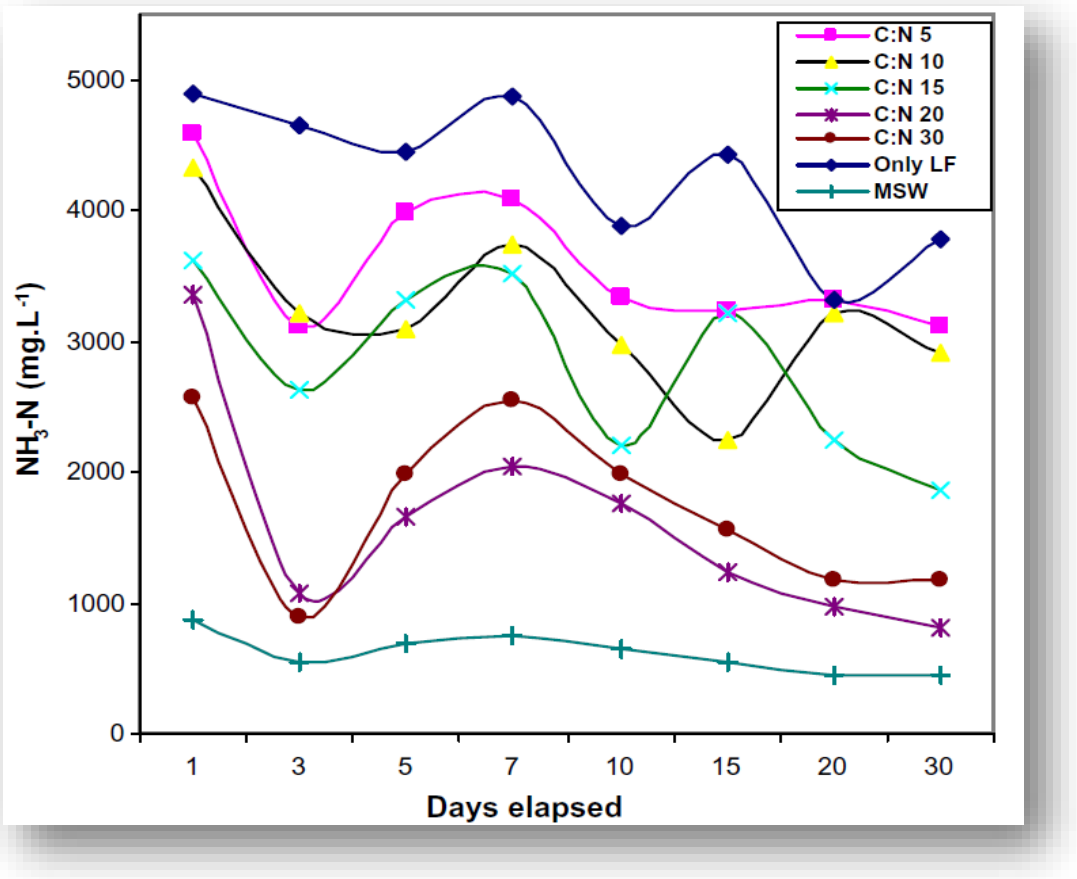

Figure 2.11. NH_3–N levels at different C:N ratio of mixed LF and MSW(Shanmugam and Horan, 2009).

2.9.2 Dilution of reactor content

Kayhanian (1994) examined the possibility of reducing the inhibitory effect of high TAN concentrations in a high-solids anaerobic digester by diluting the reactor content. The dilution of reactor content was carried out to examine whether the digestate would dilute similarly as a simple solution by comparing laboratory and pilot scale tests, as illustrated in Figure 2.12. As illustrated in Figure 2.12, the result showed that the digestate diluted similarly as a simple solution in the laboratory and as a digestate in a pilot scale digester. The dilution factor for the amount of dilution water required to dilute a digestate containing inhibitory TAN concentration can be determined at a given TAN concentration using Figure 2.12.

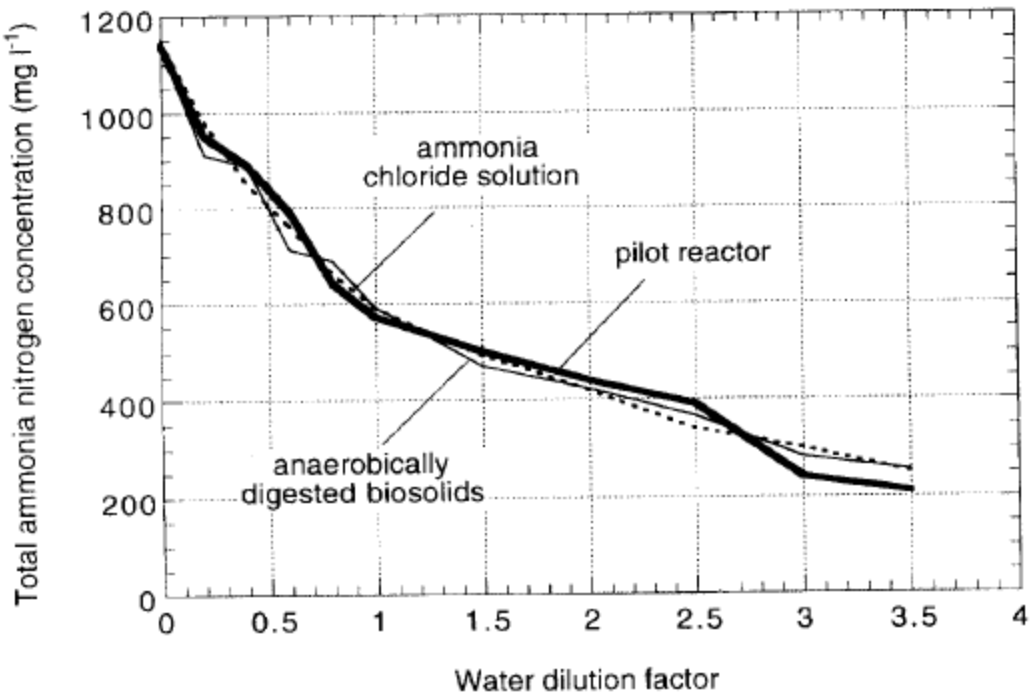

Figure 2.12: Comparism of water dilution of ammonia chloride solution, anaerobically digested biosolids, and digestate from the pilot scale reactor (Kayhanian, 1994).

While dilution method was effective for Kayhanian (1994), the author also reported that dilution of digestate caused a problem in the short term. This was because dilution caused a shift from a high-solids to a low-solids process. In order to shift to low-solids process, fewer solids were either fed to the digester and/or more digestate was removed per day, causing a reduction of the organic loading rate (OLR) or reduction of mass retention time (MRT). The reduction of the OLR and/or MRT caused a significant decrease in the digester's gas production and waste stabilization capacity and increased dewatering costs. However, gas production rates reached normal level when the OLR containing higher solids loading rate (SLR) was fed to the digester to return the digester solids content to the normal operational level, once the digester's TAN concentration reduced to less inhibitory level.

2.9.3 Acclimation of Methanogens to High Ammonia Concentration

The tolerance of microbes, most importantly the methanogens to high ammonia concentrations can be improved by acclimating the microbes to high ammonia concentrations. This can be achieved through a step-wise increase of ammonia concentration. Melbinger and Donnellon (1967) investigated the reason for the drop in biogas production capacity of one of two high-rate digesters operating under similar organic loading. Prior to the failure of the other digester, the two digesters had average biogas production of 10,640 m^3/day. After the failure of digester I, digester II had an excess biogas production of 6,400 m^3/day than digester I. The result of the investigation showed that at failure, the concentration of ammonia nitrogen in digester I had reached 1,900 mg/L compared with digester II containing ammonia nitrogen concentration of 1,700 mg/L. This conforms to the study of Alberton (1961) which placed the ammonia concentration threshold for the failure point of digesters between 1,700-1,800 mg/L. Further investigations by Melbinger and Donnellon (1967) revealed that the toxic effect of ammonia on the performance of the high-rate digesters can be greatly reduced by acclimating the methane-forming bacteria to ammonia, through gradual loading of TAN in increasing concentrations. As a result of the acclimation, the high-rate digesters operated without failure with TAN concentration up to 2,700 mg/L. Melbinger and Donnellon (1967) concluded that "it is *possible to operate at considerably higher levels; apparently, it is not the concentration of ammonia nitrogen that is critical but the rate at which it is produced.*"

2.10. Summary and Research Gap

While several studies have been conducted to examine and control these inhibitors more research is required to examine the effects of these inhibitors on the AD of OFMSW. This is because OFMSW, industrial wastewater, livestock and poultry wastes have different characteristics. For instance, poultry wastes have high ammonia concentration and the anaerobic digestion of such waste will be easily inhibited. Several studies have

been carried out to examine the inhibitory effects of ammonia on AD of wastewater however; there is limited information about the inhibitory effect of ammonia on AD of OFMSW (Yenigün and Demirel, 2013). The wide range of the reported inhibitory ammonia concentrations and the significance of ammonia toxicity on mesophilic bacteria necessitate further studies on the inhibitory effects of ammonia on AD of OFMSW.

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Chapter 3

3 Materials and Methods

3.1 Introduction

The experimental study in this research was carried out using 500 mL Kimax® glass bottles capped with butyl rubber stoppers, with sampling ports on the rubber stoppers to enhance easy sampling and to prevent the interference of oxygen on the AD process during sampling. Two sets of experiments were carried out with each set comprising different phases as explained in the latter part of this chapter. The first set of experiments involved the investigation of the inhibitory effects of ammonia on the AD of synthetic OFMSW (SW) only while the second set of experiments involved the investigation of the inhibitory effects of ammonia on the AD SW mixed with leachate (SW+L). Phase 1 of the SW experiments was biochemical methane potential (BMP), phase 2 was gradual TAN loading while phase 3 was semi-continuous reactor phase. For (SW+L), the two phases examined were the BMP phase (phase 1) and the gradual TAN loading phase (phase 2). The phases are explained in more details in the experimental set up section of the chapter.

The total working volume of the reactor contents was 300 mL in both sets of experiments, except for the semi-control reactor phase where the working volume was 200 mL. The working volumes (BMP phases) are shown in Tables 3.1 (a) and (b). For SW, the inhibitory effect of various TAN concentrations ranging from 2,500, 5,000, 7,500 to 10,000 mg/L was investigated under different pH levels of 7.5, 8.0 and 8.5. For the SW experiment, the initial TAN concentration in the control reactors was 350 mg/L while for (SW+L) experiment, the initial TAN concentration was 650 mg/L in the control reactors. The mass of NH_4Cl required to achieve the desired TAN concentration was determined by mass balance, considering initial the TAN concentrations in the reactors. For (SW+L), effect of various TAN concentrations of 7,500 and 10,000 mg/L was investigated under different pH levels of 7.5 and 8.5

Table 3.1 (a): Reactor Configuration for SW – Phase 1

	NH_4Cl added (g)
Control (30 g OFMSW + ≈ 120 mL DW + 120 mL Inoculums + 2 g buffer)	-
Control + 2500 mg/L TAN	2.46
Control + 5000 mg/L TAN	5.33
Control + 7500 mg/L TAN	8.20
Control + 10,000 mg/L TAN	11.06

Table 3.1 (b): Reactor Configuration for (SW+L) – Phase I

	NH_4Cl added (g)
Control (30 g OFMSW + 80 mL Leachate + ≈ 120 mL DW + 40 mL Inoculums + 2 g buffer)	-
Control + 7500 mg/L TAN	7.85
Control + 10,00 mg/L TAN	10.72

The operating temperature for all the AD experiments carried out in this study was 35 ^oC, in the mesophilic temperature range. All tests were carried out in conformity to the guidelines provided in the standard code of practice. All reactors were run in duplicates throughout this study. The reactors were purged for about 2 minutes with nitrogen gas, sealed with the rubber stoppers and a layer of silicone was added all around the connecting areas between the glass and the stopper, stopper with tubes to ensure gas-tightness of the reactors. All reactors were set up in duplicates. Equal portions of $NaHCO_3$ and $KHCO_3$were added to achieve an alkalinity concentration of between 4000 and 6000 mg/L as $CaCO_3$. As circumstances required, 1N Hydrochloric Acid (1N HCl) solution and 5N Sodium Hydroxide (5N NaOH) solution were added to the bottles to adjust the pH to the desired value

3.2. Organic Fraction of Municipal Solid Waste (OFMSW)

The OFMSW used in this study was simulated using food components that are representative of a typical domestic kitchen and commercial kitchen food waste. The percentages of the different components of the OFMSW had standard compositions of protein, hydrocarbon, vegetable and fat in conformity with the Canadian Food Guide, as shown in Table 3.2 (Ara, 2012). In order to obtain consistent results, each set of simulated OFMSW was prepared weekly and the remaining simulated OFMSW was frozen up at a temperature of -4 ^{o}C, to prevent the likelihood of any fermentation. The carrots, cabbage, banana, and apple used were fresh. The ground beef was cooked for 30 minutes while the rice and pasta were cooked differently for 15 minutes each in a rice cooker. Prior to being used, the OFMSW was thoroughly mixed and blended to form a slurry having a particle size ranging from 1-2 mm, using a kitchen food processor.

Table 3.2: **Configuration of the simulated OFMSW used**

OFMSW Configuration	
Composition	**Percentage weight (% w/w)**
Carrot	11
Cooked rice	18
White cabbage	10
Cooked pasta	18
Banana	11
Dog food	11
Apple	11
Cooked ground beef	10
Total	100

3.3 Inoculums and Leachate

The inoculums used in this research was obtained from the effluent of the AD digester used for the AD of sludge at the Robert O. Pickard Environmental Center (ROPEC), Ottawa, Ontario, Canada. In order to keep the inoculums alive, the inoculums were kept in suspension by agitation in a New Brunswick Scientific Controlled Environment Incubator Shaker rotating at 86 revolutions per minute (rpm) at 35°C.

Prior to being used for batch experimental studies, the OFMSW and the inoculums were characterized as shown in Table 3.3 for COD, Volatile Solids (VS), alkalinity, and Total Solids (TS). All values are averages of duplicate runs.

Table 3.3: Properties of Inoculums and OFMSW

Properties	COD mg/L	pH	TAN mg/L	Alkalinity as CaCo3 mg/L	VFAs, mg/L	Total Solids (TS) (%)	Volatile solids (VS) (%)	Organic Solid (VS/TS) (%)
OFMSW	177,000	4.5	350	2,750	9600	16.62	15.90	96
Inoculums	11,050	6.9	876.5	14,600	7500	1.73	0.96	56

Mature landfill leachate, aged between 15-25 years was obtained from a municipal landfill in Ottawa was used in this study. The leachate was stored at a temperature of 4° C until used and it was incubated at 35 °C in a Shaker for 24 hours before being used. The leachate was characterized as shown in Table 3.4 below. All values are averages of duplicate runs.

Table 3.4: Properties of Leachate

Properties	COD mg/L	pH	TAN mg/L	Alkalinity ($CaCO_3$) mg/L	VFA (acetic acid) mg/L
Leachate	4425	9.0	1878	6725	549

3.4 Experimental Setup

3.4.1 SW – Phase 1

A total of thirty six (36) bottles were set up for the BMP phase, with the inoculums batch bottles, control batch bottles, and batch bottles with each TAN concentration set up in duplicates. The BMP bottles were set to the desired pH values of 7.5, 8.0 and 8.5.

3.4.2 SW – Phase 2

The second phase of the experiment was gradual TAN loading. In order to examine whether the tolerance of the bacteria to high ammonia concentration would improve by acclimating the microbes to high ammonia concentrations, TAN concentration was increased gradually at pH 7.5, 8.0 and 8.5 using the same reactor contents as those of the BMP phase. At the beginning, TAN concentration was raised to 1250 mg/L and further increased by 1250 mg/L weekly.

3.4.3 SW – Phase 3

The third phase of the experiment was semi-continuous batch phase. In order to examine the possibility of reducing the inhibitory effect of ammonia on AD, batch reactors at pH values of 8.0 and 8.5 containing initial TAN concentrations of 7500 mg/L and 10,000 mg/L were converted to semi-continuous reactors at the end of the experiment. The volume of digestate in these reactors was adjusted to working volume of 200 mL. A hole was made in the stopper on each reactor; and was used for feeding the reactor by attaching a 5-cm piece of tubing. The reactors were purged for about 2 minutes with nitrogen gas, sealed with the rubber stoppers and a layer of silicone was added all around the connecting areas between the glass and the stopper, stopper with tubes to ensure gas-tightness of the reactors. To simulate a semi-continuous reactor process, 3 g of the digestate containing high ammonia concentration(s) was replaced with fresh substrate every 4 days, 7 days and 15 days, as shown in Figure 3.1. A total of 8 semi-continuous batch bottles were set up for this phase.

Figure 3.1: Semi-continuous Batch Phase

3.4.4 (SW + L) – Phase 1

In phase 1, BMP tests were carried out using mesophilic anaerobically digested inoculums volume of 40 mL, 80ml of anaerobic landfill leachate, 30 g of OFMSW, TAN concentrations 7,500 and 10,000 mg/L. Equal portions of $NaHCO_3$ and $KHCO_3$ were added as buffer. The total working volume of the mixture was brought to 300 mL by the addition of DW. As circumstances required, 1N Hydrochloric Acid (1N HCl) solution and 5N Sodium Hydroxide (5N NaOH) solution were used to adjust the pH to the desired value. The inoculums BMP bottles, control BMP bottles, and BMP bottles containing 7,500 and 10,000 mg/L, TAN concentrations were set up in duplicates. A total of 20 BMP bottles were set up for this phase. The pH levels were 7.5 and 8.5.

3.4.5 (SW + L) – Phase 2

The phase 2 of the experiment was gradual TAN loading to examine the possibility of adapting thermophilic bacteria to high ammonia concentration. At the beginning, TAN concentration was raised to 1000 mg/L and further increased by 1000 mg/L weekly. A total of 4 batch reactors were set up for this phase. The pH levels were 7.5 and 8.5.

As mentioned above at the beginning of the experiment and after taking samples for analysis during the experiment, the BMP bottles were sparged with nitrogen gas for about two minutes each, to expel any entrapped oxygen in the bottles. In order to ensure that the pressure in each bottle was brought to equilibrium with atmospheric pressure at beginning of the experiment, a BD 21G1½ needle connected to a U-tube manometer was inserted into each bottle.

The daily biogas production of the inoculums batch bottles were subtracted from the daily biogas production of the control BMP bottles and from each BMP bottle containing added TAN concentrations. The AD process was initiated by incubating all the batch bottles at 35 °C in a New Brunswick Scientific Controlled Environment Incubator Shaker model G-25 (shown in Figure 3.2) at 80 rpm, and to the keep bacteria and substrate in suspension. The daily biogas production of all the batch bottles were monitored and recorded daily with a BD 21G½ needle connected to a u-tube manometer, until the biogas production rates were insignificant. The VFAs, TAN, and COD of the controls were measured at the beginning of the experiment to provide a basis for comparison and analysis, midway to the end of the experiment and at the end of the experiment. Biogas composition (nitrogen, methane and carbon dioxide) was analyzed during and after the end of test while pH was monitored every three days to ensure the pH values were in the desired ranges. The composition of the biogas production of the batch bottles were also analyzed in duplicates.

Figure 3.2: Reactors in New Brunswick Scientific Controlled Environment Incubator Shaker

3.5.1 Analytical Equipment and Methods

Biogas composition was analyzed using thermal conductivity gas chromatograph (series 400, Gow-Mac Instrument Co., USA). The operating temperatures of the column, detector, and injection port temperatures were 120^0C 130^0C, and 130^0C, respectively. The equipment has two sampling columns namely, column A and column B as shown in Figure 3.3. Column A measures the percentages of N_2, O_2 and CH_4while column B measures the percentages N_2, CH_4 and CO_2 in the biogas sample analyzed. Column B was used for the analysis of the biogas composition. The carrier gas used for the separation of the different biogas compositions was helium, operated at inflow rate of 30 mL/min. Biogas samples (0.5 mL) were taken in duplicates and injected into the column through the injection port.

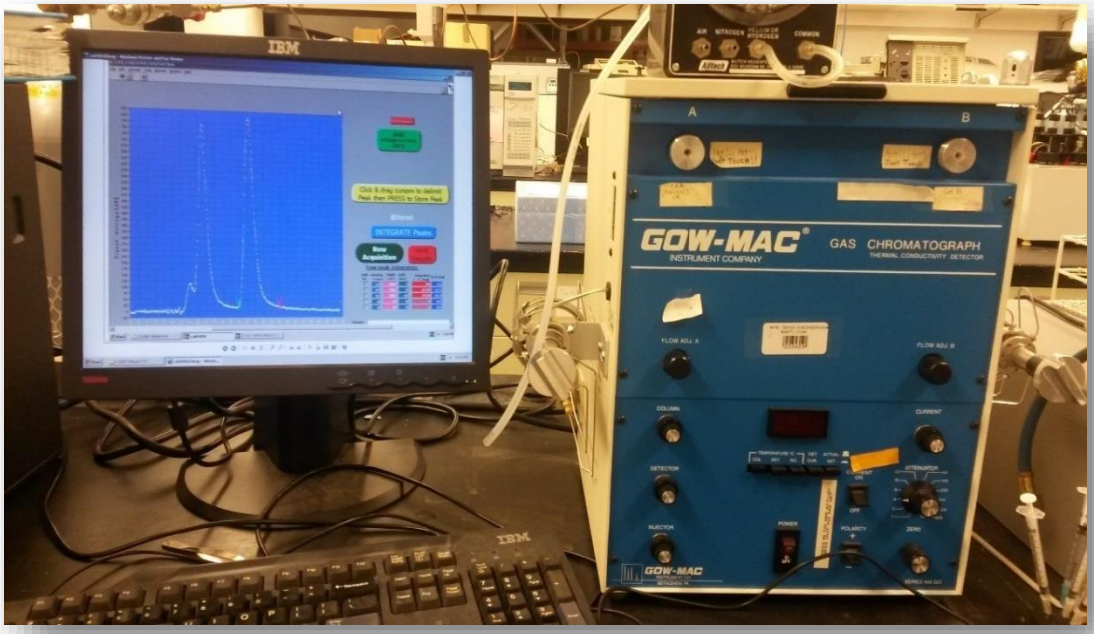

Figure 3.3: Thermal conductivity gas chromatograph (series 400, Gow-Mac Instrument Co., USA).

3.5.2 pH

Fisher Accumet model XL25 dual channel pH/ion meter (shown in Figure 3.4) equipped with a glass electrode was used to measure the pH of all inoculums, substrates (OFMSW) and batch samples. Before being used for measuring pH, electrode of the pH meter which was stored in a pH 7 buffer solution was removed, rinsed with distilled water, and dried with Kim wipes. Samples were thoroughly mixed in Pyrex beaker with the aid of a magnetic stirring rod and placed on a Thermix stirrer model 120MR. Sample pH values were then measured by inserting the electrode of the pH/ion meter into the samples. The electrode was returned to the buffer solution, rinsed again with distilled water and dried between each pH measurement.

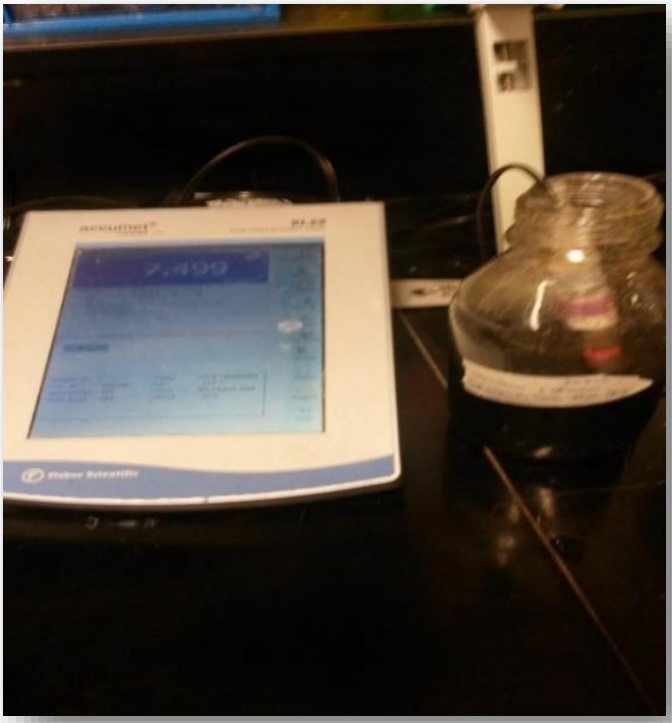

Figure 3.4: Fisher Accumet model XL25 dual channel pH/ion meter

3.5.3 COD, TAN, VFA and Alkalinity

Hach TNT plus™ 822 high range reagent vials (Method 8000) were used to measure the chemical oxygen demand (COD). Samples were first homogenized and well mixed to form a slurry paste using the Brinkmann Polytron PT 3000 homogenizer shown in Figure 3.5. Samples taken for COD analyses were diluted using serial dilution method until COD measured fell within the range of the 20 to 1500 mg/L COD high range reagent vials used. Sample CODs were measured according to the procedure shown in Appendix A 1. The digital reactor block RB200 was used to heat and digest the Hach TNT 822 test vials for 2 hours as shown in Figure 3.6.

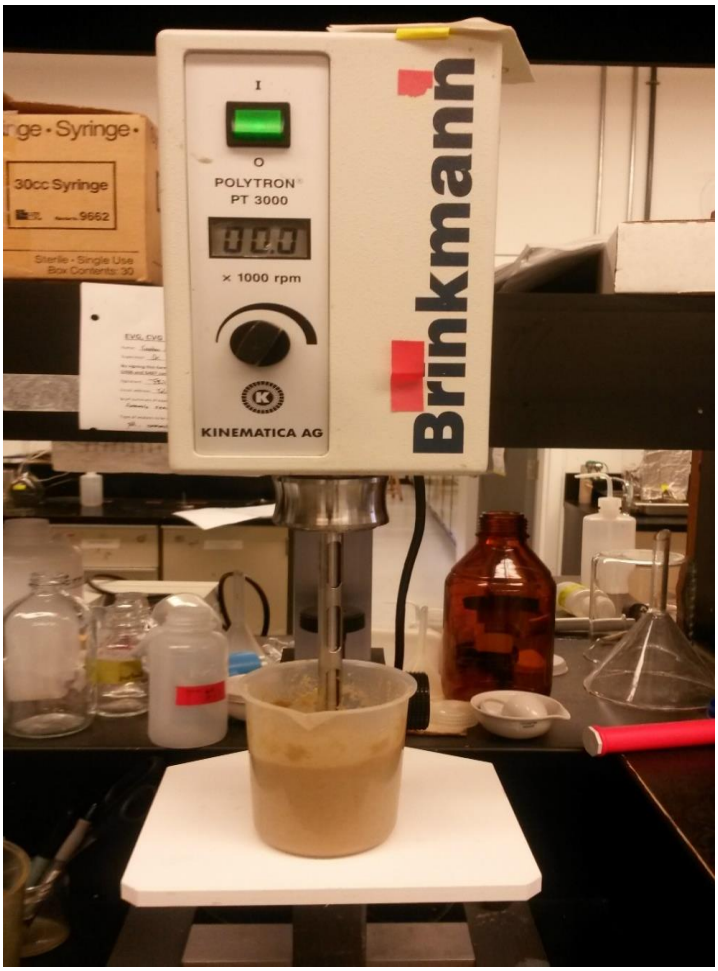

Figure 3.5: BrinkmannPolytron PT 3000 Homogenizer

Prior to the analyses of TAN, VFA and total alkalinity, samples were centrifuged in a Thermoscientific Sorvall Legend T+ model centrifuge at 10,000 rpm for 30 minutes. Once centrifuged, the supernatant was poured onto and filtered through filters with nominal pore size of 0.45 µm filters, using a Fisher Scientific pump (Wilkinson, 2011). TAN was measured with the use of TNT plus™ 832 reagent vials (Method 10205, Hach, USA). Total alkalinity determination was carried out with using TNTplus™870 reagent vials with alkalinity measuring range of 25 to 400 mg/L $CaCO_3$ (Method 10239, Hach USA). VFA was measured as acetic acids using TNT plus™ 872 with measuring range of 50 to 2,500 mg/L Acetic Acid. The concentrations of the COD, TAN, VFA and total alkalinity were measured using the UV-VIS spectrophotometer shown in Figure 3.7.

Figure 3.6: RB200: Digital Reactor Block

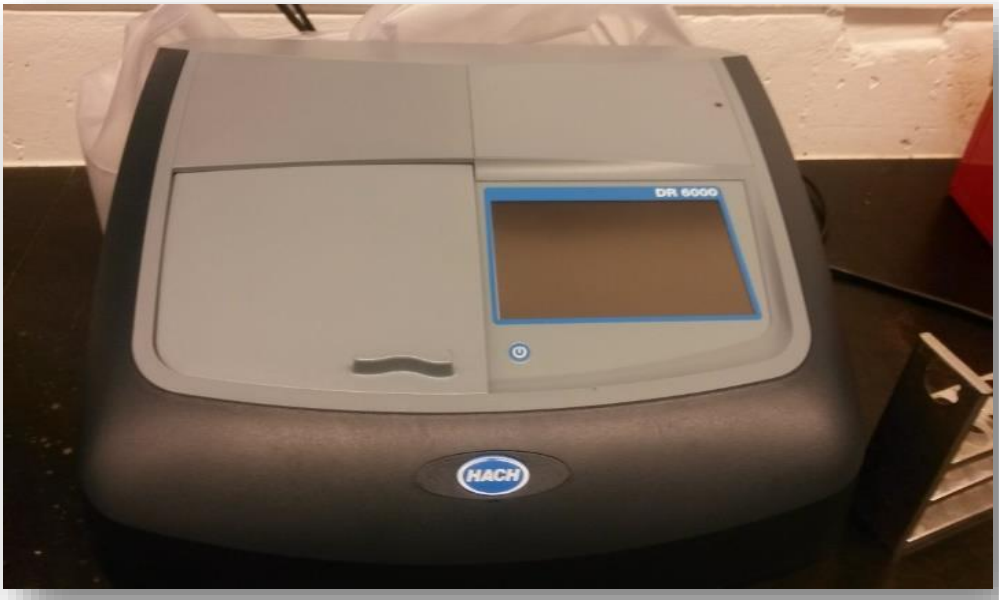

Figure 3.7: UV-VIS Spectrophotometer

3.5.4 Total and Volatile Solids

The analysis of the VS and TS was carried out using the Standard Method 2540G. Prior to TS and VS analyses, the aluminium weighing pans used were placed in a Precision mechanical convection oven model 23 maintained at 105 degrees Celsius. The pans were placed in desiccators to cool completely for about 10 minutes (Wilkinson, 2011). The mass of each aluminium weighing pan was measured on a balance and the mass was recorded as (A). Each thoroughly mixed sample was poured into the aluminium weighing pan and the combined mass of the aluminium weighing pan and the sample was recorded as (B). The aluminium weighing pan containing the sample was heated in Precision mechanical convection oven at 105 ^{o}C for 24 hours. After drying, the sample was placed in a desiccator for 1 hour. After 1 hour, the samples were weighed and the new mass (C) was recorded. After being weighed, the oven-dried samples were placed in a muffle furnace operated at 550 ^{o}C and incinerated for 1 hour. After 1 hour, the samples were placed in desiccators to cool for 1 hour. Finally, the mass of the incinerated sample was weighed and recorded as (D). All samples were analyzed in duplicates. The TS and VS were determined using following Equations (3) and (4) below:

$$\%\ \text{Total Solids (TS)} = \frac{(C - A)}{(B - A)} * 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (3)}$$

$$\%\ \text{Volatile Solids (VS)} = \frac{(C - D)}{(B - A)} * 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (4)}$$

where A = Mass of empty aluminium pan (g); B = mass of the aluminium weighing pan plus sample (g); C = mass of the aluminium weighing pan plus sample after drying at 105 ^{o}C for 24 hours (g); D = mass of the aluminium weighing pan plus sample after incineration at 550^{o}C for 1 hour (g).

3.5.5 Biogas Measurement

In order to measure the daily biogas production, a BD 21G1½ needle connected to a U-tube manometer (show in Appendix C) was inserted into each bottle to release the biogas.

Before inserting the needle in the reactors, the water levels in the u-tube manometer are equal and in equilibrium with the atmospheric pressure as shown in the schematic diagram in Figure 3.8.

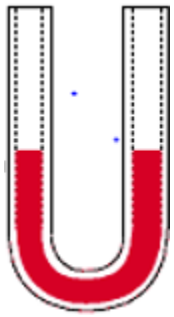

Figure 3.8: U-tube manometer section showing the initial water levels at equilibrium with atmospheric pressure

As the needle is inserted into the reactors to release the biogas, air pressure from the released biogas pushes the water down one side of the manometer and up the other side. The difference in height (H) is found by subtracting the initial height from the final height. The volume of the biogas is then determined using the formula for the volume of a cylindrical section as describe below:

$$V = \frac{\pi D^2 H}{4} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (5)}$$

where V = volume of biogas (cm^3); D = diameter of u-tube = 2.54 cm; H = difference between final height and the initial height of water level in the reference tube, after the release of biogas into the u-tube; $\pi = 3.142$. Note: 1 cm^3 = 1 mL

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Chapter 4

TECHNICAL PAPER I

The Toxicity Effects of Ammonia on Anaerobic Digestion of Synthetic Organic Fraction of Municipal Solid Waste

Akinwumi Abiodun Akindele, Majid Sartaj

Abstract

The effect of ammonia on the anaerobic digestion of synthetic organic fraction of municipal solid waste (OFMSW) was examined under different total ammonia nitrogen (TAN) concentrations of 2500 mg/L, 5000 mg/L, 7500 mg/L, and 10,000 mg/L. The study was conducted at pH 7.5, 8.0 and 8.5. Results obtained from this study confirmed that ammonia is inhibitory to methanogenic activity. Reduction in Cumulative Biogas Production (CBP) compared with control reactors was as much as 43 %, 64 % and 77 % in reactors containing 7500 mg/L TAN at pH 7.5, pH 8.0 and pH 8.5. CBP reduced to 80-85 % in reactors containing 10,000 mg/L TAN across all the pH ranges examined. The operating pH influenced the level of toxicity and the composition of TAN. At high pH (i.e. 8.5), FAN component of TAN was about 26 % and was inhibitory to the methanogens. Also, replacing 3g of digestate containing high TAN concentrations of 7500 mg/L and 10,000 mg/L with 3 g fresh enhanced the activity of the mesophilic bacteria. Lastly, results also showed that mesophilic bacteria could be adapted to a TAN concentration of about 5000 mg/L at pH 7.5 through gradual TAN loading.

Keywords: total ammonia nitrogen, free ammonia nitrogen, organic fraction of municipal solid waste

4.1 Introduction

Municipal Solid Waste (MSW) generation rate has been increasing with increasing world population and is therefore a global concern (Staley and Barlaz, 2009). It was reported that global production of MSW is projected to reach 3 billion metric tonnes per year by 2025 (Ara et al. 2014). In the same vein, the annual global cost of waste disposal is expected to increase from $ 205 billion to $ 375 billion (World Bank, 2015).

Anaerobic digestion (AD) is the most widely used process for the treatment of wastewater containing high organic content and can be an acceptable alternative to the current disposal strategies for organic fraction of MSW (OFMSW) (Ara et al, 2015). AD as a sustainable process converts the organic matter present in MSW into energy resource and reduces the volume of landfilled wastes (El Hadj et al., 2009). However, the AD process is susceptible to influence of pH, sulphate reducing bacteria, temperature and high ammonia concentration which accumulates during the process (Angelidaki and Ahring, 1994; Hansen et al., 1999; Shanmugam and Horan 2009; Nair et al., 2014). Ammonia is the by-product resulting from the AD of proteins present in OFMSW and it has been reported as a major inhibitor of microbial activities during AD process (Jung et al., 2004).

Ammonia exists in two forms as ionized ammonia or ammonium (NH_4^+) and unionized ammonia or free ammonia (NH_3). The combination of these two forms of ammonia is expressed as total ammonia nitrogen (TAN). The percentages of these two forms of ammonia in TAN vary with temperature and pH (Ding and Sartaj, 2015). Also, previous research has shown that toxicity in aqueous solutions is mainly due to NH_3 form (Dong and Sartaj, 2015).

The inhibitory effects of ammonia on the AD of high and low organic matters at various operating temperatures and pH values have been reported (Pfeffer, 1974: Braun et al., 1981; Webb, & Hawkes 1985; Hashimoto, 1986; Blomgren et al., 1990; Angelidaki & Ahring, 1993). Kayhanian (1994) studied the impact of high ammonia concentrations on the biodegradation of the OFMSW using varying ammonia concentrations. It was reported that an instant inhibition occurred at 1000 mg/L ammonia concentration, followed by a 50% inhibition and a complete bioreactor failure occurring at 1500, and

2500 mg/L ammonia concentrations respectively. Using various ammonia concentrations of 48.8, 73.8, 98.8, 148.8, 248.8, 448.8, and 848.8 mg/L, Sossa et al. (2004) studied the inhibitory effects of ammonia on anaerobic film enriched by methylaminotrophic methane producing Archaea. The authors reported that maximum methanogenic activity occurred at 48.8 mg/L ammonia concentration. However, methanogenic activity was significantly inhibited at 848.8 mg/L ammonia concentration.

When present in high concentration, ammonium can directly inhibit biogas production during AD however; free ammonia nitrogen (FAN) has been reported as the major inhibitor of AD process at high pH and temperature. This is because FAN has the capability of penetrating through the cell membrane of bacteria (Sung & Liu 2003). McCarty & McKinney (1991) reported that FAN was more responsible for AD process inhibition than TAN. Bhattacharya and Parkin (1989) reported a threshold FAN concentration of 55 ± 11 mg/L–NH_3 while Braun et al., 1981 reported a slightly higher FAN concentration threshold of 80 mg/L–NH_3. Methanogenic activity was inhibited when FAN concentration reached 150 mg/L–NH_3 (McCarty and McKinney, 1991)

The two main groups of methanogens involved in AD are acetoclastic methanogens and hydrogenotrophic methanogens, each accounting for 70% and 30% of methane production respectively (Speece, 1983). Acetoclastic methanogens consume acetate or acetic acids to produce methane while hydrogenotrophic methanogens consume hydrogen and carbon dioxide to produce methane. Varying effects of FAN on acetoclastic methanogens and hydrogenotrophic methanogens have been reported (Koster & Lettinga, 1984; Wiegant & Zeeman, 1986; Angelidaki et al., 1993). The reason for the various thresholds of inhibitory ammonia concentration reported can be attributed to the various initial ammonia concentrations examined, process temperature, operating pH, organic loading rate and acclimation of inoculums. While several studies have been carried out to examine the inhibitory effects of ammonia on AD of wastewater, there is only limited information about the inhibitory effects of ammonia on AD of OFMSW (Yenigün and Demirel, 2013). This research was focused on the toxicity effects of ammonia on AD of the OFMSW and research objectives include:

- To examine the possible inhibitory effect(s) of different ammonia concentrations on the mesophilic AD of the OFMSW, under different operating pH levels of 7.5, 8.0 and 8.5, at similar operating temperature of 35 ºC.
- To examine the possibility of reducing the inhibitory effect(s) of ammonia on AD by acclimating the bacteria to high ammonia concentrations, through gradual loading of influent ammonia concentrations.
- To examine the possibility of running the reactors under the most severe ammonia toxicity in the semi-continuous AD reactor by replacing the digestate containing high ammonia concentration(s) with fresh substrate.

4.2 Materials and Methodology

This experimental study was carried out in three phases, the Biochemical Methane Potential (BMP) with instantaneous addition of TAN (phase 1), the BMP with gradual TAN loading (phase 2) and the semi-continuous batch reactors (phase 3). All tests were carried using 500 mL Kimax® glass bottles capped with butyl rubber stoppers in duplicates.

For each set of BMP arrangements (at pH of 7.5, 8.0 and 8.5 each) in phase 1, mesophilic anaerobically digested inoculums volume of 120 mL, 30 g of synthetic OFMSW, and various TAN concentrations ranging from 2,500, 5,000, 7,500 to 10,000 mg/L were added to the reactors. The total working volume of the mixture was brought to 300 mL by the addition of distilled water (DW). A total of 36 BMP bottles were set up for this experiment, with the inoculums BMP bottles, control BMP bottles, and BMP bottles with each TAN concentration set up in duplicates.

The second phase of the experiment was gradual TAN loading. In order to examine whether the tolerance of the bacteria to high ammonia concentration would improve by acclimating the microbes to high ammonia concentrations, TAN concentration was increased gradually (at pH 7.5, 8.0 and 8.5), using the same reactor contents as those of the phase 1. At the beginning, TAN concentration was raised to 1250 mg/L and further increased by 1250 mg/L weekly, until it reached 10,500 mg/L on the 8th week. A total of 6 batch bottles were set up for this phase.

The third phase of the experiment was Semi-continuous mode. In order to examine the possibility of operating under continuous mode and assessthe inhibitory effect of ammonia on AD, batch reactors at pH values of 8.0 and 8.5 containing initial TAN concentrations of 7500 mg/L and 10,000 mg/L were converted to semi-continuous reactors at the end of the phase 1. The volume of digestate in these reactors was adjusted to working volume of 200 mL. A hole was made in the stopper on each reactor; and was used for feeding the reactor by attaching a 5-cm piece of tubing. To simulate a semi-continuous reactor process, 3 g of the digestate containing high ammonia concentration(s) was replaced with fresh substrate, at the end of every 4 days, 7 days and 15 days.

For all 3 phases the reactors were purged for about 2 minutes with nitrogen gas, sealed with the rubber stoppers and a layer of silicone was added all around the connecting areas between the glass and the stopper, ensure gas-tightness of the reactors. Equal portions of $NaHCO_3$ and $KHCO_3$ were added as buffer. As circumstances required, 1N Hydrochloric Acid (1N HCl) solution and 5N Sodium Hydroxide (5N NaOH) solution were used to adjust the pH to the desired value. The daily biogas production of the inoculums BMP bottles was subtracted from the daily biogas production of the control BMP bottles and from BMP bottles containing TAN concentrations of 2,500, 5,000, 7,500 to 10,000 mg/L when necessary.

The OFMSW used in this study was synthesized using food components that are representative of a typical domestic kitchen and commercial kitchen food waste. The percentages of the different components of the OFMSW had standard compositions of protein, hydrocarbon, vegetable and fat in conformity with the Canadian Food Guide, as shown in Table 4.1 (Ara, 2012). In order to obtain consistent results, each set of simulated OFMSW was prepared weekly and the remaining modeled OFMSW was frozen up at a temperature of -4 ^{o}C, to prevent the likelihood of any fermentation. The carrots, cabbage, banana, and apple used were fresh. The ground beef was cooked for 30 mins while the rice and pasta were cooked differently for 15 minutes each. Prior to being used, the OFMSW was thoroughly mixed and blended to form a slurry having a particle size ranging from 1-2 mm, using a kitchen food processor.

Table 4.1: Configuration of the simulated OFMSW used

OFMSW Configuration	
Composition	**Percentage weight (% w/w)**
Carrot	11
Cooked rice	18
White cabbage	10
Cooked pasta	18
Banana	11
Dog food	11
Apple	11
Cooked ground beef	10
Total	100

The inoculums used in this research was obtained from the effluent of the AD digester used for the AD of sludge at the Robert O. Pickard Environmental Center (ROPEC), Ottawa, Ontario, Canada. In order to keep the inoculums alive, the inoculums was kept in suspension by agitation in a New Brunswick Scientific Controlled Environment Incubator Shaker rotating at 86 revolutions per minute (rpm), at 35°C. Prior to being used for experimental studies, the OFMSW and the inoculums were characterized as shown in Table 4.2 for COD, Volatile Solids (VS), Alkalinity, and Total Solids (TS). All values are averages of duplicate runs.

Table 4.2: Properties of inoculums and OFMSW

Properties	**COD mg/L**	**pH**	**TAN mg/L**	**Alkalinity as CaCo3 mg/L**	**VFAs (Acetic acid) mg/L**	**Total Solids (TS) (%)**	**Volatile solids (VS) (%)**	**Organic Solid (VS/TS) (%)**
OFMSW	177,000	4.5	350	2,750	9600	16.62	15.90	96
Inoculums	11,050	6.9	876.5	14,600	7500	1.73	0.96	56

4.2.1 Analytical Methods and Equipment

Hach TNT plus™ 822 high range reagent vials (Method 8000) were used to measure the chemical oxygen demand (COD). Samples were first homogenized and well mixed to form a slurry paste using the Brinkmann Polytron PT 3000 homogenizer before COD concentrations were measured. TAN was measured using the salicylate method, with the use of TNT plus™ 832 reagent vials (Method 10205, Hach, USA). Total alkalinity determination was carried out according colorimetric method using TNT plus™ 870 reagent vials with alkalinity measuring range of 25 to 400 mg/L $CaCO_3$ (Method 10239, Hach USA). VFA was measured as acetic acids using TNT plus™ 872 with measuring range of 50 to 2,500 mg/L Acetic Acid. Prior to the analyses of TAN, VFA and total alkalinity, samples were centrifuged in a Thermoscientific Sorvall Legend T+ model centrifuge at 10,000 rpm for 30 minutes. Once centrifuged, the supernatant was poured onto and filtered through filters with nominal pore size of 0.45 µm filters using a Fisher Scientific pump (Wilkinson, 2011).

The daily biogas production was monitored and recorded daily with a BD 21G½ needle connected to a u-tube manometer, until the biogas production rates were insignificant. Biogas composition of the biogas produced was analyzed using thermal conductivity gas chromatograph (series 400, Gow-Mac Instrument Co., USA). Fisher Accumet model XL25 dual channel pH/ion meter equipped with a glass electrode was used to measure the pH of all inoculums, substrates (OFMSW) and BMP samples. The carrier gas used for the separation of the different biogas compositions was helium, operated at inflow rate of 30 mL/min. Biogas samples (0.5 mL) were taken in duplicates and injected into the column through the injection port. Spectrophotometer was used to measure the COD, VFA, TAN, and Total Alkalinity concentrations.

4.3 Results and Discussions

The results of the various batch tests conducted showed that the TAN could have significant inhibitory effects on the AD of the OFMSW. This is particularly observable in

the difference between the amount of biogas produced by the control reactors and reactors containing various TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L under identical pH values of 7.5, 8.0 and 8.5, in phase 1 of the experiment. Similar trends were also observed in phases 2 and 3.

Also, the results of the experimental studies conducted confirmed that pH plays a major role on the severity of the inhibitory effect of TAN on the AD of the OFMSW. However, the effect of pH was not very significant on control batch reactors. As discussed earlier, the percentage of FAN in TAN varies with pH and temperature. Under identical temperature and TAN concentrations and different pH levels, the FAN content of TAN varied significantly and this had a resultant effect on the variation in the biogas produced by batch reactors containing similar TAN concentrations.

Experimental studies on the effect of gradual TAN loading indicated that the gradual increase of TAN allowed methanogenic bacteria to become acclimated to high TAN concentrations as much as 5000 mg/L at pH 7.5. Lastly, results from semi-continuous reactors indicated that replacing equal amount of digestate containing high TAN concentration with equal amount of OFMSW fresh substrate enhanced the performance of the reactors.

4.3.1 Phase 1 (SW)

4.3.1.1 The Effect of TAN at pH 7.5

Figure 4.1 shows the average of the cumulative biogas productions (CBP) from control BMP reactors (CB-reactors) and reactors containing TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L, under similar operating pH of 7.5. The results of the study show that TAN inhibits methanogenic activity and the extent of waste biodegradation even at the lowest TAN concentration studied, with increasing effect with increasing TAN concentrations. On the first day of the experiment, BMP reactors containing 7,500 mg/L and 10,000 mg/L had no biogas production. As seen in Figure 4.1, CB-reactors produced CBP of 2843 mL while BMP reactors containing TAN

concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L produced 2627 mL, 2363 mL, 1616 mL, and 560 mL CBP respectively, within 62 days of incubation.

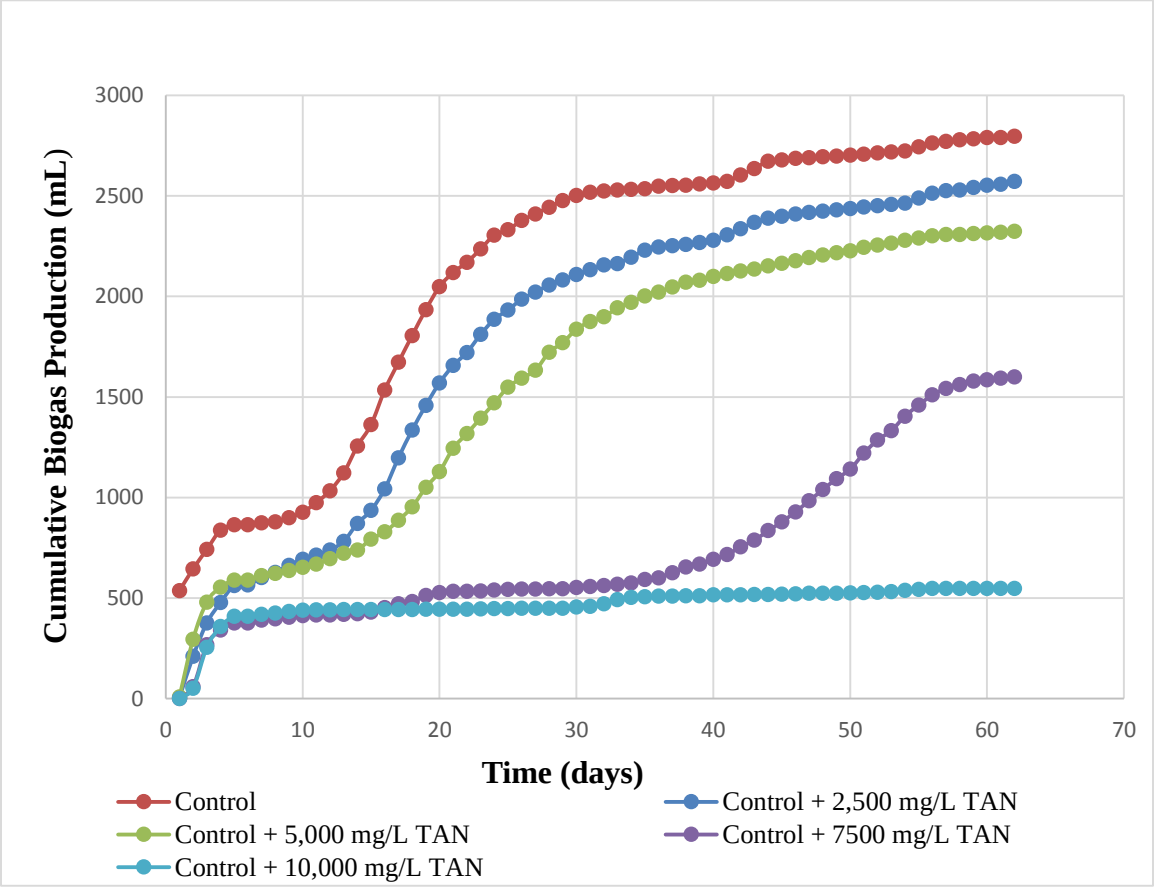
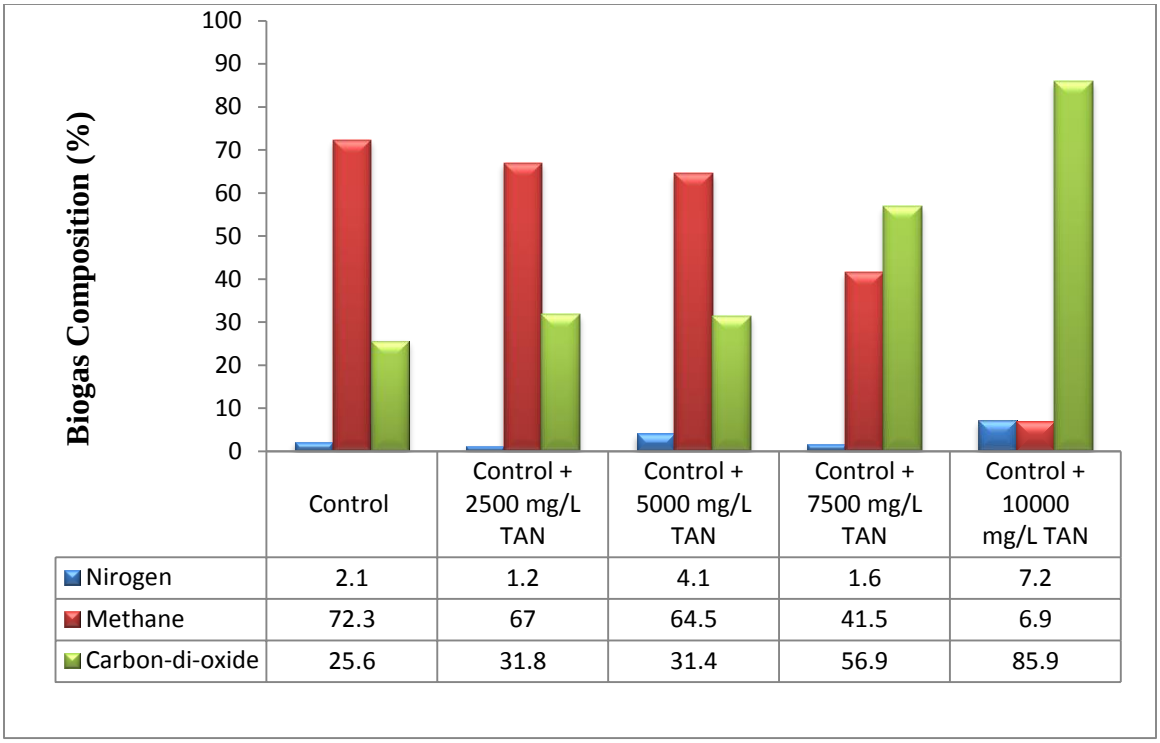


Figure 4.1: Biogas production from BMP reactors under different TAN concentrations and similar operating pH of 7.5 (phase 1)

BMP reactors containing TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L had 7%, 17%, 43% and 80% reduction in CBP respectively, compared with CB-reactors. Biogas composition analyses (shown in Figure 4.2) carried out during day 30 of the incubation period showed that the percentage of methane in the biogas produced reduced significantly as the concentration of TAN increased. This is consistent with the study of Sawayama et al., (2004) and Strik et al., (2006).

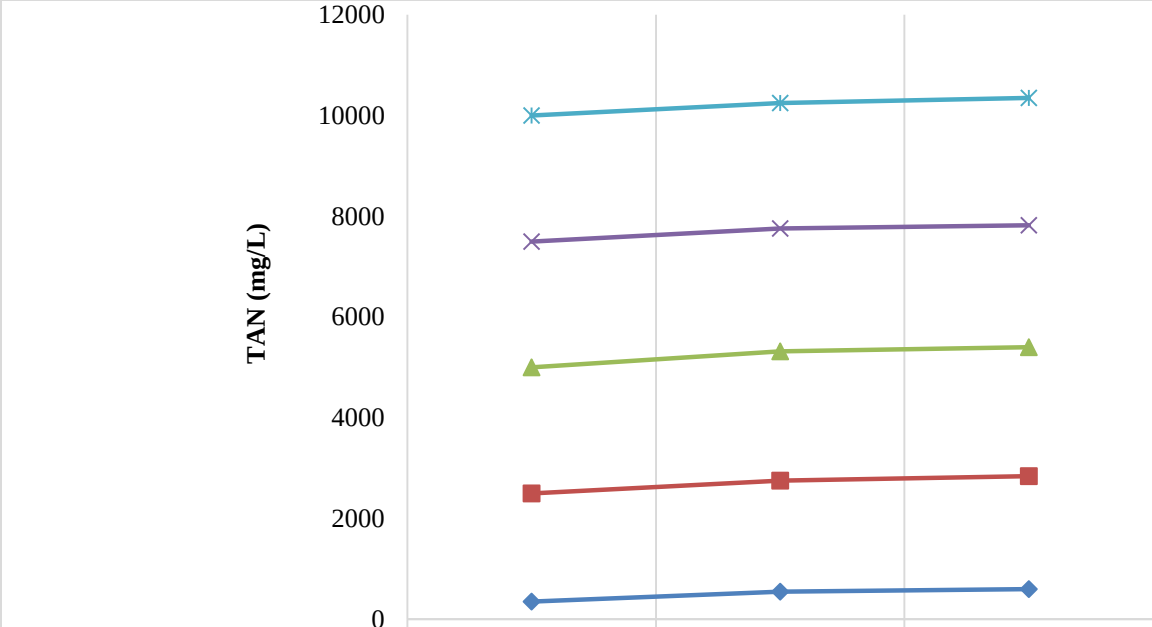


	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
Nirogen	2.1	1.2	4.1	1.6	7.2
Methane	72.3	67	64.5	41.5	6.9
Carbon-di-oxide	25.6	31.8	31.4	56.9	85.9

Figure 4.2: biogas composition from BMP reactors under different TAN concentrations and similar operating pH of 7.5 (phase 1)

It can be observed from Figure 4.2 that ammonia has inhibitory effect on methanogenic bacteria, especially at higher TAN concentrations. Reactors containing 10,000 mg/L TAN had the least methane percentage (6.9%). At the end of the incubation period of 62 days the biogas composition was also analyzed. Methane percentage had reduced to as low as 0% in reactors containing 10,000 mg/L while nitrogen accounted for as much as 90%, indicating a complete methanogenic inhibition.

The analysis of the TAN concentrations in the reactors at the beginning of the experiment, after 30 days and after 62 days is presented in Figure 4.3. For the CB-reactors, the initial TAN concentration was 350 mg/L. It can be observed from Figure 4.3 that after 30 days and 62 days of incubation, the TAN concentrations in all the reactors had increased.

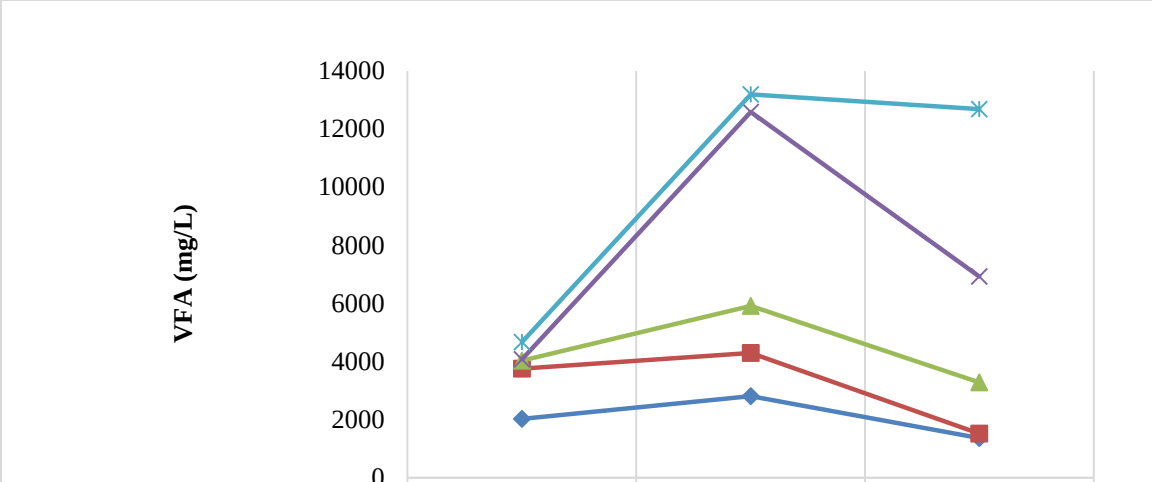


	TAN (mg/L) at Start	TAN (mg/L) at day 30	TAN (mg/L) at day 60
Control	350	550	600
Control + 2500 mg/L TAN	2500	2750	2840
Control + 5000 mg/L TAN	5000	5320	5400
Control + 7500 mg/L TAN	7500	7760	7820
Control + 10000 mg/L TAN	10000	10250	10350

Figure 4.3: TAN concentration at the beginning, mid-way and end of incubation period, at operating pH of 7.5 (phase 1)

At the end of the incubation period, TAN concentration increased from 350 mg/L to 550 mg/L for CB reactors while for other reactors, TAN concentrations increased from 2500 mg/L to 2840mg/L, 5000 mg/L to 5400 mg/L, 7500 mg/L to 7820 mg/L, 10,000 mg/L to 10,350 mg/L. This increase can be attributed to the biodegradation of the proteins in the waste mixture (Nair et al., 2014).

Figure 4.4 shows the analysis of the VFA at the beginning, midway and end of the digestion period. The VFA was measured as acetic acid. The analysis of the VFA on day 30 showed that CB-reactors had a VFA concentration of 2810 mg/L while reactors containing 2,500 mg/L, 5,000 mg/L, 7,500 mg/L and 10,000 mg/L of TAN had VFA concentrations of 4298 mg/L, 5919 mg/L, 12,590 mg/L, and 13,190 mg/L respectively.

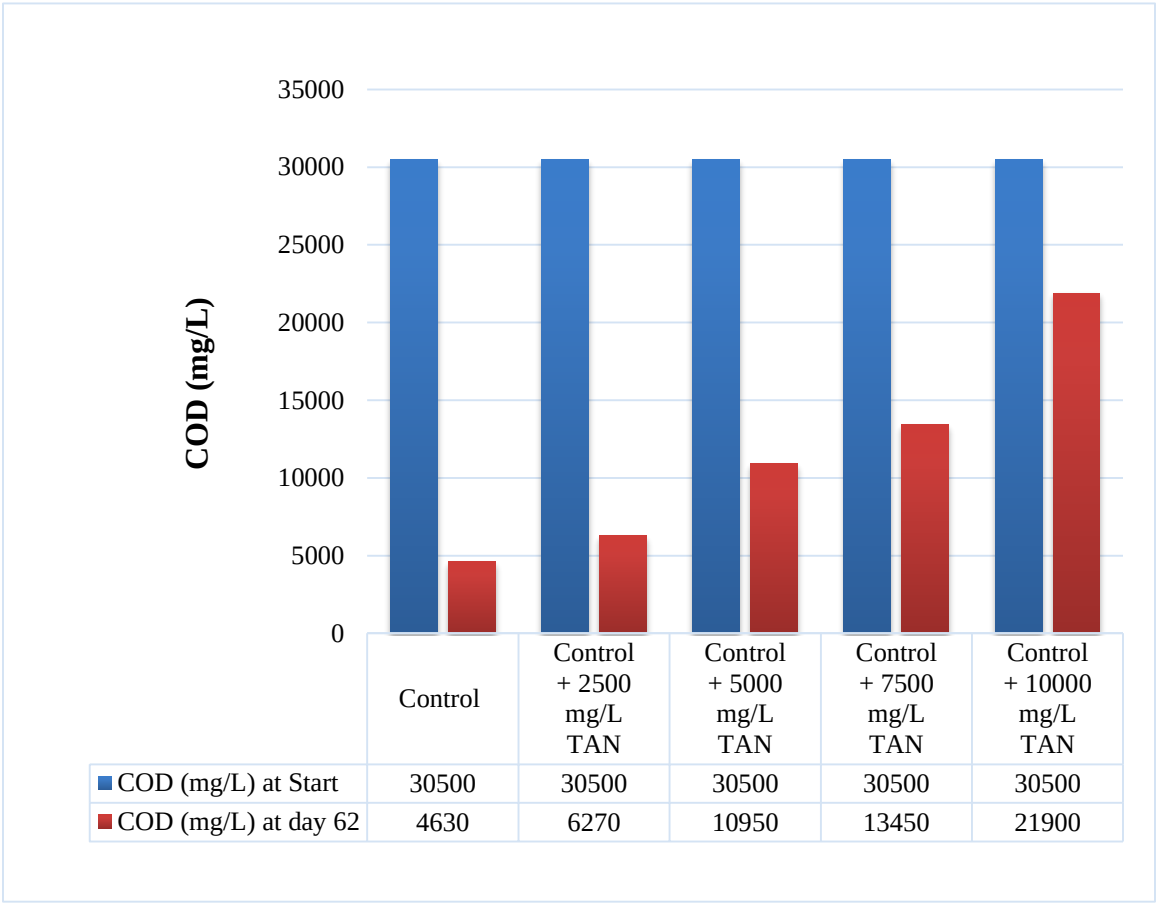


	VFA as Acetic Acid (mg/L) at Start	VFA as Acetic Acid (mg/L) at day 30	VFA as Acetic Acid (mg/L) at day 60
Control	2030	2810	1370
Control + 2500 mg/L TAN	3760	4298	1520
Control + 5000 mg/L TAN	4033	5919	3286
Control + 7500 mg/L TAN	4077	12590	6928
Control + 10000 mg/L TAN	4672	13190	12690

Figure 4.4: VFA concentration at the beginning, mid-way and end of incubation period, at operating pH of 7.5 (phase 1)

At the end of the digestion period, the VFA was also analyzed. The VFA concentrations in CB-reactors and reactors containing 2500 mg/L TAN had decreased from 2810 to 1370 mg/L and from 4298 to 1520 mg/L, respectively. For reactors containing 5,000 mg/L, 7,500 mg/L and 10,000 mg/L of TAN, VFA reduced from 5919 mg/L to 3286 mg/L, 12,590 mg/L to 6928 mg/L and from 13,190 mg/L to 12,690 mg/L, respectively. The low concentrations of the VFA obtained in CB-reactors and in reactors containing 2,500 mg/L TAN indicate that most of the acetic acids produced in these reactors have been used up by the methanogens to produce biogas. However, high VFA concentrations obtained in reactors containing 5,000 mg/L, and 7,500 mg/L and 10,000 mg/L of TAN show that not much of the acetic acetate produced has been consumed by the methanogens due to ammonia inhibition, consistent with the study of Chen et al.(2008). Also, the high VFA concentrations obtained even at high TAN concentration of 10,000 mg/L indicate that the acidogens and acetogens have high resistances to high TAN concentration.

The COD analysis at the beginning and end of the incubation period is presented in Figure 4.5. The average initial COD concentration from all the reactors at the beginning of the experiment was 30,500 mg/L.



	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
COD (mg/L) at Start	30500	30500	30500	30500	30500
COD (mg/L) at day 62	4630	6270	10950	13450	21900

Figure 4.5: COD concentration at the beginning, and end of incubation period, at operating pH of 7.5 (phase 1)

The analysis of the COD concentrations at the end of the AD process in all the reactors shows that COD concentration reduced to 4630 mg/L in CB-reactors, while it reduced to 6270 mg/L, 10,950 mg/L, 13,450 mg/L, and 21,900 mg/L in reactors containing TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L respectively. This indicates that the utilization of COD by mesophilic bacteria reduced with increased TAN concentrations.

4.3.1.2 The Effect of TAN at pH 8.0

CBP at operating pH of 8.0 is presented in Figure 4.6. Similar to what was observed at pH 7.5, biogas production reduced with increasing TAN concentration.

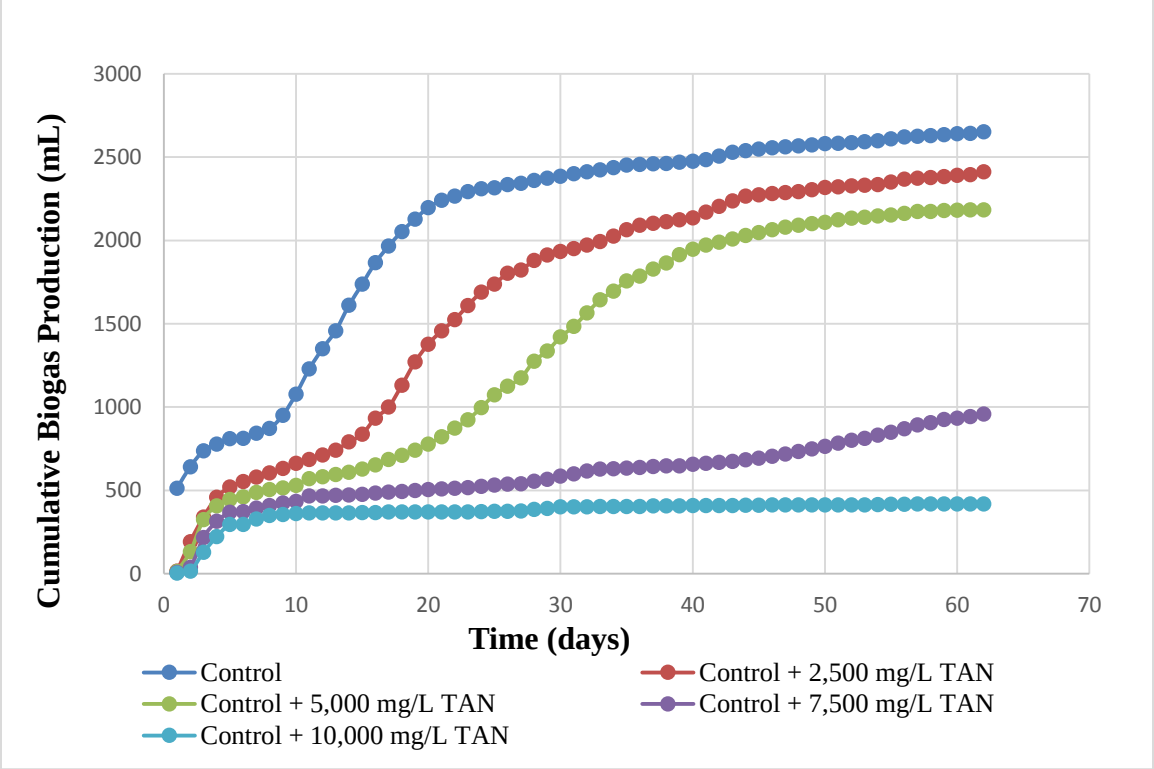
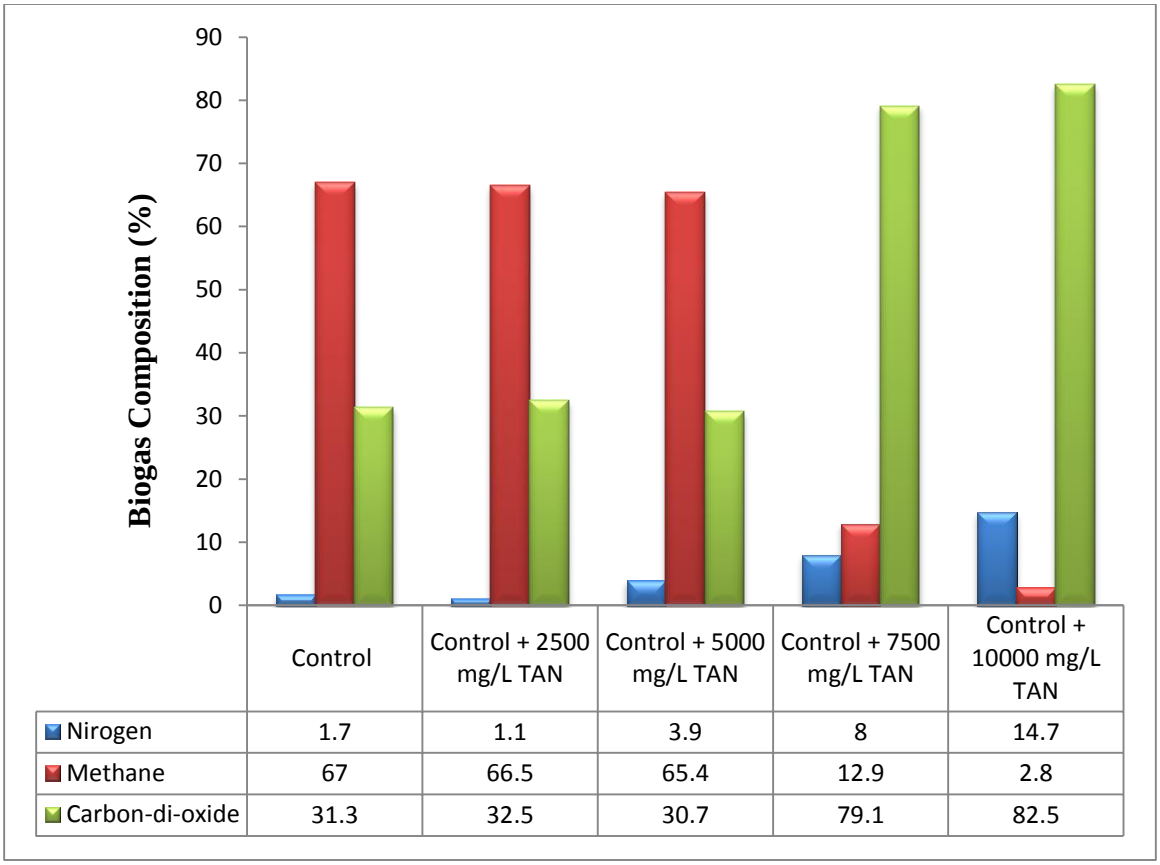


Figure 4.6: Biogas production from BMP reactors under different TAN concentrations and similar operating pH of 8.0 (phase 1)

CB-reactors had CBP of 2651 mL of biogas compared with CBP of 2413 mL, 2184 mL, 960 mL, and 420 mL produced by reactors containing TAN concentration of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L, respectively. This implies that reactors containing TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L had 9%, 18%, 64% and 84% reduction in CBP respectively, compared with CB-reactor.

The analysis of the biogas composition during the test is presented in Figure 4.7 below. Similar to what was observed at pH 7.5, ammonia has inhibitory effect on methanogenic bacteria.

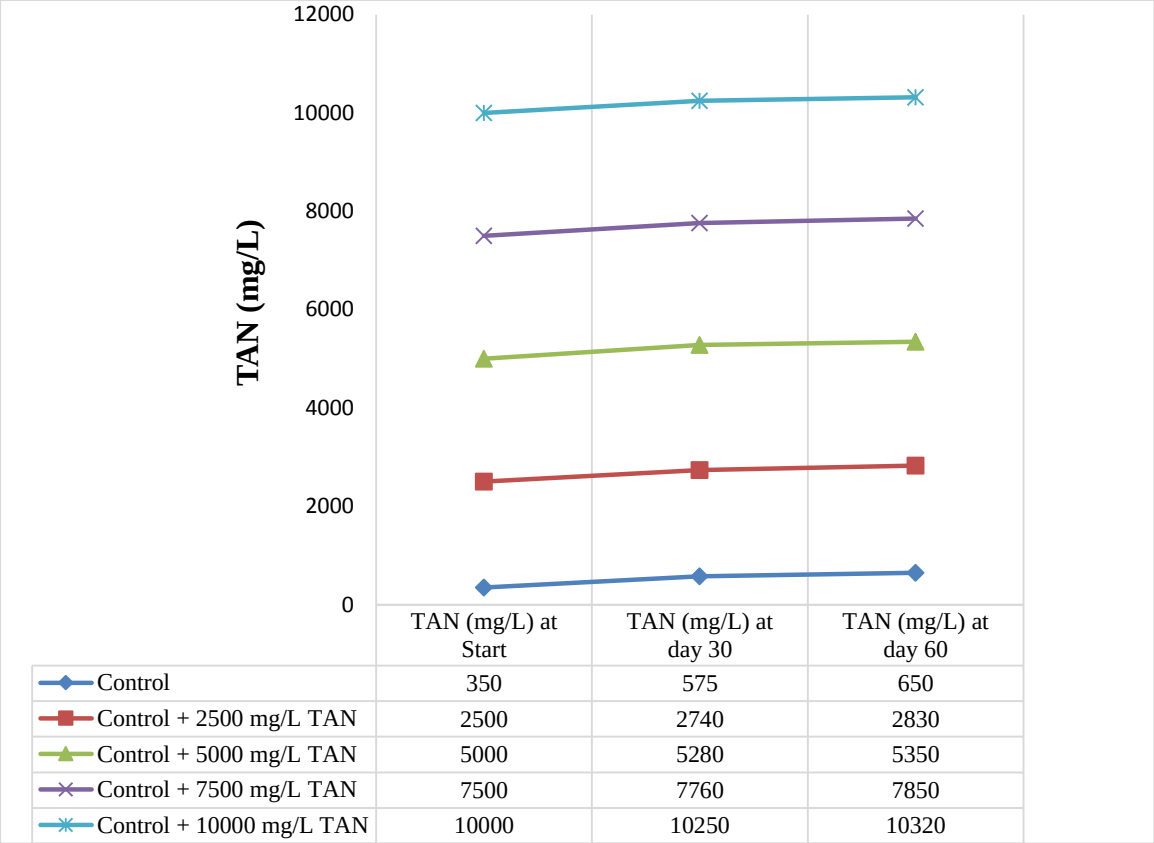


	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
Nirogen	1.7	1.1	3.9	8	14.7
Methane	67	66.5	65.4	12.9	2.8
Carbon-di-oxide	31.3	32.5	30.7	79.1	82.5

Figure 4.7: Biogas composition from BMP reactors under different TAN concentrations and similar operating pH of 8.0 (phase 1)

Also, the inhibitory effect was very significant especially at 7,500 mg/L and 10,000 mg/L TAN as shown in Figure 4.7. At the end of the test, biogas analysis showed that the methanogenic bacteria could not recover from the ammonia inhibition. Methane percentage had reduced to as low as 0% in reactors containing 7,500 mg/L and 10,000 mg/L TAN, while nitrogen accounted for more than 90% of the biogas.

TAN analysis conducted during the test is presented in Figure 4.8. In similar trend with pH 7.5, the TAN concentrations in all the reactors had increased after 30 days and at the end of the 62 days of incubation.

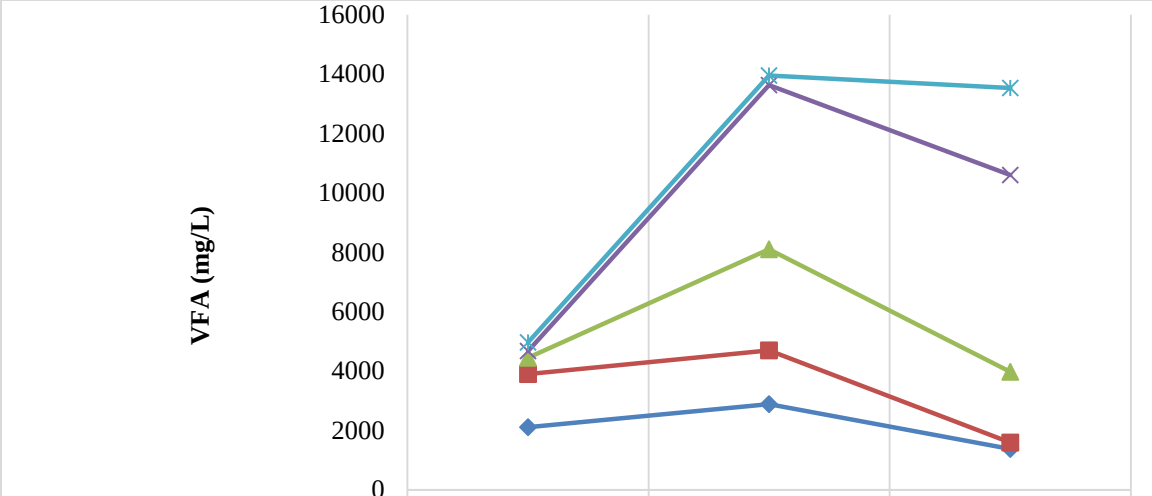


	TAN (mg/L) at Start	TAN (mg/L) at day 30	TAN (mg/L) at day 60
Control	350	575	650
Control + 2500 mg/L TAN	2500	2740	2830
Control + 5000 mg/L TAN	5000	5280	5350
Control + 7500 mg/L TAN	7500	7760	7850
Control + 10000 mg/L TAN	10000	10250	10320

Figure 4.8: TAN concentration at the beginning, mid-way and end of incubation period, at operating pH of 8.0 (phase 1)

At the end of 62 days, TAN concentration increased from 350 mg/L to 650 mg/L for CB-reactors while for other reactors, TAN concentrations increased from 2500 mg/L to 2830 mg/L, 5000 mg/L to 5350 mg/L, 7500 mg/L to 7850 mg/L, 10,000 mg/L to 10,320 mg/L. The observed increase in TAN concentration is attributed to the hydrolysis of the proteins in the waste mixture as explained earlier.

Figure 4.9 shows the measured VFA at the beginning, midway and at the end of the incubation period, at operating pH of 8.0. On day 30, analysis carried out indicated that CB-reactors had a VFA concentration of 2893 mg/L while reactors containing 2500 mg/L TAN had a VFA of 4705 mg/L. However, VFA concentrations in reactors containing TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L were 8102 mg/L, 13,630 mg/L, and 13,950 mg/L respectively. At the end of 62 days, the VFA was also analyzed.

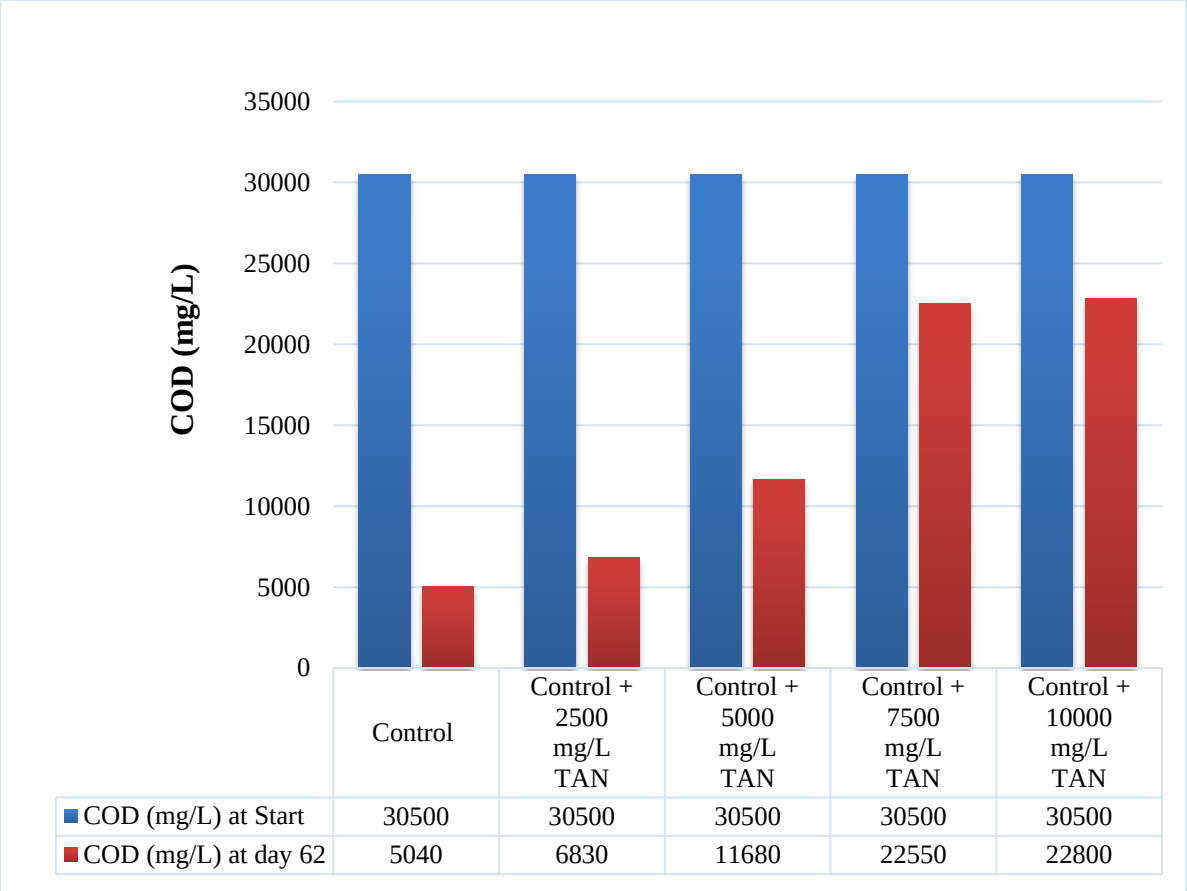


	VFA as Acetic Acid (mg/L) at Start	VFA as Acetic Acid (mg/L) at day 30	VFA as Acetic Acid (mg/L) at day 60
Control	2120	2893	1380
Control + 2500 mg/L TAN	3900	4705	1600
Control + 5000 mg/L TAN	4450	8102	3975
Control + 7500 mg/L TAN	4676	13630	10600
Control + 10000 mg/L TAN	4965	13950	13530

Figure 4.9: VFA concentration at the beginning, mid-way and end of incubation period, at operating pH of 8.0 (phase 1)

The VFA concentrations in CB-reactors and reactors containing 2500 mg/L TAN had decreased from 2893 to 1380 mg/L and from 4705 to 1520 mg/L, respectively. For reactors containing 5,000 mg/L, 7,500 mg/L and 10,000 mg/L of TAN, VFA reduced from 8102 mg/L to 3975 mg/L, 13,630 mg/L to 10,600 mg/L and from 13,950 mg/L to 13,530 mg/L, respectively. As explained earlier, the low concentrations of the VFA obtained in CB-reactors and in reactors containing 2,500 mg/L TAN indicate that most of the acetic acids produced in these reactors have been used up by the methanogens to produce biogas. However, due to ammonia inhibition, not much of the VFA produced was consumed by the methanogens in reactors containing 5,000 mg/L, and 7,500 mg/L and 10,000 mg/L of TAN.

The COD analysis at the beginning and at the end of 62 days is presented in Figure 4.10. At the end of 62 days, the CB-reactors had COD concentration of 5040 mg/L while reactors containing 2500 mg/L TAN had a COD of 6830 mg/L.



	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
COD (mg/L) at Start	30500	30500	30500	30500	30500
COD (mg/L) at day 62	5040	6830	11680	22550	22800

Figure 4.10: COD concentration at the beginning and end of incubation period, at operating pH of 8.0 (phase 1)

The COD concentrations in reactors containing TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L were11,680 mg/L, 22,550 mg/L, and 22,800 mg/L respectively. The low COD concentrations observed in CB-reactors and in reactors containing 2500 mg/L TAN concentration indicate that most of the COD have been used up by mesophilic bacteria as reflected in the biogas produced by these reactors. However, the high COD concentrations observed in reactors containing TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L indicate that the toxic effect of ammonia prevented the utilization of the COD by mesophilic bacteria.

4.3.1.3 The Effect of TAN at pH 8.5

At operating pH of 8.5, the average CBP from the reactors is presented in Figure 4.11. Consistent with the results obtained in other operating pH values, the CBP reduced as the

concentration of TAN increased. On the first day of the experiment, while CB-reactors produced 258 mL of biogas, there was no biogas production from reactors containing 2,500, 5,000 mg/L, 7,500 mg/L and 10,000 mg/L TAN.

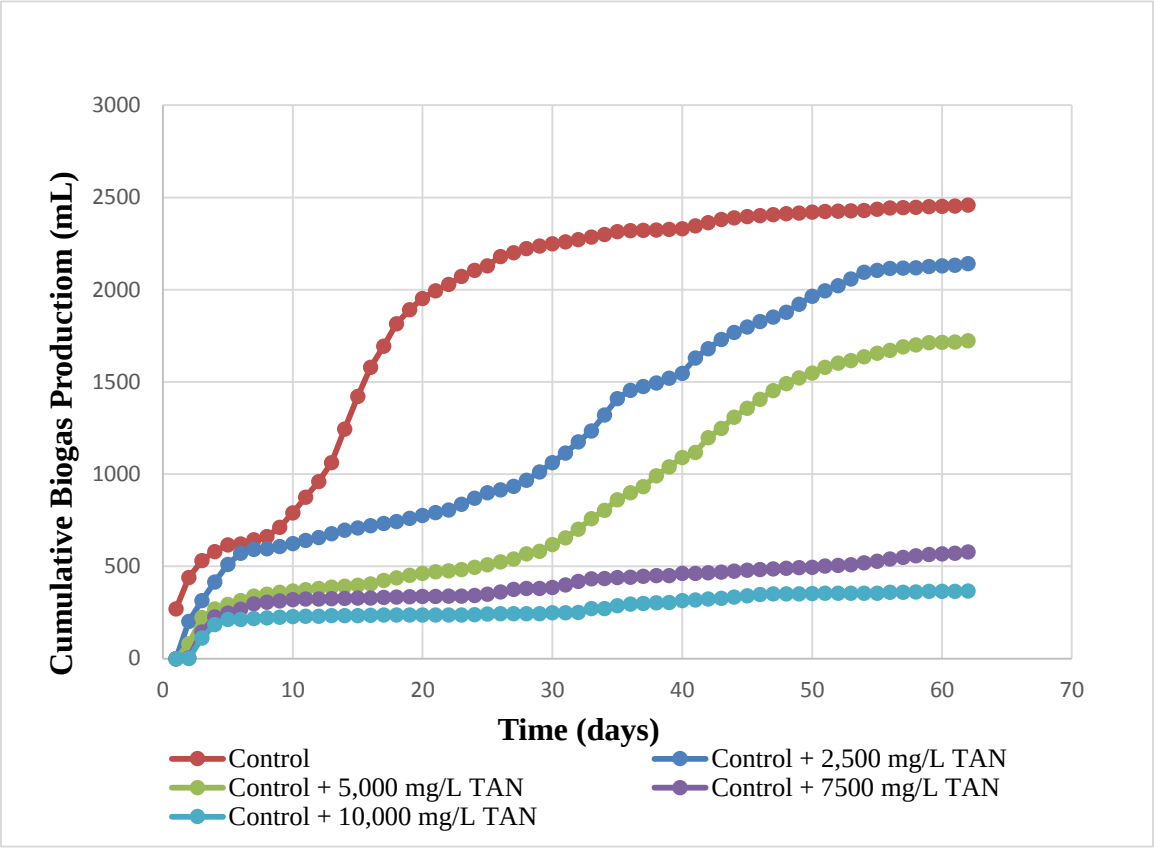
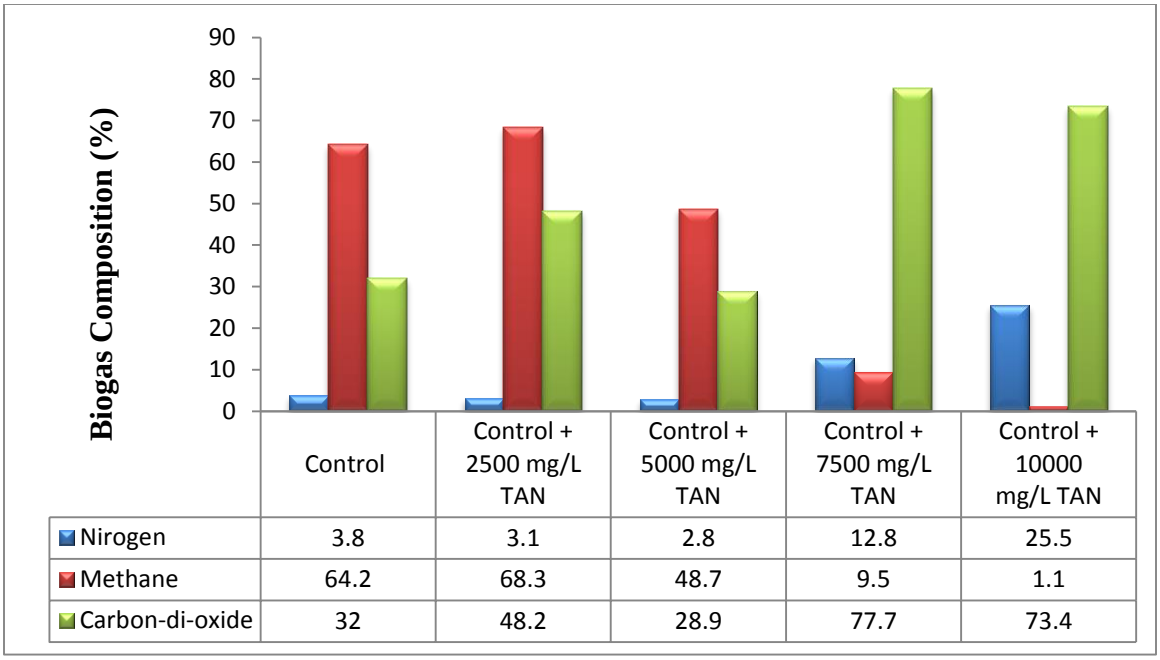


Figure 4.11: Biogas production from BMP reactors under different TAN concentrations and similar operating pH of 8.5 (phase 1)

While CB-reactors had CBP of 2459 mL of at the end of 62 days of incubation, the CBPs from reactors containing TAN concentrations of 2,500 mg/L, 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L were 2142 mL, 1724 mL, 579 mL, and 365 mL, respectively.

Biogas composition analysis on day 30 at pH 8.5 is presented in Figure 4.12. Also as in previous operating pH values, the percentage of methane in the biogas produced reduced as the concentration of TAN increased.

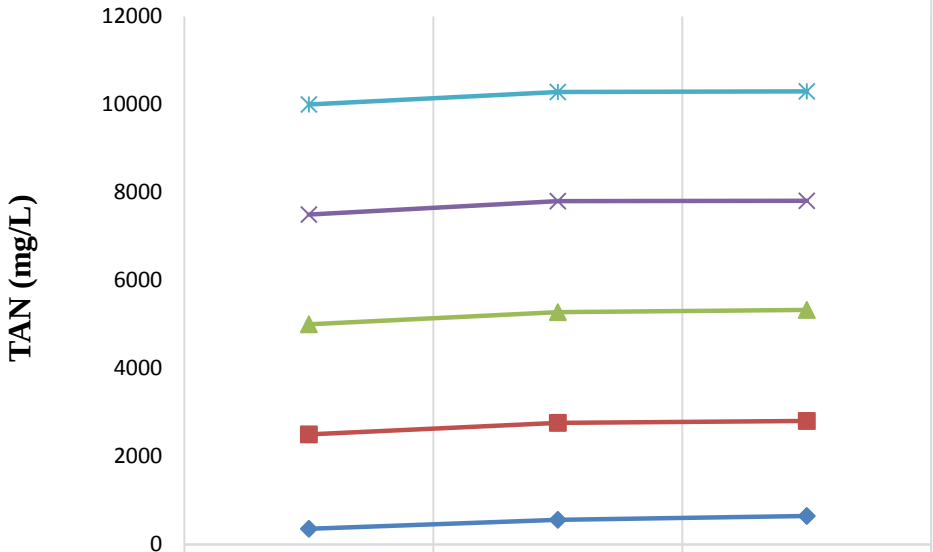


	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
Nirogen	3.8	3.1	2.8	12.8	25.5
Methane	64.2	68.3	48.7	9.5	1.1
Carbon-di-oxide	32	48.2	28.9	77.7	73.4

Figure 4.12: Biogas composition from batch reactors under different TAN concentrations and similar operating pH of 8.5 (phase 1)

From Figure 4.12 above, it is evident that ammonia has inhibitory effect on methanogenic bacteria. The inhibitory effect was mostly felt at 7,500 mg/L TAN and 10,000 mg/L TAN. The analysis of the biogas composition at the end of the test was consistent with the results obtained earlier at pH 7.5 and 8.5. Methane percentage in the biogas produced reduced to as low as 0% in reactors containing 7,500 mg/L TAN and 10,000 mg/L TAN while nitrogen accounted for as much as 90%. This indicates a complete methanogenic inhibition.

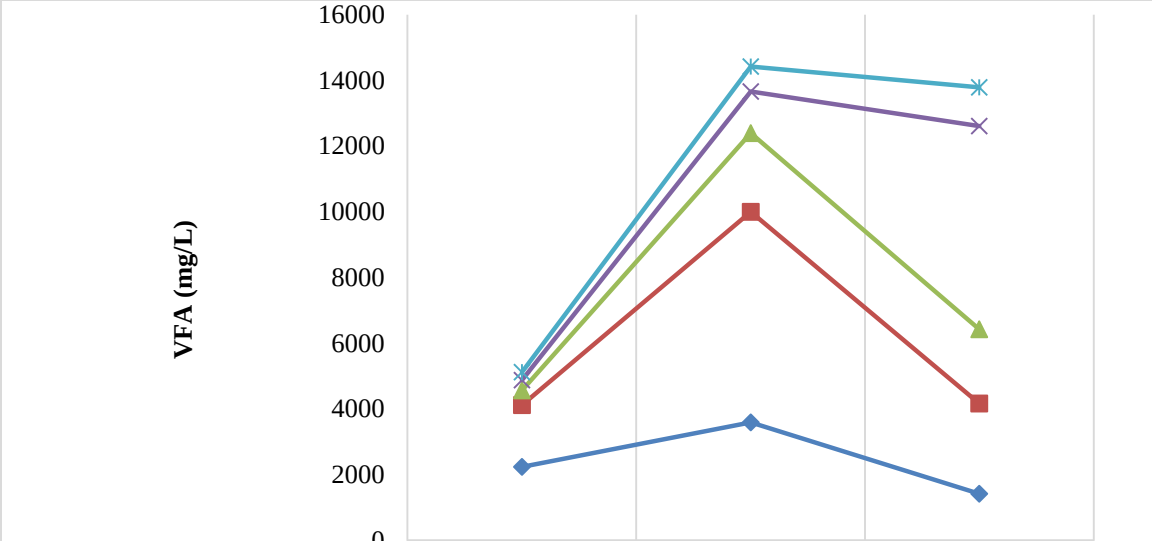
TAN analysis conducted after 30 days and at the end of 62 days is presented in Figure 4.13. In similar trend to other pH values, TAN concentrations in all the reactors increased due to the hydrolysis of the proteins in the waste mixture as explained earlier. The concentration of TAN increased from 350 mg/L to 648 mg/L for CB reactors while for other reactors, TAN concentrations increased from 2500 mg/L to 2810 mg/L, 5000 mg/L to 5330 mg/L, 7500 mg/L to 7810 mg/L, 10,000 mg/L to 10,300 mg/L.



	TAN (mg/L) at Start	TAN (mg/L) at day 30	TAN (mg/L) at day 60
Control	352	560	648
Control + 2500 mg/L TAN	2500	2760	2810
Control + 5000 mg/L TAN	5000	5280	5330
Control + 7500 mg/L TAN	7500	7800	7810
Control + 10000 mg/L TAN	10000	10280	10300

Figure 4.13: TAN concentration at the beginning, midway and end of incubation period, at operating pH of 8.5 (phase 1)

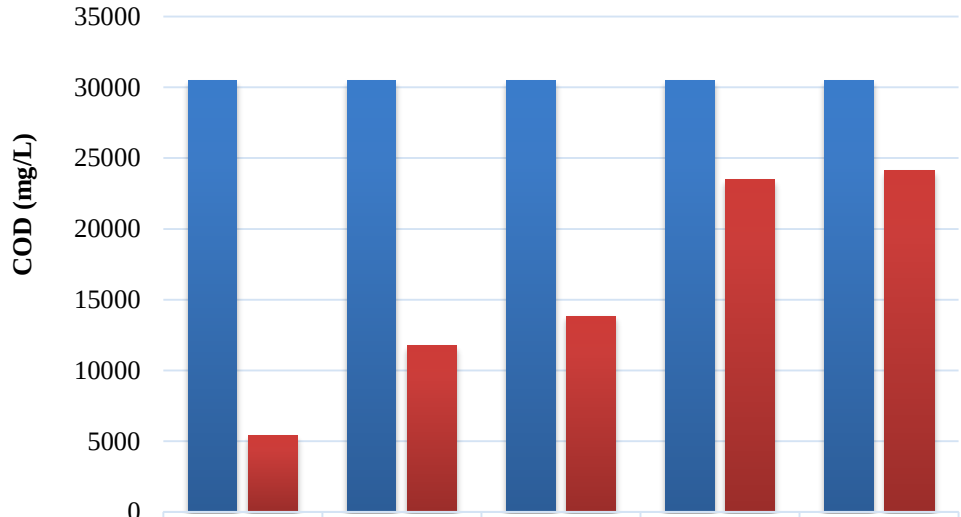
The VFA analysis of the digestate in the reactors midway to and the end of the AD period is presented in Figure 4.14. At the end of 62 days, CB-reactors had a VFA concentration of 1420 mg/L while VFA concentration was 4175 mg/L in reactors containing 2500 mg/L TAN. The concentration of VFA in reactors containing TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L was 6431 mg/L, 12,616 mg/L, and 13,787 mg/L respectively. The high TAN concentration in other reactors was very toxic to methanogenic bacteria, preventing the uptake of VFA for biogas production.



	VFA as Acetic Acid (mg/L) at Start	VFA as Acetic Acid (mg/L) at day 30	VFA as Acetic Acid (mg/L) at day 60
Control	2245	3591	1420
Control + 2500 mg/L TAN	4120	10006	4175
Control + 5000 mg/L TAN	4565	12403	6431
Control + 7500 mg/L TAN	4879	13665	12616
Control + 10000 mg/L TAN	5120	14421	13787

Figure 4.14: VFA concentration at the beginning and end of incubation period, at operating pH of 8.5 (phase 1)

The COD analysis at the beginning and at the end of 62 days is presented in Figure 4.15 At the end of 62 days, COD concentration in CB-reactors was 5390 mg/L while reactors containing 2500 mg/L TAN had a COD of 11,790 mg/L. For reactors containing TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L, the COD was 13,850 mg/L, 23,500 mg/L, and 24,100 mg/L respectively. As explained earlier, the low COD concentrations observed in CB-reactors and in reactors containing 2500 mg/L TAN concentration was an indication that most of the COD has been used up by mesophilic bacteria for biogas production.



	Control	Control + 2500 mg/L TAN	Control + 5000 mg/L TAN	Control + 7500 mg/L TAN	Control + 10000 mg/L TAN
COD (mg/L) at Start	30500	30500	30500	30500	30500
COD (mg/L) at day 62	5390	11790	13850	23500	24100

Figure 4.15: TAN concentration at the beginning and end of incubation period, at operating pH of 8.5 (phase 1)

However, for other reactors containing high TAN concentrations of 5,000 mg/L, 7,500 mg/L, and 10,000 mg/L, COD concentration was very high, indicating that the toxic effect of ammonia prevented the utilization of the COD by mesophilic bacteria.

The results of the alkalinity analyses conducted at all the pH values used is provided in Table 4.3 and Figure4.16. The results show that alkalinity increases with increase in TAN concentrations and pH. Alkalinity concentration reached as much as 37,790 mg/L in reactors containing 10,000 mg/L TAN at pH 8.5.

Table 4.3: Initial and Final Alkalinity at pH 7.5, 8.0 and 8.5

pH	BMP Configuration	Initial Alkalinity (mg/L)	Final Alkalinity (mg/L)
pH 7.5	Control	5400	4815
	Control + 2500 mg/L TAN	12350	11120
	Control + 5000 mg/L TAN	17350	16650
	Control + 7500 mg/L TAN	20230	19020
	Control + 10,000 mg/L TAN	22360	21910
pH 8.0	Control	5565	5750
	Control + 2500 mg/L TAN	12840	12970
	Control + 5000 mg/L TAN	18750	18940
	Control + 7500 mg/L TAN	22070	22292
	Control + 10,000 mg/L TAN	24590	24840
pH 8.5	Control	6100	6356
	Control + 2500 mg/L TAN	14270	12840
	Control + 5000 mg/L TAN	20840	21880
	Control + 7500 mg/L TAN	24520	26970
	Control + 10,000 mg/L TAN	27330	32790

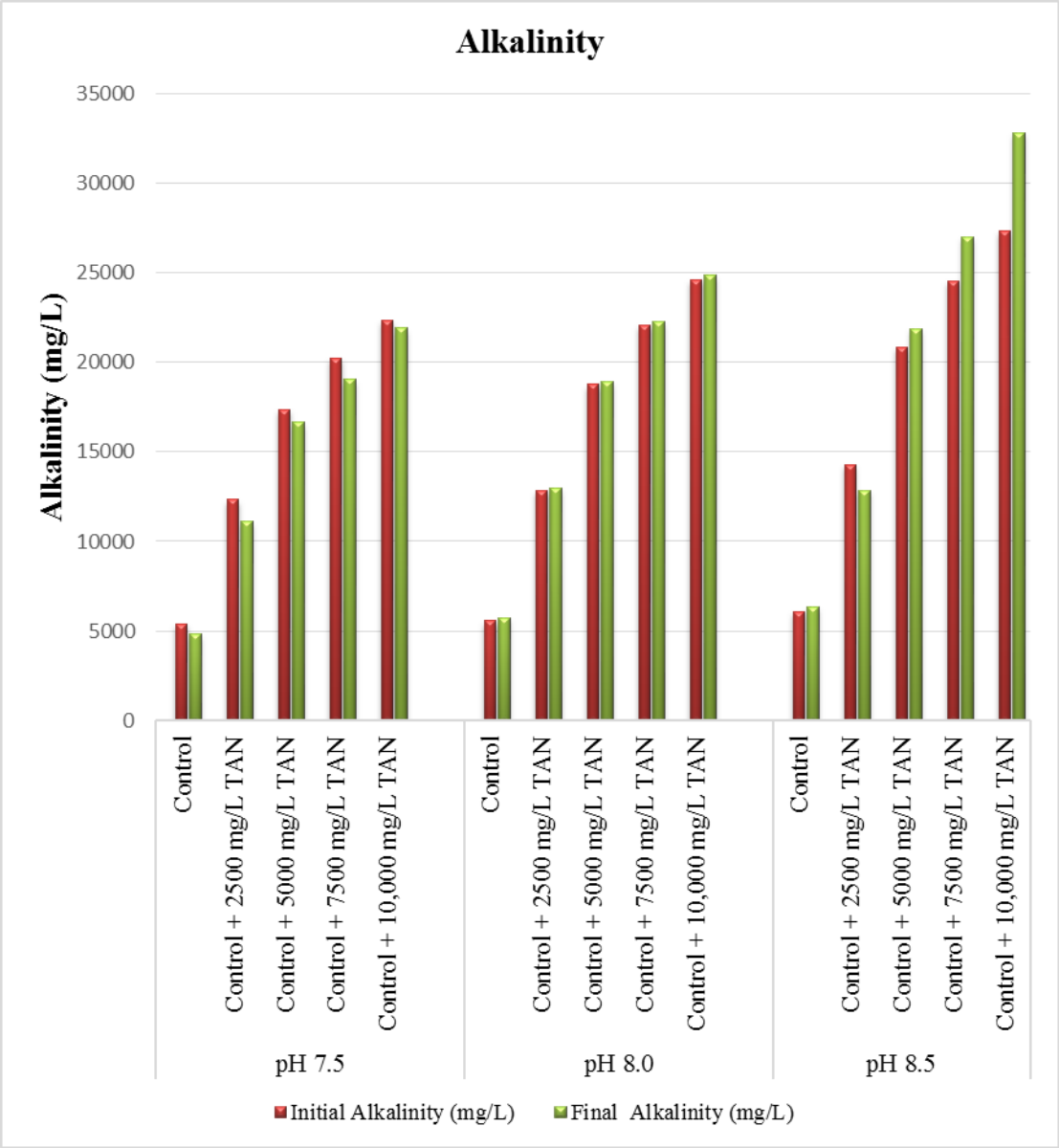


Figure 4.16: Alkalinity at pH 7.5, 8.0 and 8.5 (phase 1)

4.3.3 The Effects of pH and FAN

In order to examine the effect of pH on biogas production, CBP from reactors having similar initial TAN concentrations were compared, at different pH values of 7.5, 8.0, and 8.5. Figure 4.17 shows the cumulative biogas productions from CB-reactors, at

pH values of 7.5, 8.0 and 8.5. The cumulative biogas production at pH values of 7.5, 8.0 and 8.5 was 2835 mL, 2651 mL, and 2459 mL respectively. CB-reactors at pH 8.0 and 8.5 had 7 %, and 13 % lesser than CB-reactors at pH 7.5. This shows that the operating pH had a significant influence on methanogenic activity. This is understandable because Mohan et al. (2008) reported that methanogenic bacteria require a favourable pH range of 6.5-7.5.

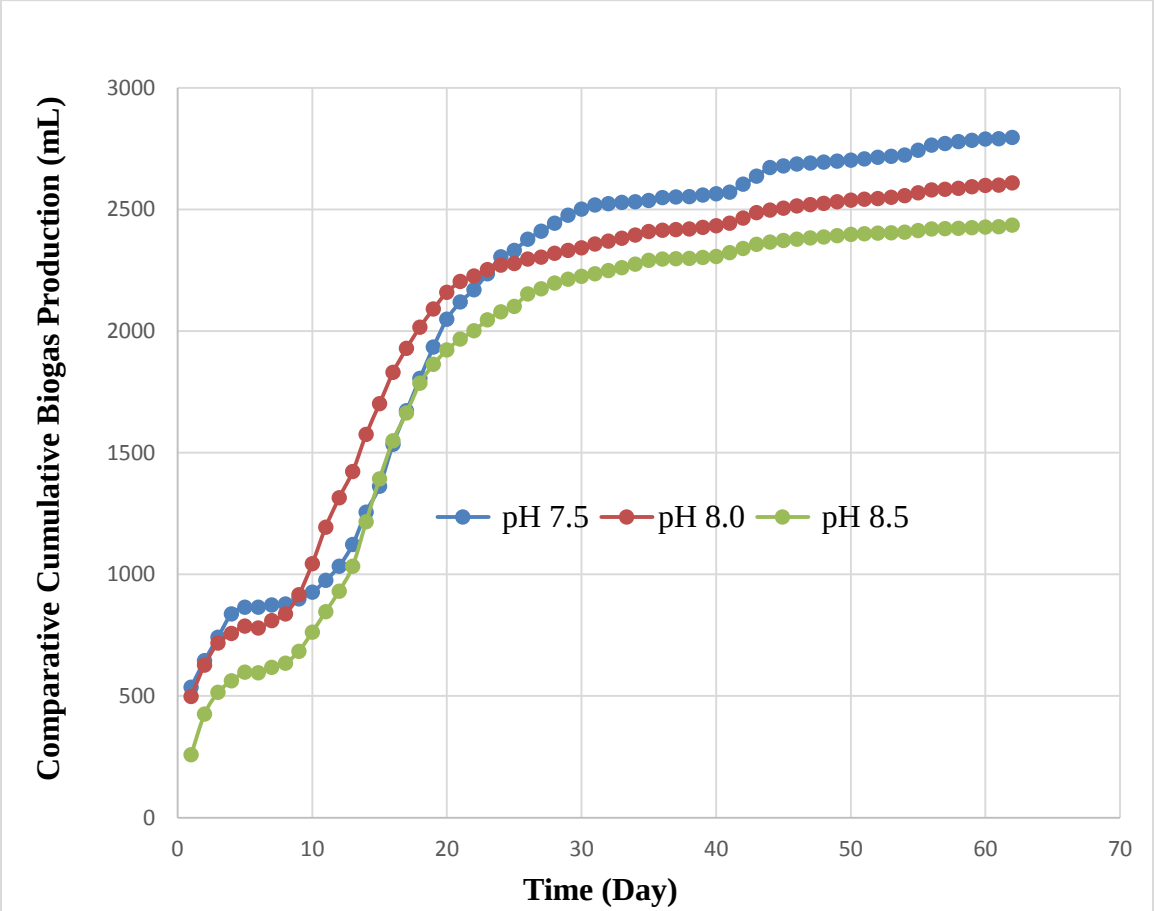


Figure 4.17: Cumulative biogas production from CB-reactors under different pH values and similar initial TAN concentration of 350 mg/L (phase 1)

Another possible reason for the difference in CBP is the difference in composition of the FAN in TAN concentrations at the different operating pH values used in this study. The percentage composition of FAN in TAN at pH from 7.5-8.5 under mesophilic temperature of 35 ^{o}C is shown in Figure 4.18 and in Table 4.4. The initial TAN

concentration of 350 mg/L TAN in CB-reactors increased to approximately 633 mg/L on day 62, an average of the TAN concentrations at of all the pH values. At the end of the test, the FAN concentrations were 20 mg/L, 69 mg/L and 163 mg/L at pH 7.5, 8.0 and 8.5 respectively. However, this cannot be ascertained because the TAN concentration and the FAN component of the CB-reactors are not very significant.

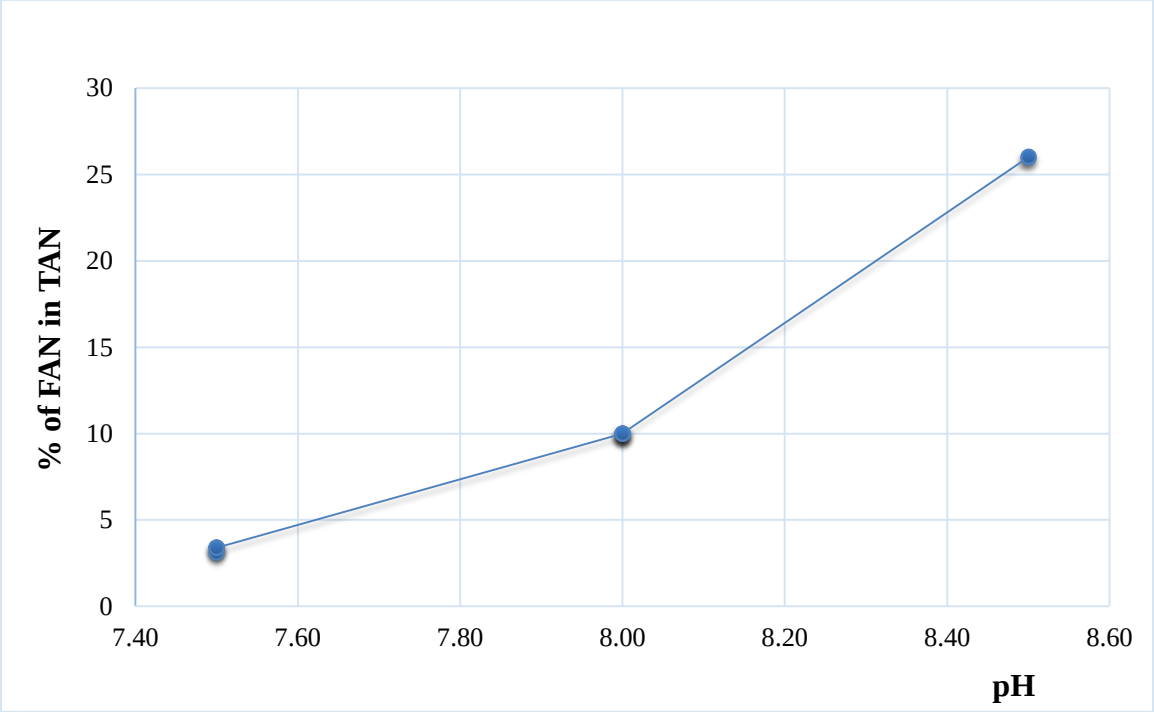


Figure 4.18: Percentage of FAN in TAN as a function of pH (7.5 – 8.5) and temperature (35 ºC)

Table 4.4: Percentage of FAN in TAN

Initial TAN (mg/L)	pH	Initial FAN (mg/L)	Initial % of FAN in TAN	Final TAN (mg/L)	pH	Final FAN (mg/L)	Final % of FAN in TAN
350	7.50	11	3	600	7.52	20	3
2500	7.50	85	3	2840	7.51	92	3
5000	7.50	170	3	5400	7.48	192	4
7500	7.50	255	3	7820	7.50	297	4
10000	7.50	340	3	10350	7.51	344	3
350	8.00	35	10	650	7.98	69	11
2500	8.00	250	10	2830	8.01	302	11
5000	8.00	501	10	5350	7.99	606	11
7500	8.00	751	10	7850	8.02	770	10
10000	8.00	1001	10	10320	8.00	1077	10
350	8.50	91	26	648	8.50	163	25
2500	8.50	651	26	2810	8.51	707	25
5000	8.50	1302	26	5330	8.48	1387	26
7500	8.50	1952	26	7810	8.50	2068	26
10000	8.50	2603	26	10300	8.47	2774	27

The comparative cumulative biogas production from reactors containing 2500 mg/L TAN at pH 7.5, 8.0 and 8.5 is presented in Figure 4.19. CBP at pH 7.5 was 2627 mL while at pH 8.0 and 8.5, 2413 mL, and 2142 mL were produced respectively. The difference is biogas production is attributed to the difference in FAN concentrations. This implies 8 %, and 19 % lesser than reactors operating at pH 7.5. The reason for the difference in CBP can be linked with the difference in FAN composition at pH 7.5, 8.0 and 8.5.

As shown in Table 4.4 above, FAN composition of TAN was 3 % at 7.5 while at pH 8.0 and 8.5, it increased to 11 % and 22 % respectively. This shows that FAN concentration was responsible for the difference in CBP. The FAN concentrations were calculated

using the Hansen et al., (1998) equation (Equation (2)), based on the assumption that FAN escaping the system as nitrogen gas is negligible.

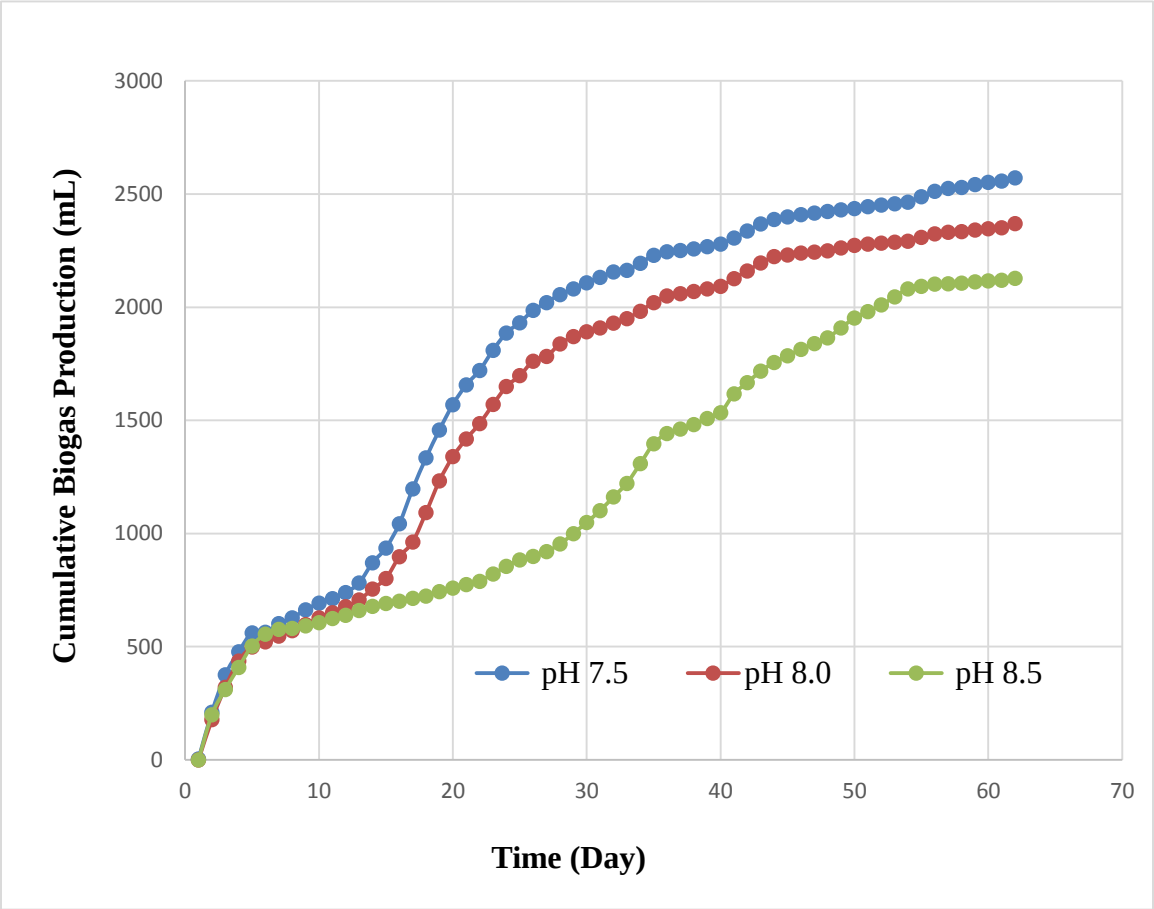


Figure 4.19: Cumulative biogas production under different pH values and similar initial TAN concentration of 2500 mg/L (phase 1)

Figure 4.20 shows the CBP from reactors containing 5000 mg/L TAN at pH 7.5, 8.0 and 8.5. As explained earlier, the difference in FAN concentrations at the various pH used was responsible for the observed difference in biogas production. A shown in Table 4.4above, FAN composition of TAN varied as much as 4% at 7.5, 11 % at pH 8.0 and 26 % at pH 8.5. This shows that FAN concentration was responsible for the difference in CBP.

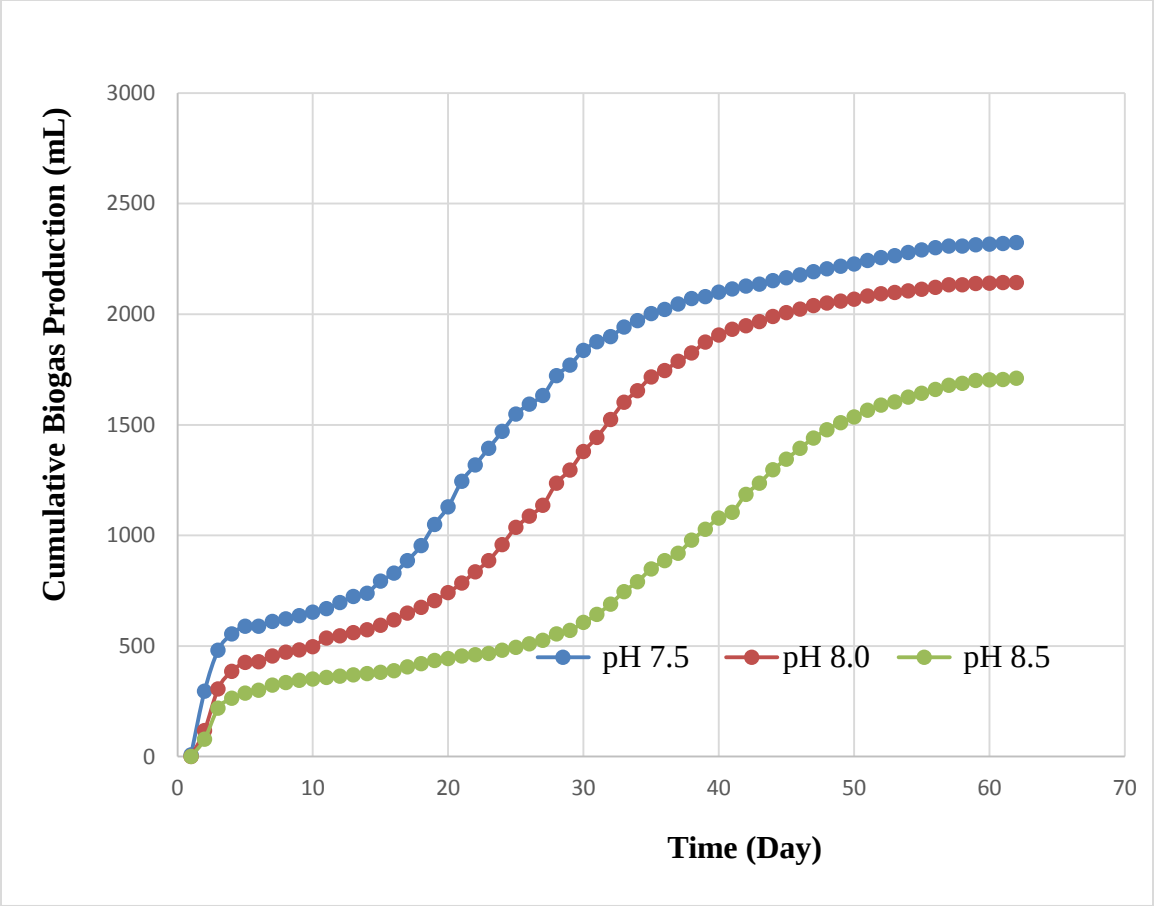


Figure 4.20: Cumulative biogas production from batch reactors under different pH values and similar TAN concentrations of 5000 mg/L

Similar trends were observed in reactors having initial TAN concentrations of 7500 mg/L and 10,000 mg/L as shown in Figures 4.21 and 4.22. For reactors containing 7500 mg/L and operated at pH 8.0 and 8.5, CBP was 40 %, and 64 % lesser than the CBP from reactors operated at pH 7.5. Also, at 10,000 mg/L TAN, CBP was 25%, and 34 % lesser than the CBP from reactors operated at pH 7.5.The percentage of FAN in TAN varied as much as 4 % at 7.5, 11 % at pH 8.0 and 26 % at pH 8.5 as previously discussed. The difference in CBP is attributable to the difference in the percentage of FAN in TAN at each pH value.

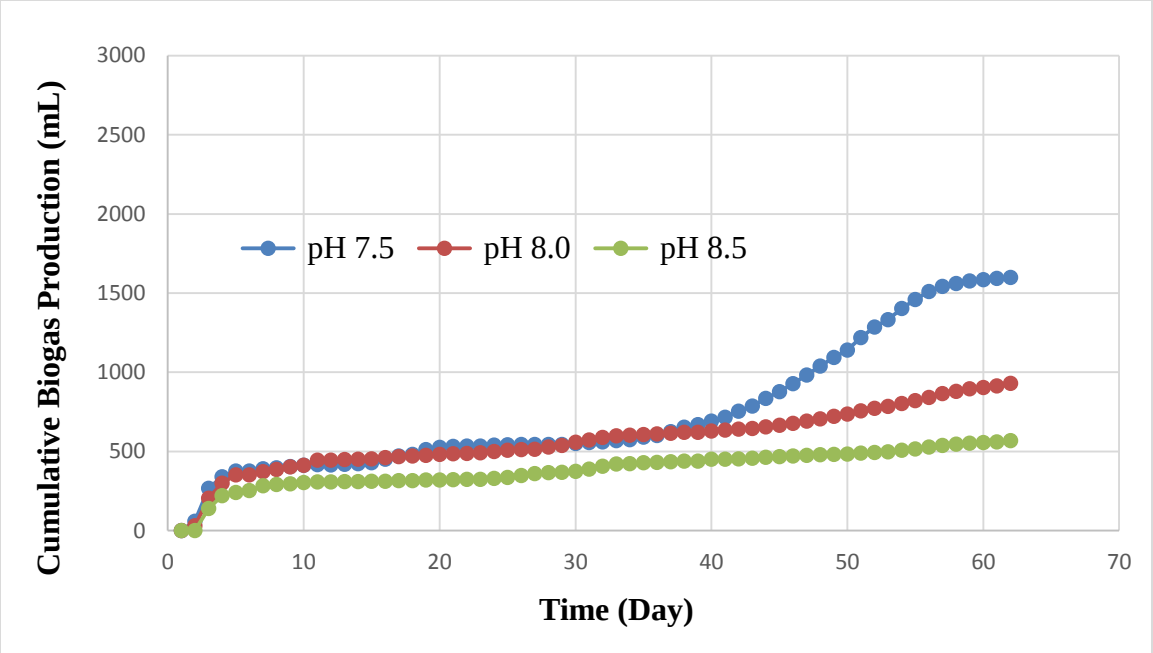


Figure 4.21: Cumulative biogas production from reactors at different pH values and similar TAN concentration of 7,500 mg/L (Phase 1)

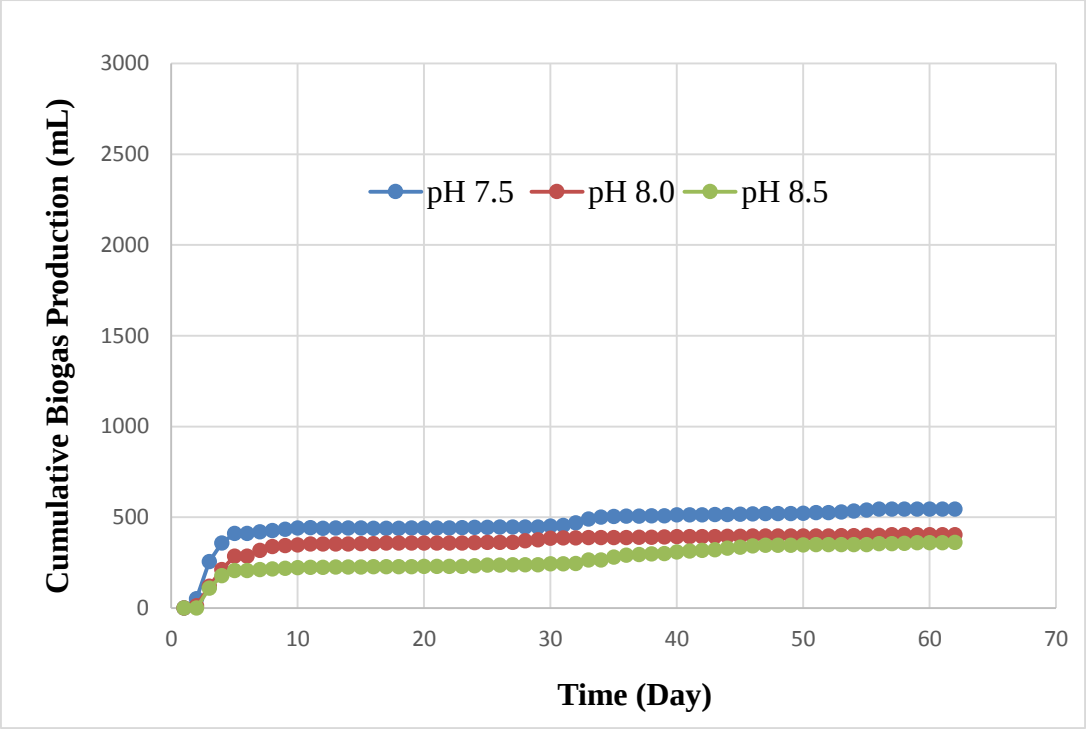


Figure 4.22: Cumulative biogas production from reactors at different pH values and similar TAN concentration of 10,000 mg/L (Phase 1)

Table 4.5 shows the volume of methane produced per gram of COD degraded. The volume of methane produced per gram of COD degraded (COD_d) decreased as the TAN and FAN concentrations in the systems increased, across all the pH levels examined throughout the research. For CB-reactors and reactors containing TAN concentrations up to 5000 mg/L, although the volumes of methane produced per of COD obtained in this research are lesser than the standard 350 mL CH_4 /g COD, the volume of methane produced per gram of COD still fall in the ranges reported in the literature (Alqaralleh et al., 2015). Methane produced per gram of COD_d was 264 mL CH_4 /g COD_d for CB-reactors at pH 7.5 while reactors containing TAN concentrations of 2500 mg/L, 5000 mg/L and 7500 mg/L and 10,000 mg/L had 227 mL CH_4 /g COD_d, 196 mL CH_4 /g COD_d, 86 mL CH_4 /g COD_d, and 5 mL CH_4 /g COD_d. Similar trends were observed in other pH levels as shown in Table 4.5. Across all pH levels, reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L had the least methane gas produced per gram of COD_d.

Table 4.5: Methane production per gram of COD degraded

BMP	pH 7.5	pH 8.0	pH 8.5
	mL CH_4 / g COD_d	mL CH_4 / g COD_d	mL CH_4 / g COD_d
Control	264	229	203
Control + 2500 mg/L TAN	227	207	188
Control + 5000 mg/L TAN	196	184	108
Control + 7500 mg/L TAN	86	16	7
Control + 10000 mg/L TAN	5	2	1

4.3.4 Phase 2 - Gradual TAN Loading Phase - SW

The CBP under gradual TAN loading is presented below in Figure 4.23. The inhibitory effect of TAN on biogas production was insignificant in the early days. The average daily biogas production (DBP) from the reactors when TAN loading was 1250 mg/L in the first week were 118 mL/d, 96 mL/d and 77 mL/d at pH 7.5, 8.0 and 8.5 respectively. This

corresponded to a CBP of 825 mL, 675 mL and 540 mL at pH 7.5, 8.0 and 8.5 respectively at the end of week one.

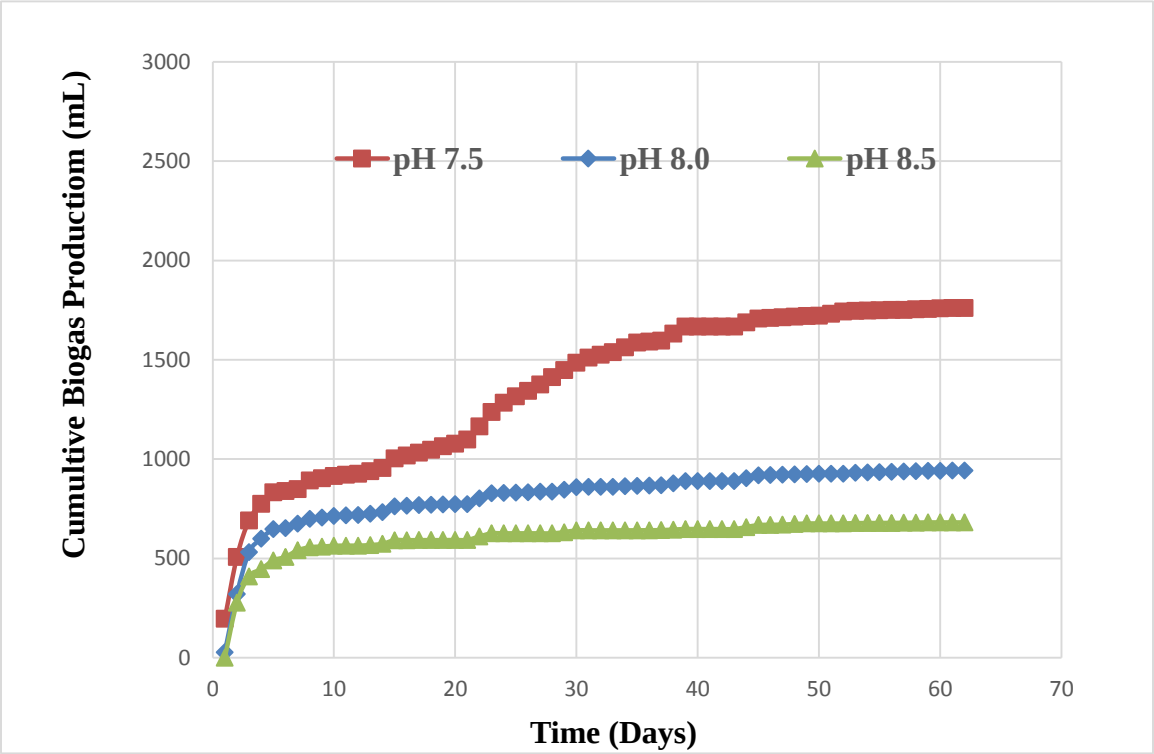


Figure 4.23: Cumulative biogas production under gradual TAN loading up to 10,000 mg/L (Phase 2)

This shows that TAN concentration of 1250 mg/L is not significantly inhibitory to methanogenic activity. By the end of the fourth week, with gradual TAN concentration reaching 5305 mg/L, average DBP was 41 mL/d, 9 mL/d and 5 mL/d at pH 7.5, 8.0 and 8.5 respectively. In the fifth week (TAN concentration was 6650 mg/L) and until TAN concentration reached 10,500 mg/L after the 8th week, daily biogas production reduced below 1% of the CBP. This suggests that methanogenic bacteria could not adapt to TAN concentration beyond 5000-6000 mg/L, in the case of this study.

A comparison was made between CBP under sudden TAN loading of 10,000 mg/L and gradual TAN loadings, at each operating pH value of 7.5, 8.0, and 8.5 as shown in Figures 4.24 (a), (b) and (c). Under gradual TAN loading, a CBP of 1761 mL was produced at pH 7.5, with 943 mL and 682 mL produced at pH 8.0 and 8.5 respectively,

meanwhile under sudden TAN loading; CBP of 560 mL was produced at pH 7.5, with 420 mL and 365 mL produced at the end of 61 days.

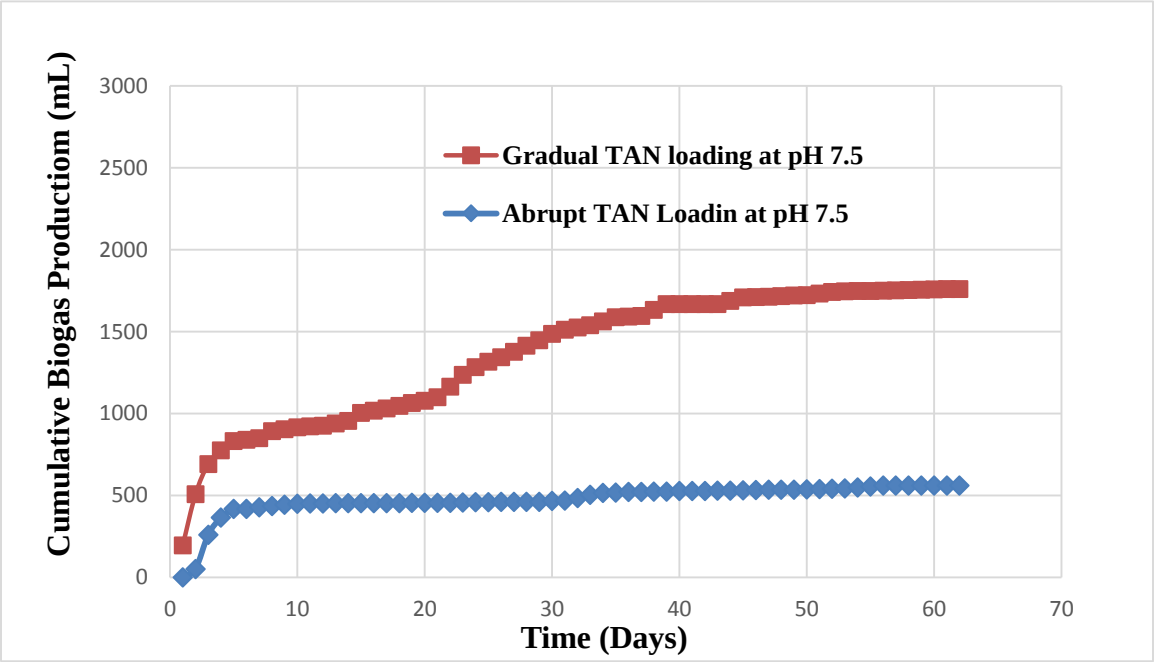


Figure 4.24 (a): Cumulative biogas production under gradual TAN loading up to 10,000 mg/L vs abrupt TAN loading of 10,000 mg/L at pH 7.5 (Phase 2)

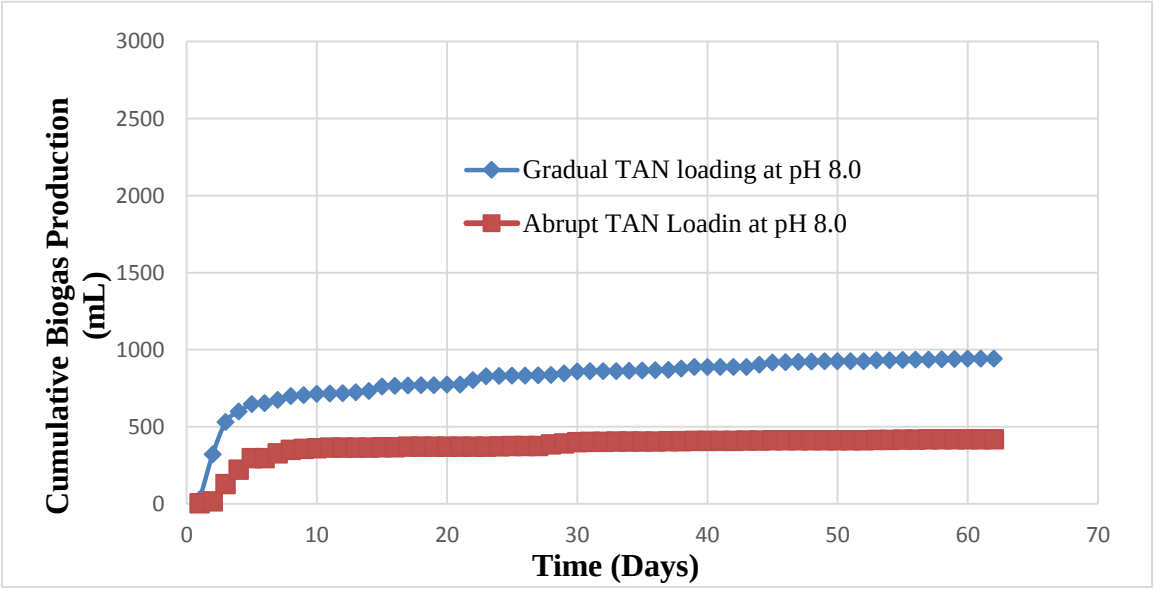


Figure 4.24 (b): Cumulative biogas production under gradual TAN loading up to 10,000 mg/L vs abrupt TAN loading of 10,000 mg/L at pH 8.0 (phase 2)

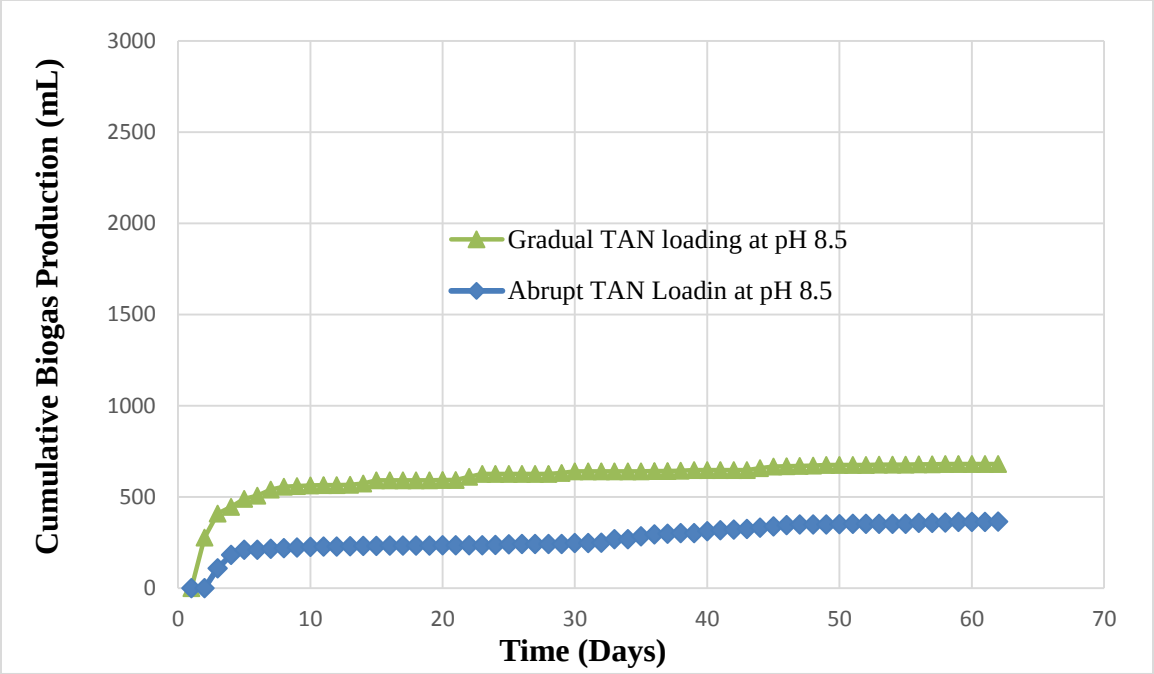
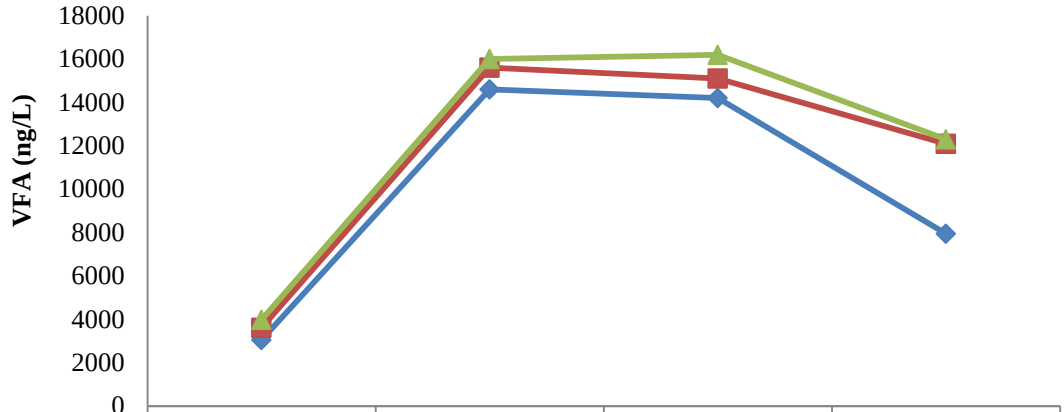


Figure 4.24 (c): Cumulative Biogas production under gradual TAN loading up to 10,000 mg/L vs abrupt TAN loading of 10,000 mg/L at pH 8.5 (phase 2)

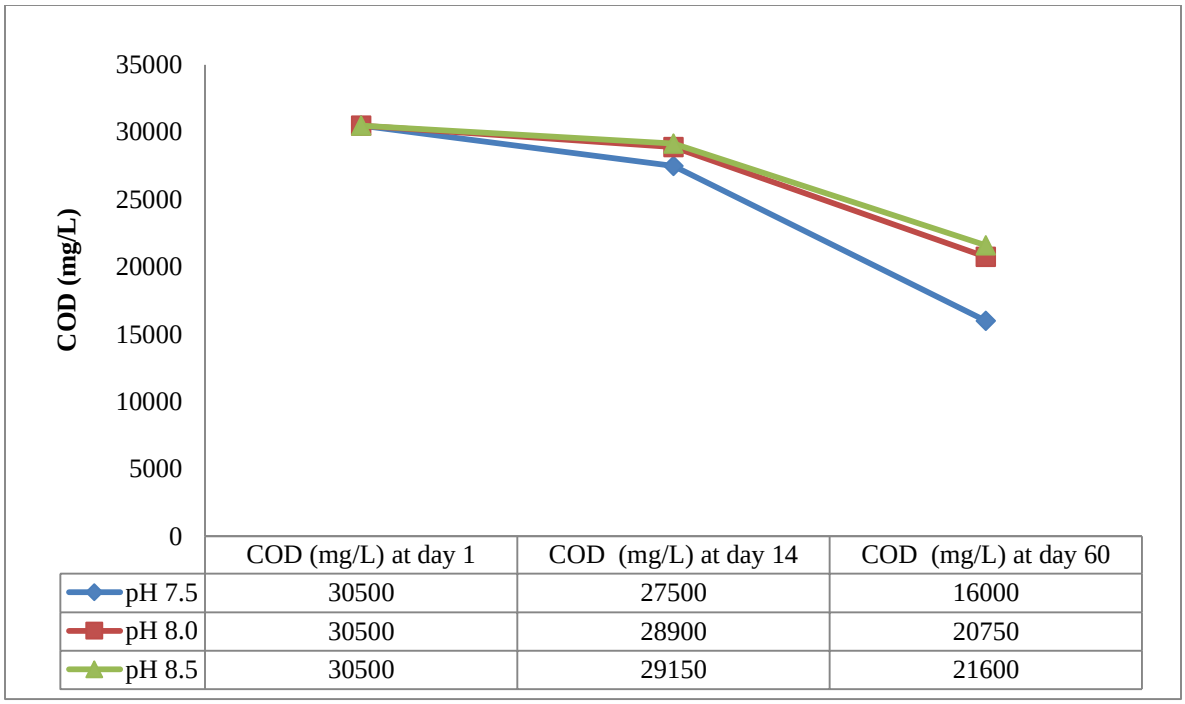
The analysis of the VFA under gradual TAN loading is presented in Figure 4.25. At the beginning of the experiment, VFA concentrations in the reactors were 3045 mg/L, 3610 mg/L and 3976 mg/L at pH of 7.5, 8.0 and 8.5 respectively. On day 7, the VFA concentrations in the reactors had increased to 14600 mg/L, 15600 mg/L and 16000 mg/L at pH of 7.5, 8.0 and 8.5 respectively. With gradual TAN loading up to 10,500 mg/L at the end of the experiment, VFA concentration reduced to 7935 mg/L, 12,100 mg/L and 12,300 mg/L at pH 7.5, 8.0 and 8.5, respectively. This shows that at the lowest pH of 7.5, the methanogens were more able to consume most the VFA for biogas production than at pH 8.0 and 8.5. This can also be attributed to the fact the FAN concentration at pH of 8.0 and 8.5 caused the accumulation VFA and prevented the utilization of the VFA for biogas production as previously discussed.



	VFA as Acetic Acid (mg/L) on day 1	VFA as Acetic Acid (mg/L) on day 7	VFA as Acetic Acid (mg/L) on day 14	VFA as Acetic Acid (mg/L) on day 62
pH 7.5	3045	14600	14200	7940
pH 8.0	3610	15600	15100	12100
pH 8.5	3980	16000	16200	12300

Figure 4.25: VFA consumption under gradual TAN loading (phase 2)

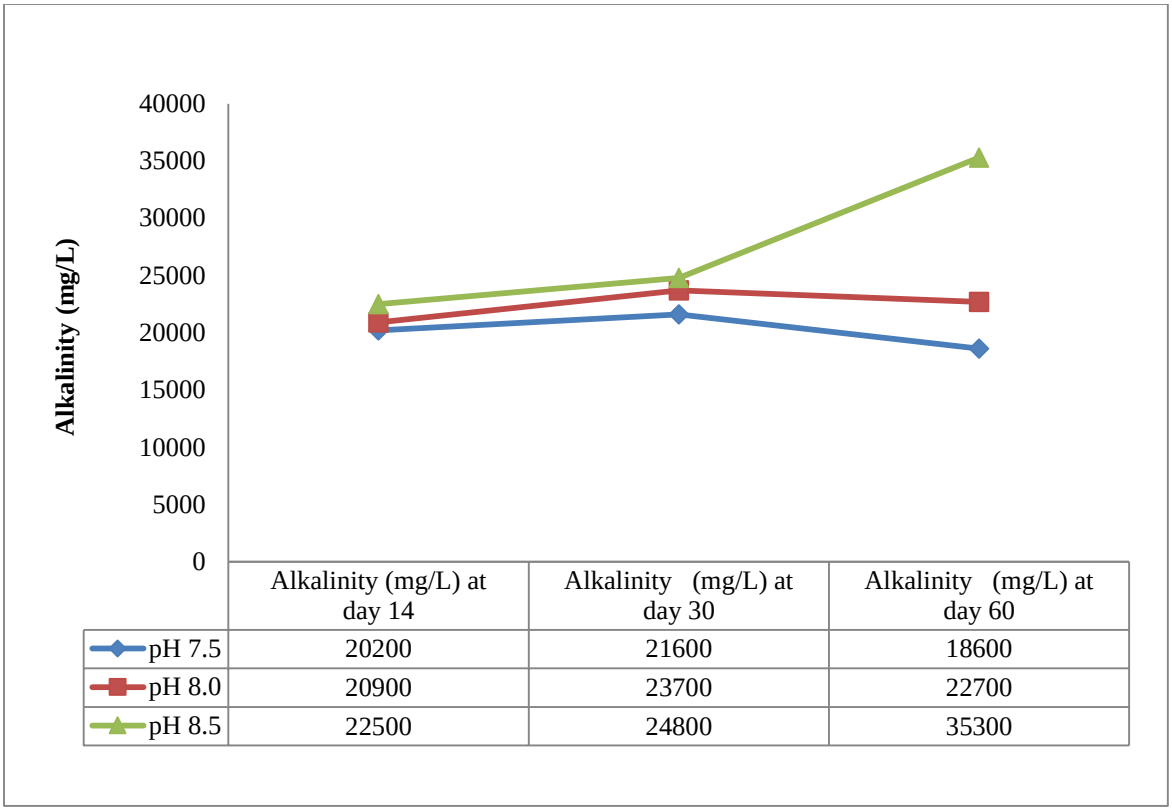
The COD analysis under gradual TAN loading is presented in Figure 4.26. The study shows that the COD consumption rate decreased as the concentration of TAN added increased, especially at pH of 8.0 and 8.5. At the end of the study, COD concentrations in in the reactors reduced to 16,000 mg/L, 20,750 mg/L and 21,600 mg/L at pH 7.5, 8.0 and 8.5 respectively. The observed difference in the effluent COD concentrations can be attributed to the fact FAN concentration inhibited the utilization of the COD for biogas production by the methanogens, especially at pH 8.0 and 8.5.



	COD (mg/L) at day 1	COD (mg/L) at day 14	COD (mg/L) at day 60
pH 7.5	30500	27500	16000
pH 8.0	30500	28900	20750
pH 8.5	30500	29150	21600

Figure 4.26: COD consumption under gradual TAN Loading (Phase 2)

The alkalinity analysis under gradual TAN loading is presented in Figure 4.28. On day 14 of the experiment, alkalinity concentrations were 20,200 mg/L, 20,900 mg/L and 22,500 mg/L at pH 7.5, 8.0 and 8.5 respectively. For reactors operated at pH 7.5, the alkalinity concentration reduced to 18,600 mg/L at the end of the experiment. However, for reactors operated at pH 8.0 and 8.5, alkalinity concentration increased to 22,700 mg/L and 35,300 mg/L respectively, at the end of the experiment. This shows that although alkalinity is good for buffering the system, it can be inhibitory to the AD process at very high concentrations, FAN concentrations and high operating pH values.



	Alkalinity (mg/L) at day 14	Alkalinity (mg/L) at day 30	Alkalinity (mg/L) at day 60
pH 7.5	20200	21600	18600
pH 8.0	20900	23700	22700
pH 8.5	22500	24800	35300

Figure 4.28: Alkalinity under gradual TAN loading (Phase 2)

4.35 Phase 3 - Semi-continuous Mode - SW

Under semi-continuous reactor phase, 3 g of the digestate containing high ammonia concentration was replaced with fresh substrate. Figure 4.29 shows the DBP from the batch reactors converted to semi-continuous reactors. With the injection of fresh substrates mesophilic bacteria were to resume into activity as seen in DBP shown in Figure 4.29. Although biogas production started with replacement of the digestate with fresh substrate, biogas production reduced drastically after the first 3 days. This may possibly be attributed to the fact that the bacteria were only able to consume the readily biodegrade portion of the fresh substrate coming into the system.

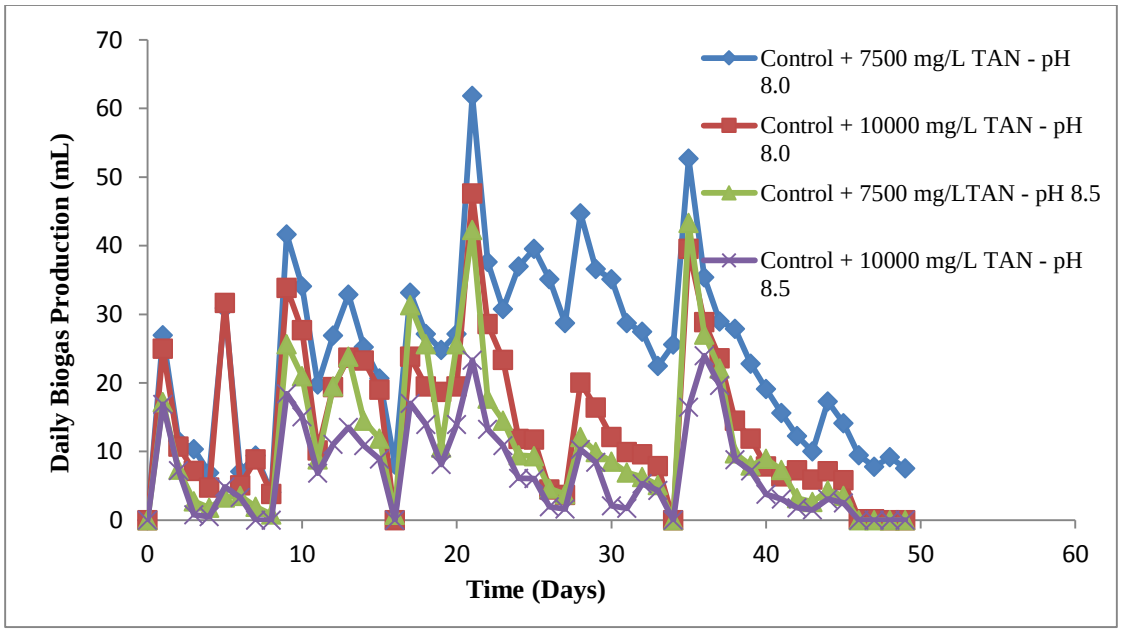


Figure 4.29: Daily biogas production under semi-continuous mode (Phase 3)

Reactors containing TAN concentration of 7,500 mg/L (786 mg/L-FAN) and 10,000 (380 mg/L-FAN) mg/L at pH 8.0 had the highest DBP under the semi-continuous mode at each of the HRTs tested. This is because these reactors have the lowest FAN concentrations. Under pH 8.5, reactors containing TAN concentrations of 7,500 mg/L (2073 mg/L-FAN) and 10,000 mg/L (2800 mg/L-FAN) had the least DBP. It should be noted that based on the loading rate of the fresh substrate injected into the system, the HRTs will be 267 days, 467 days and 1000 days. However in reality, HRT should not be less than 10-20 days. The purpose was to examine if the methanogens would return to activity after the injection of fresh substrate.

4.4 Conclusion

The effect of ammonia on AD of OFMSW was studied under TAN concentrations of 2500 mg/L, 5000 mg/L, 7500 mg/L, and 10,000 mg/L at pH 7.5, 8.0 and 8.5. Results confirmed that ammonia is inhibitory to methanogenic bacteria. CBP reduced with increasing TAN concentration as shown in Figure 4.32 activity especially at 7,500 mg/L and 10,000 mg/L. Percentage reduction in CBP compared with CB-reactors was as much

43 %, 64 % and 77 % in reactors containing 7500 mg/L TAN at pH 7.5, pH 8.0 and pH 8.5. CBP reduced to 80-85 % in reactors containing 10,000 mg/L TAN across the pH examined, as shown in Table 4.6.

Table 4.6: % Reduction in Cumulative Biogas Production compared to CB-reactors

% Reduction in Cumulative Biogas Production			
BMP	pH 7.5	pH 8.0	pH 8.5
Control + 2500 mg/L TAN	7	9	13
Control + 5000 mg/L TAN	17	18	30
Control + 7500 mg/L TAN	43	64	77
Control + 10000 mg/L TAN	80	84	85

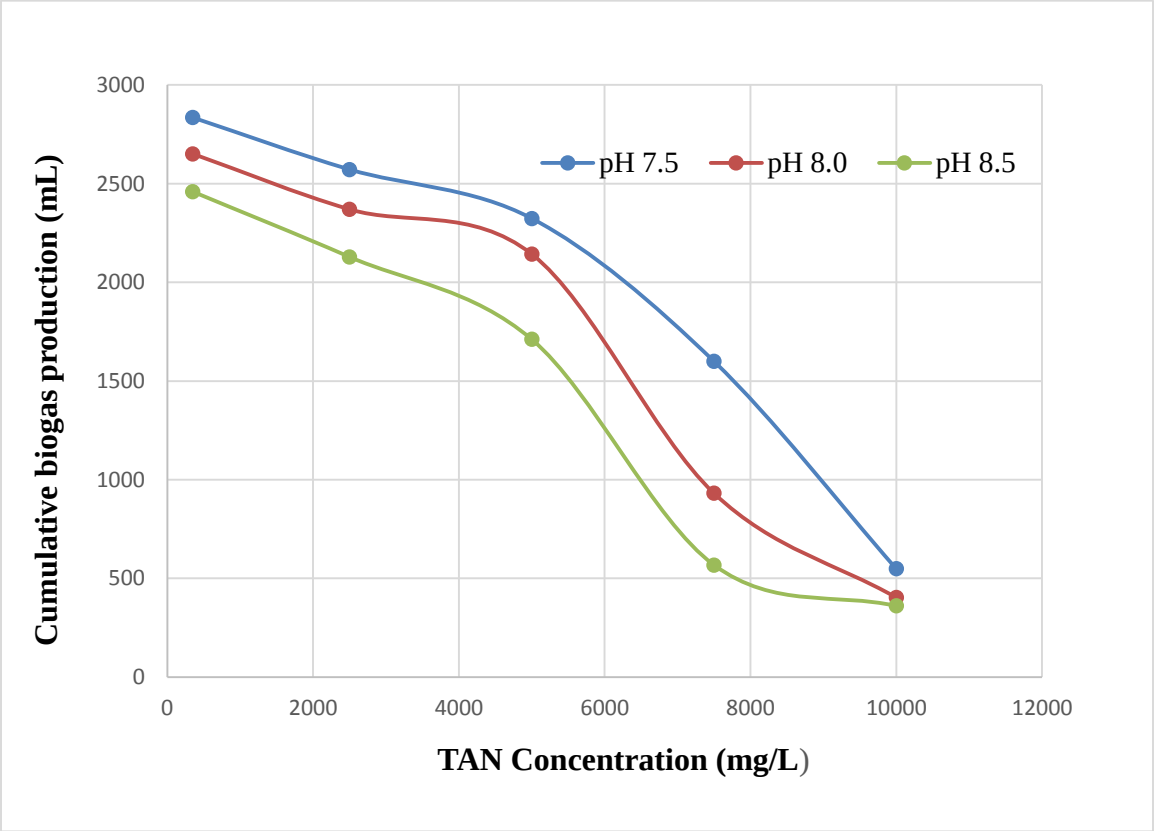


Figure 4.30: Cumulative biogas production under various TAN concentrations.

The study also showed that pH influenced the inhibitory capacity and the FAN component of TAN. At high pH (i.e. 8.5), FAN component of TAN was about 26 % and was inhibitory to the methanogens. Results also showed that mesophilic bacteria could be adapted to a TAN concentration of about 5000 mg/L at pH 7.5 through gradual TAN loading. Also, the reactors under gradual TAN loading had 1200 mL of CBP than reactors under sudden TAN loading of 10,000 mg/L at pH 7.5. Replacing 3g of digestate containing high TAN concentrations of 7500 mg/L and 10,000 mg/L with 3 g fresh substrate enhanced the return to activity of the methanogens as the observed in the semi-continuous mode.

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Chapter 5

TECHNICAL PAPER II

The Toxicity Effects of Ammonia on Anaerobic Digestion of Organic Fraction of Municipal Solid Waste Mixed with Real Landfill Leachate

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Abstract

The effect of ammonia on the anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) mixed with real landfill leachate to simulate an anaerobic bioreactor landfill was investigated in this study. The tests were conducted under different total ammonia nitrogen (TAN) concentrations of 7500 mg/L, 10,000 mg/L and pH 7.5 and 8.5. Results confirmed that ammonia is toxic to anaerobic digestion, as Cumulative Biogas Production (CBP) reduced as the TAN concentration increased. Compared with control reactors, reactors containing 7500 mg/L TAN at pH 8.0 and pH 8.5 had 61 % and 80 % reduction in CBP. Likewise, reactors containing 10,000 mg/L TAN at pH 8.0 and pH 8.5 had 68 % and 85 % reduction in CBP, compared with control reactors. The operating pH influenced the level of toxicity and composition of Total Ammonia Nitrogen (TAN). At high pH (i.e. 8.5), FAN component of TAN was about 26 % and was inhibitory to the methanogens. Lastly, results also showed that mesophilic bacteria could be adapted to a TAN concentration of about 5000 mg/L at pH 7.5 through gradual TAN loading.

Keywords: total ammonia nitrogen, free ammonia nitrogen, organic fraction of municipal solid waste

5.1 Introduction

Municipal Solid Waste (MSW) is a combination of wastes generated from residential households and apartment buildings, commercial and institutional establishments, construction and demolition waste, municipal services, and treatment plants (Tchobanoglous, et al., 1993; Staley and Barlaz, 2009). MSW generation rate is a global concern because the generation rate increases with increasing world population. The World Bank recently published that about 1.3 billion tons of MSW is generated globally per year and projected waste generation rate to increase by 70 % by the year 2025. Also, the annual global cost of waste disposal is expected to increase from $ 205 billion to $ 375 billion (World Bank, 2015). In year 2008, Statistics Canada published that over 25 million tonnes of MSW was deposited into landfills across Canada. Out of the 25 million tonnes of waste landfilled, household wastes accounted for about 13 million tonnes (Statistics Canada, 2008). The anaerobic digestion (AD) of MSW in anaerobic bioreactor landfills enhances waste degradation and the biogas generated in the process can be used for energy recovery. Leachate recirculation in anaerobic bioreactor landfills leads to an increase in waste density, resulting in a better utilization of the landfill capacity (Benson et al., 2007). However, leachate recirculation as practiced in anaerobic bioreactor landfills increases the rate of ammonification, resulting in accumulation of higher levels of ammonia, which intensifies the toxicity of the leachate. This is due to the fact that there is no degradation pathway for ammonia in anaerobic systems (Nair et al., 2014). High concentrations of ammonia in recirculated leachate could undermine the anaerobic digestion process performance by inhibiting microorganisms and result in low methane production. Ammonia exists in two forms as ionized ammonia or ammonium (NH_4^+) and unionized ammonia or free ammonia (NH_3). The combination of these two forms of ammonia is expressed as total ammonia nitrogen (TAN). The percentages of these two forms of ammonia in TAN vary with temperature and pH (Ding and Sartaj, 2015; Dong and Sartaj 2015).

The inhibitory effects of ammonia on the AD of high and low organic matters at various operating temperatures and pH values have been reported (Pfeffer, 1974; Braun et al., 1981; Webb and Hawkes 1985; Hashimoto, 1986; Blomgren et al., 1990; Angelidaki and Ahring, 1993). Kayhanian (1994) studied the impact of high ammonia concentrations on the biodegradation of the OFMSW using varying ammonia

concentrations. It was reported that an instant inhibition occurred at 1000 mg/L ammonia concentration, followed by a 50% inhibition and a complete bioreactor failure occurring at 1500, and 2500 mg/L ammonia concentrations respectively. Using varying ammonia concentrations of 48.8, 73.8, 98.8, 148.8, 248.8, 448.8, and 848.8 mg/L, Sossa et al. (2004) studied the inhibitory effects of ammonia on anaerobic film enriched by methylaminotrophic methane producing Archaea. The authors reported that maximum methanogenic activity occurred at 48.8 mg/L ammonia concentration. However, methanogenic activity was significantly inhibited at 848.8 mg/L ammonia concentration.

When present in high concentration, ammonium can directly inhibit biogas production during AD however; free ammonia (FAN) has been reported as the major inhibitor of AD process at high pH and temperature. This is because FAN has the capability of penetrating through the cell membrane of bacteria (Sung & Liu, 2003). McCarty & McKinney (1991) reported that FAN was more responsible for AD process inhibition than TAN, and methanogenic activity was inhibited when FAN concentration reached 150 mg/L– NH_3. Bhattacharya and Parkin (1989) reported a threshold FAN concentration of 55 ± 11 mg/L–NH_3 while Braun et al., 1981 reported a slightly higher FAN concentration threshold of 80 mg/L–NH_3.

The reason for the various thresholds of inhibitory ammonia concentration reported can be attributed to the various initial ammonia concentrations examined, process temperature, operating pH, organic loading rate and acclimation of inoculums. While several studies have been carried out to examine the inhibitory effects of ammonia on AD of wastewater, there is only limited information about the inhibitory effects of ammonia on AD of OFMSW (Yenigün and Demirel, 2013).

This research focuses on the effects of ammonia on AD OFMSW and research objectives are summarized below:

- To examine the possible inhibitory effect(s) of different ammonia concentrations on the mesophilic AD of the OFMSW, under different operating pH levels of 7.5, and 8.5, at similar operating temperature of 35 ^{o}C.
- To examine the possibility of reducing the inhibitory effect(s) of ammonia on AD by acclimating the bacteria to high ammonia concentrations, through gradual loading of influent ammonia concentrations.

5.2 Materials and Methodology

This experimental study was carried out in two phases, the Biochemical Methane Potential (BMP) phase, and the gradual TAN loading phase.

The first phase was the Biochemical Methane Potential (BMP) test phase. In the first phase, the effect of ammonia on anaerobic digestion of OFMSW with real landfill leachate was studied, to simulate an anaerobic bioreactor landfill. The 500 mL Kimax® glass bottles use were capped with butyl rubber stoppers. The BMP test was carried out using mesophilic anaerobically digested inoculums volume of 40 mL, 80ml of anaerobic landfill leachate, 30 gm of OFMSW, TAN concentrations 7,500 and 10,000 mg/L. Equal portions of $NaHCO_3$ and $KHCO_3$ were added as buffer. The total working volume of the mixture was brought to 300 mL by the addition of distilled water (DW).As circumstances required, 1N Hydrochloric Acid (1N HCl) solution and 5N Sodium Hydroxide (5N NaOH) solution were used to adjust the pH to the desired value. The inoculums BMP bottles, control BMP bottles, and BMP bottles containing 7,500 and 10,000 mg/L, TAN concentrations were set up in duplicates. A total 20 BMP bottles were set up for this phase.

The second phase of the experiment was gradual TAN loading. This was carried out to examine the possibility of adapting thermophilic bacteria to high ammonia concentration. At the beginning, TAN concentration was raised to 1000 mg/L and further increased by 1000 mg/L weekly. The OFMSW used in this study was synthesized using food components that are representative of a typical domestic kitchen and commercial kitchen food waste in Canada. The percentages of the different components of the OFMSW had standard compositions of protein, hydrocarbon, vegetable and fat in conformity with the Canadian Food Guide, as shown in Table 5.1 (Ara, 2012). In order to obtain consistent results, each set of simulated OFMSW was prepared weekly and the remaining modeled OFMSW was frozen up at a temperature of -4 ^{o}C, to prevent the likelihood of any fermentation. The carrots, cabbage, banana, and apple used were fresh. The ground beef was cooked for 30 mins while the rice and pasta were cooked differently for 15 minutes each in a rice cooker. Prior to being used, the OFMSW was thoroughly mixed and blended to form a slurry having a particle size ranging from 1-2 mm, using a

kitchen food processor. The inoculums used in this research was obtained from the effluent of the AD digester used for the AD of sludge at the Robert O. Pickard Environmental Center (ROPEC), Ottawa, Ontario, Canada. In order to keep the inoculums alive, the inoculums were kept in suspension by agitation in a New Brunswick Scientific Controlled Environment Incubator Shaker rotating at 86 revolutions per minute (rpm) at 35°C. Mature landfill leachate, aged between 15-25 years was obtained from a municipal landfill in Ottawa was used in this study. The leachate was stored at a temperature of 4° C until used and it was incubated at35 °C in a Shaker for 24 hours before being used.

Table 5.1: Configuration of the simulated OFMSW used

OFMSW Configuration	
Composition	**Percentage Weight (% w/w)**
Carrot	11
Cooked rice	18
White cabbage	10
Cooked pasta	18
Banana	11
Dog food	11
Apple	11
Cooked ground beef	10
Total	100

Prior to being used for batch experimental studies, the OFMSW and the inoculums were characterized as shown in Table 5.2 for COD, Volatile Solids (VS), alkalinity, and Total Solids (TS).

Table 5.2: Properties of inoculums, leachate and OFMSW

Properties	COD mg/L	pH	TAN mg/L	Alkalinity as CaCo3 mg/L	VFA (acetic acid) mg/L
OFMSW	177,000	4.5	350	2,750	9600
Inoculums	11050	6.9	877	14,600	7500
Leachate	4425	9.0	1878	6725	549

5.2.1 Materials and Equipment

Hach TNT plus™ 822 high range reagent vials (Method 8000) were used to measure the chemical oxygen demand (COD). Samples were first homogenized and well mixed to form a slurry paste using the Brinkmann Polytron PT 3000 homogenizer before COD concentrations were measured. TAN was measured using the salicylate method, with the use of TNT plus™ 832 reagent vials (Method 10205, Hach, USA).Total alkalinity determination was carried out according colorimetric method using TNTplus™870 reagent vials with alkalinity measuring range of 25 to 400 mg/L $CaCO_3$ (Method 10239, Hach USA).VFA was measured as acetic acids using TNT plus™ 872 with measuring range of 50 to 2,500 mg/L Acetic Acid. The UV-VIS. Prior to the analyses of TAN, VFA and total alkalinity, samples were centrifuged in a ThermoscientificSorvall Legend T+ model centrifuge at 10,000 rpm for 30 minutes. Once centrifuged, the supernatant was poured onto and filtered through filters with nominal pore size of 0.45 µm filters using a Fisher Scientific pump (Wilkinson, 2011).

The daily biogas production was recorded daily with a BD 21G½ needle connected to a u-tube manometer, until the biogas production rates were less than 1 % of the cumulative biogas produced. The daily biogas production from reactors containing leachate and inoculums only was subtracted from the daily biogas production from CB-reactors and reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L. Biogas composition of the biogas produced was analyzed using thermal conductivity gas

chromatograph (series 400, Gow-Mac Instrument Co., USA). Fisher Accumet model XL25 dual channel pH/ion meter equipped with a glass electrode was used to measure the pH of all inoculums, substrates (OFMSW) and BMP samples. The carrier gas used for the separation of the different biogas compositions was helium, operated at inflow rate of 30 mL/min. Biogas samples (0.5 mL) were taken in duplicates and injected into the column through the injection port. Spectrophotometer was used to measure the COD, VFA, TAN, Total and Alkalinity concentrations.

5.3 Results and Discussions

The results of the various batch tests conducted showed that the TAN and FAN have significant effects on the AD of the OFMSW. This is particularly observable in the difference between the amount of biogas produced by control batch reactors and batch reactors containing various TAN concentrations of 7,500 mg/L, and 10,000 mg/L under identical pH values of 7.5, and 8.5.

Also, the results of the experimental studies conducted confirmed that pH has a significant effect on the severity of the inhibitory effect of TAN on the AD of the OFMSW. However, the effect of pH was not very significant on control batch reactors. As discussed earlier, the percentage of FAN in TAN varies with pH. Under identical TAN concentrations and different pH levels, the FAN content of TAN varied significantly and this had a resultant effect on the variation in the biogas produced by batch reactors containing similar TAN concentrations.

Experimental studies on the effect of gradual TAN loading indicated that the gradual increase of TAN allowed methanogenic bacteria to become acclimated to high TAN concentration of about 5000 mg/L.

5.3.1 Phase 1 – (SW+L)

5.3.1.1 The Effect of TAN at pH 7.5

The cumulative biogas production (CBP) from control BMP reactors (CB-reactors), reactors containing leachate and inoculums only and reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L, under similar operating pH of 7.5 is

shown in Figure 5.1. The daily biogas production from reactors containing leachate and inoculums only was subtracted from the daily biogas production from CB-reactors and reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L. Methanogenic activity inhibition by ammonia was evident from the first day of the experiment. On day 1 of the experiment, (CB-reactors) produced an average daily biogas of 152 ml while reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L produced 23 mL and 13 mL respectively. At the end of the experiment, CB-reactors produced CBP of 2853 mL while BMP reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L produced 1108 mL, 904 mL respectively. This implies reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L has 61 % and 68 % reductions in biogas respectively, compared with CB-reactors.

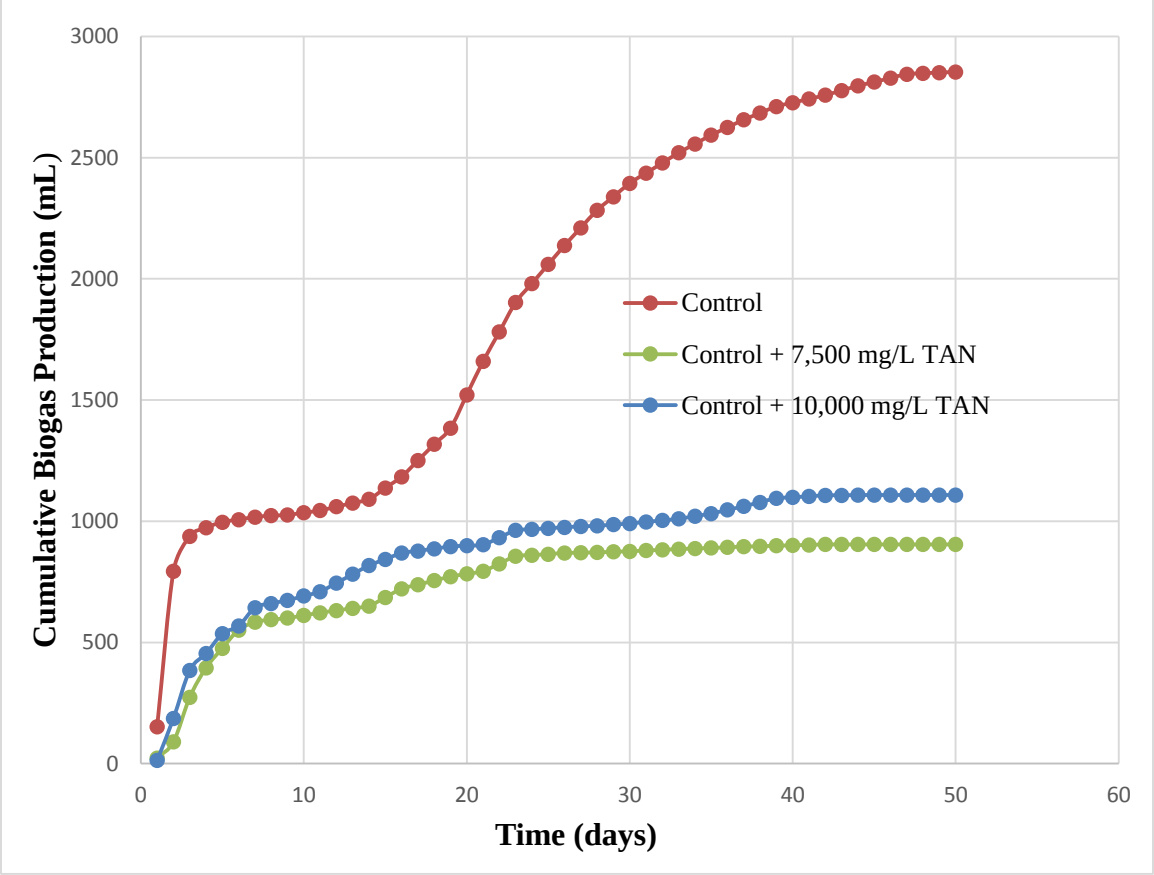
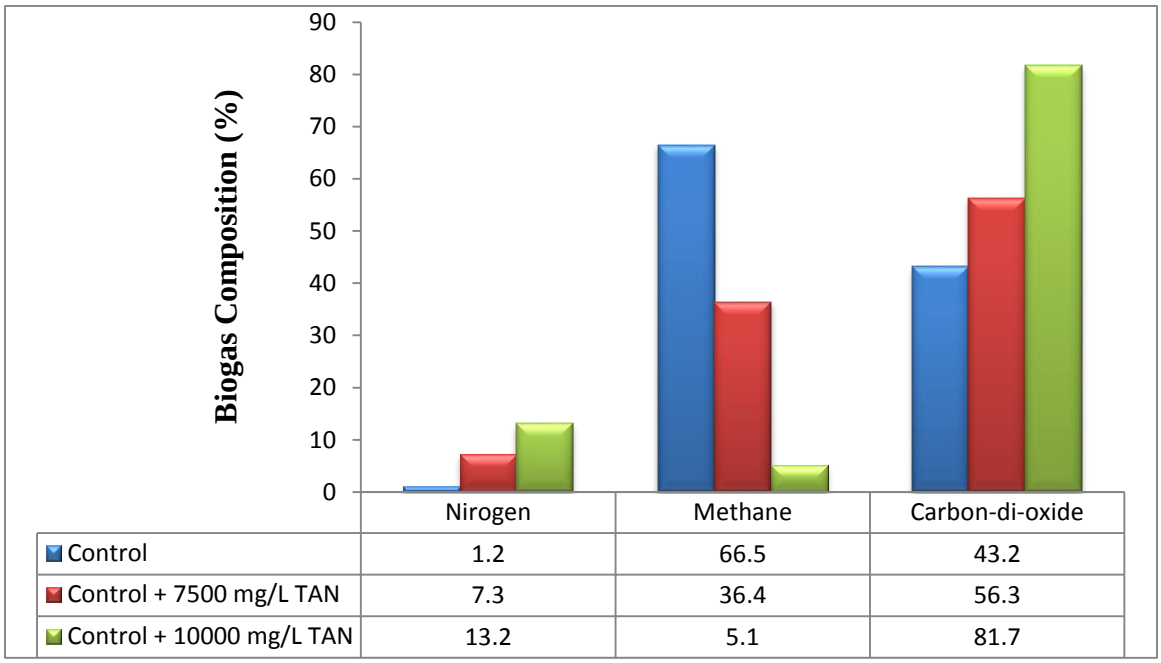


Figure 5.1: Biogas production from BMP reactors under different TAN concentrations and similar operating pH of 7.5 (Phase 1)

Consistent with the study of Sawayama et al., (2004) and Strik et al., (2006), biogas composition analyses showed that the percentage of methane in the biogas reduced with increasing TAN concentration. Methane percentage was negligible in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L, compared with CB-reactors. As shown in Figure 5.2, the percentages of methane in the biogas analyzed in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L were 36.4 % and 5.1 %, respectively. However, the average methane percentage of the biogas produced by CB-reactors was 66.5 %. There was no noticeable increase in methane percentage of the biogas produced by reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L throughout the experiment, due to ammonia inhibition.

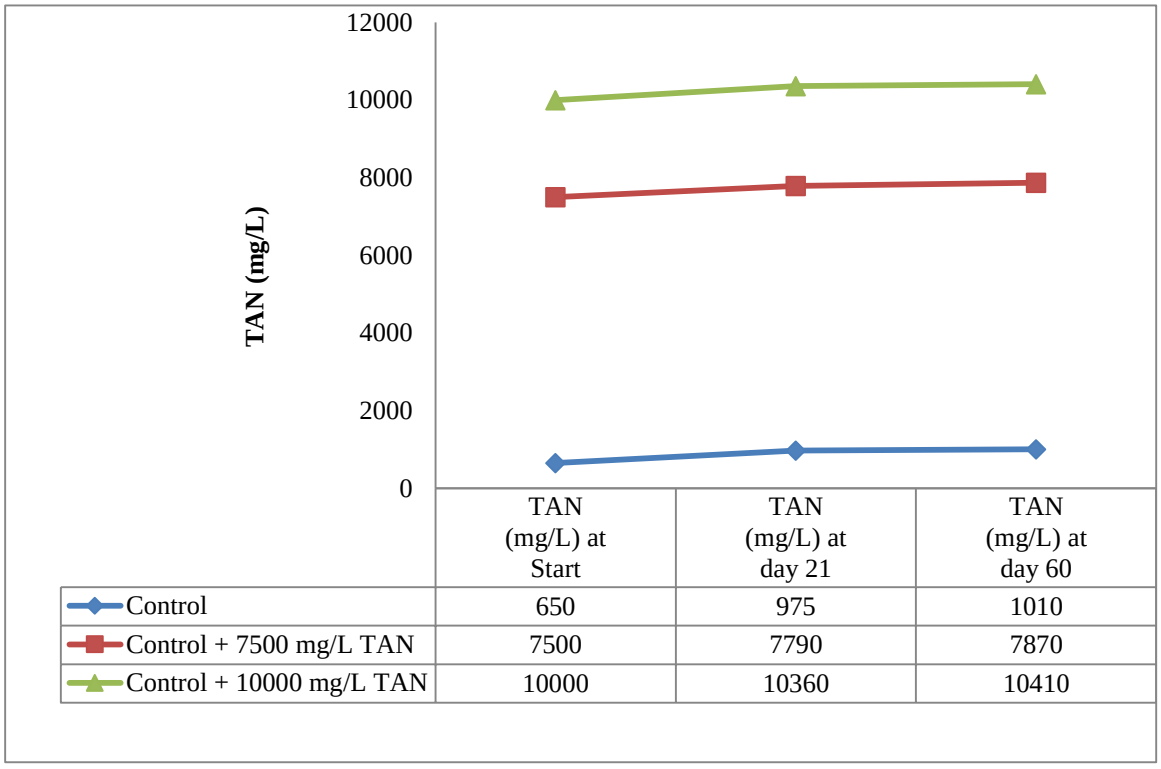


	Nirogen	Methane	Carbon-di-oxide
Control	1.2	66.5	43.2
Control + 7500 mg/L TAN	7.3	36.4	56.3
Control + 10000 mg/L TAN	13.2	5.1	81.7

Figure 5.2: Biogas Composition at pH 7.5 (Phase 1)

TAN concentrations increased in the reactors as evident in the TAN analyses carried out in the course of the experiment as shown in Figure 5.3. For the CB-reactors, the TAN concentration increased from 650 mg/L to 1010 mg/L. TAN concentrations increased from 7500 mg/L to 7870 mg/L, 10,000 mg/L to 10,410 mg/L in reactors containing initial TAN concentrations of 7500 mg/L and 10,000 mg/L respectively. This

increase can be attributed to the biodegradation of the proteins in the waste mixture (Nair et al., 2014).

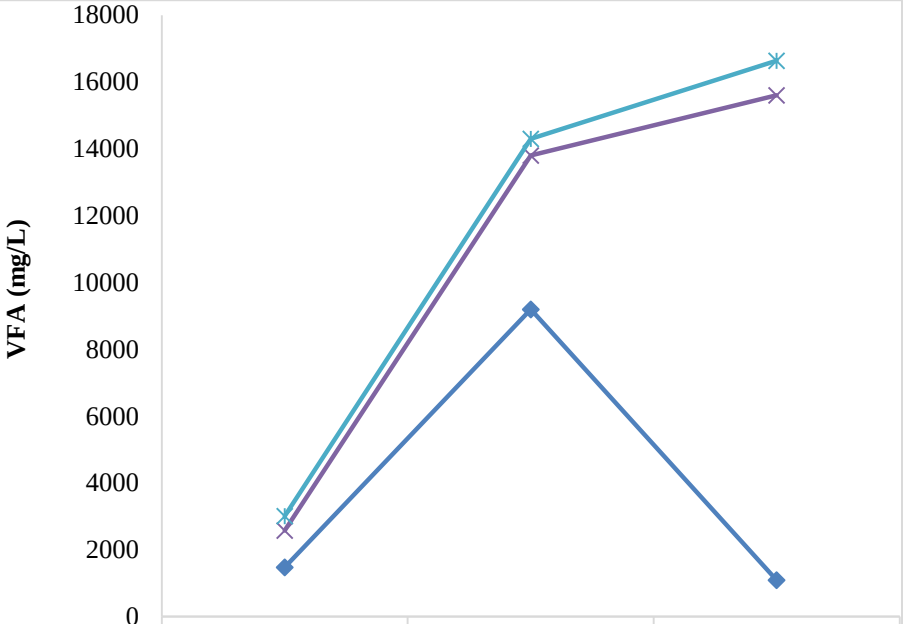


	TAN (mg/L) at Start	TAN (mg/L) at day 21	TAN (mg/L) at day 60
Control	650	975	1010
Control + 7500 mg/L TAN	7500	7790	7870
Control + 10000 mg/L TAN	10000	10360	10410

Figure 5.3: TAN concentration under pH 7.5 (Phase 1)

Figure 5.4 shows the VFA analyses carried out throughout the experiment. The VFA was measured as acetic acids. The average initial VFA concentration in the CB-reactors was approximately 1480 mg/L. The VFA was measured as acetic acids. Reactors containing 7,500 mg/L TAN and 10,000 mg/L TAN had initial VFA concentrations of 2575 and 3010 respectively. The VFA concentrations in all the reactors increased during the experiment as shown in Figure 5.4. On day 21, VFA concentration increased to 9,200 mg/L, 13,800 mg/L and 14,300 mg/L in CB-reactors and in reactors containing 7,500 mg/L and 10,000 mg/L of TAN, respectively. The increase can be attributed to the breakdown of the fatty acids, sugars and amino acids in the waste mixture (Gujer and Zehnder 1983). At the end of the experiment, CB-reactors had a VFA concentration of

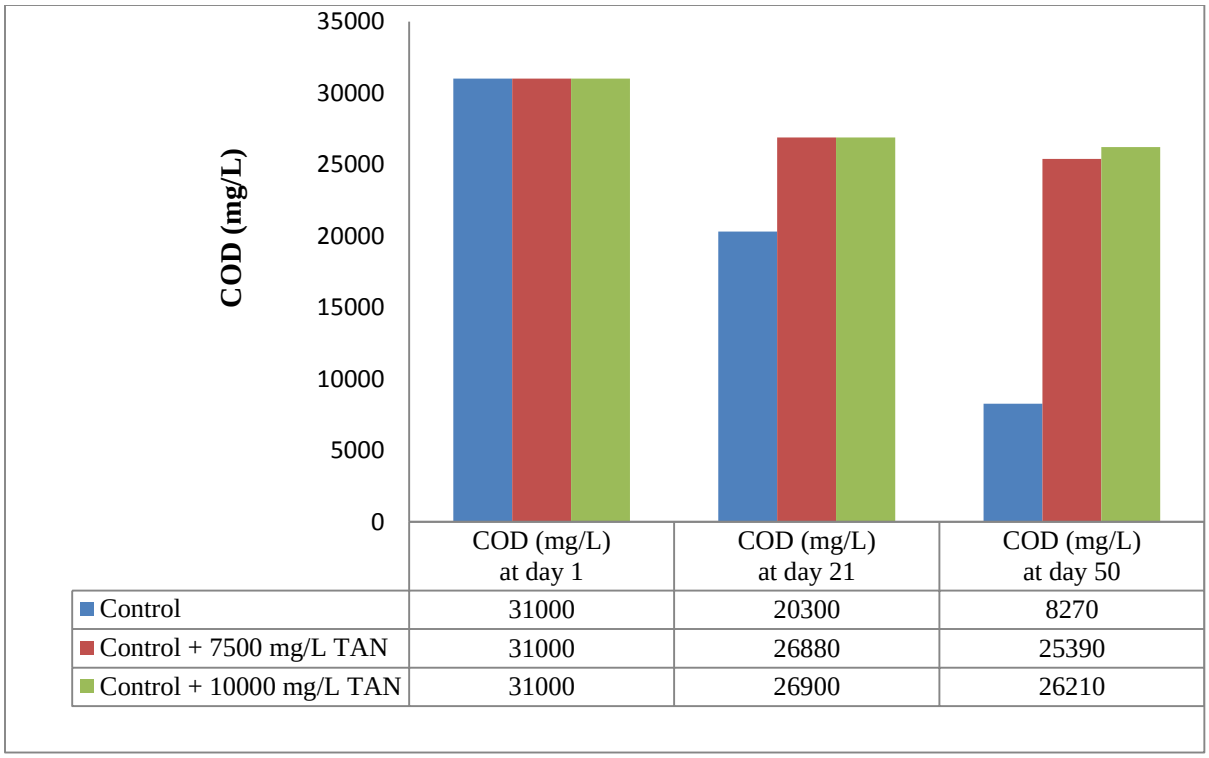
1090 mg/L while reactors containing 7,500 mg/L and 10,000 mg/L of TAN had VFA concentrations of 15,600 mg/L, and 16,640 mg/L respectively. The low acetic acids concentration observed in CB-reactors indicate that the bulk of the VFA produced had been used up by the methanogens to produce biogas. Whereas VFA accumulated in reactors containing 7,500 mg/L and 10,000 mg/L of TAN due to ammonia inhibition, preventing the utilization of the acetic acids produced for biogas production by the methanogens.



	VFA as Acetic Acid (mg/L) at Start	VFA as Acetic Acid (mg/L) at day 21	VFA as Acetic Acid (mg/L) at day 60
Control	1480	9200	1090
Control + 7500 mg/L TAN	2575	13800	15600
Control + 10000 mg/L TAN	3010	14300	16640

Figure 5.4: VFA concentration under pH 7.5 (Phase 1)

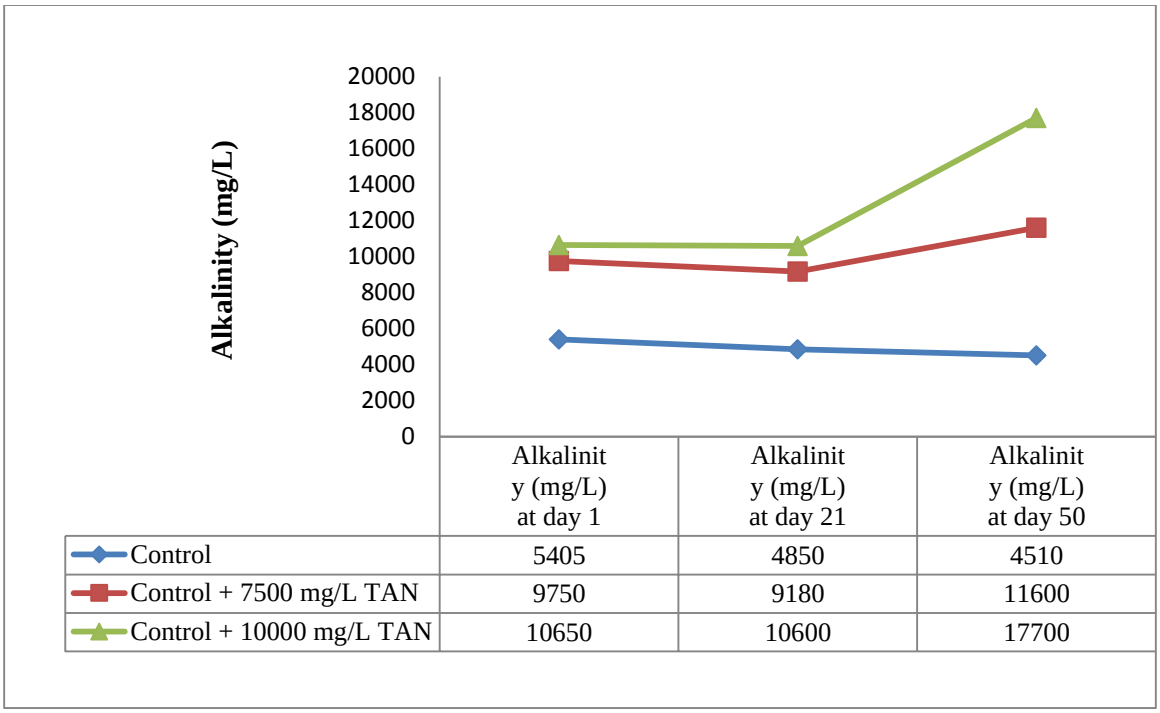
The analysis of the COD is presented in Figure 5.5. At the end of the experiment, CB-reactors had COD concentration of 8270 mg/L while reactors containing 7500 mg/L TAN and 10,000 mg/L TAN had a COD of 25,390 mg/L and 26,210 mg/L respectively. Due to ammonia toxicity, not much of the COD was used in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L.



	COD (mg/L) at day 1	COD (mg/L) at day 21	COD (mg/L) at day 50
Control	31000	20300	8270
Control + 7500 mg/L TAN	31000	26880	25390
Control + 10000 mg/L TAN	31000	26900	26210

Figure 5.5: COD concentration under pH 7.5 (Phase 1)

Alkalinity analyses carried out during the test showed that alkalinity increases as the concentration of TAN increases, as shown in Figure 5.6. The alkalinity increased at the end of the experiment, with alkalinity reaching 11,600 mg/L and 17,700 mg/L in reactors containing 7,500 mg/L and 10,000 mg/L of TAN. The low biogas production in reactors having high alkalinity can be attributed to alkalinity toxicity caused by high TAN concentrations in these reactors.



	Alkalinity (mg/L) at day 1	Alkalinity (mg/L) at day 21	Alkalinity (mg/L) at day 50
Control	5405	4850	4510
Control + 7500 mg/L TAN	9750	9180	11600
Control + 10000 mg/L TAN	10650	10600	17700

Figure 5.6: Alkalinity concentration under pH 7.5 (Phase 1)

5.3.1.2 The Effect of TAN at pH 8.5

Figure 5.7 shows the CBP at pH 8.5. As observed at pH 7.5, CBP reduced as the concentration of TAN increased. Similar to pH 7.5, the daily biogas production from reactors containing leachate and inoculums only was subtracted from the daily biogas production from CB-reactors and reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L. However, the effect of TAN on biogas was significant at the operating pH of 8.5. Methanogenic activity inhibition by ammonia was evident from the first day of the experiment. Reactors containing TAN concentrations of 7,500 mg/L and 10,000 mg/L had no biogas production on day 1, from the second to the third week and completely stopped producing biogas from day 38 to the end of the experiment. At the end of the experiment, CBP of 1624 mL was produced by CB-reactors while reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L produced 322 mL, 245 mL respectively. The CBP produced by reactors containing leachate and inoculums only was 23 mL.

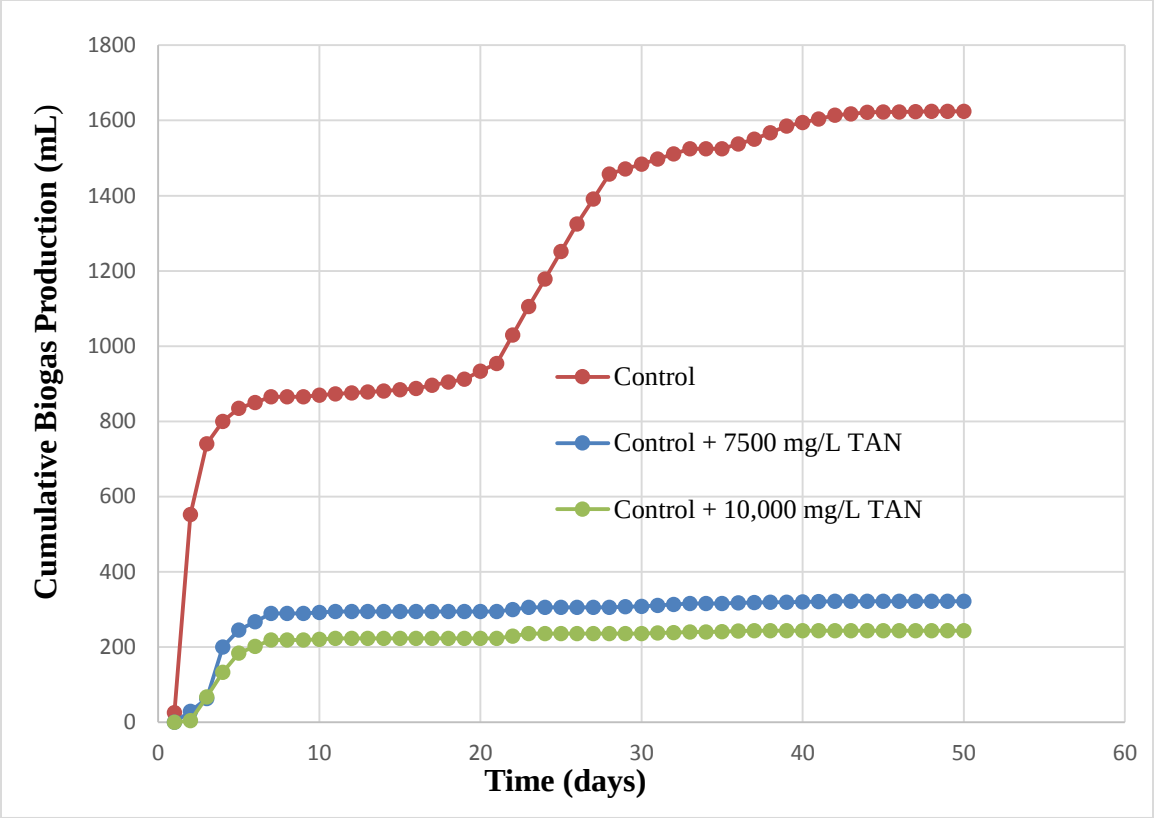
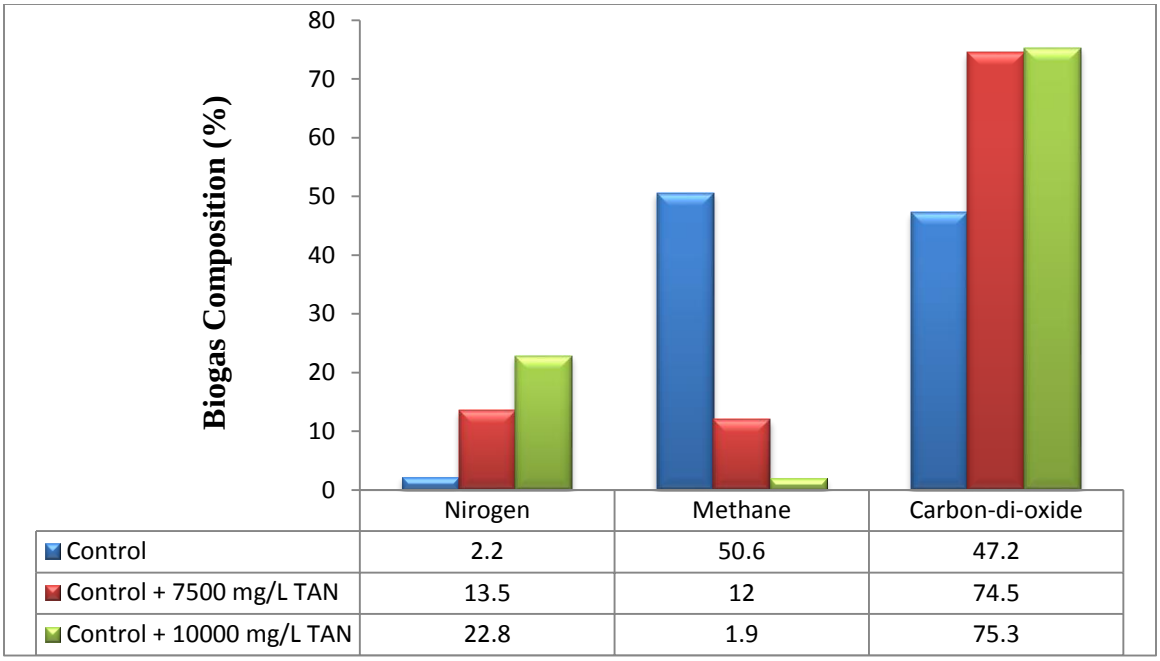


Figure 5.7: Cumulative biogas production under pH 8.5 (Phase 1)

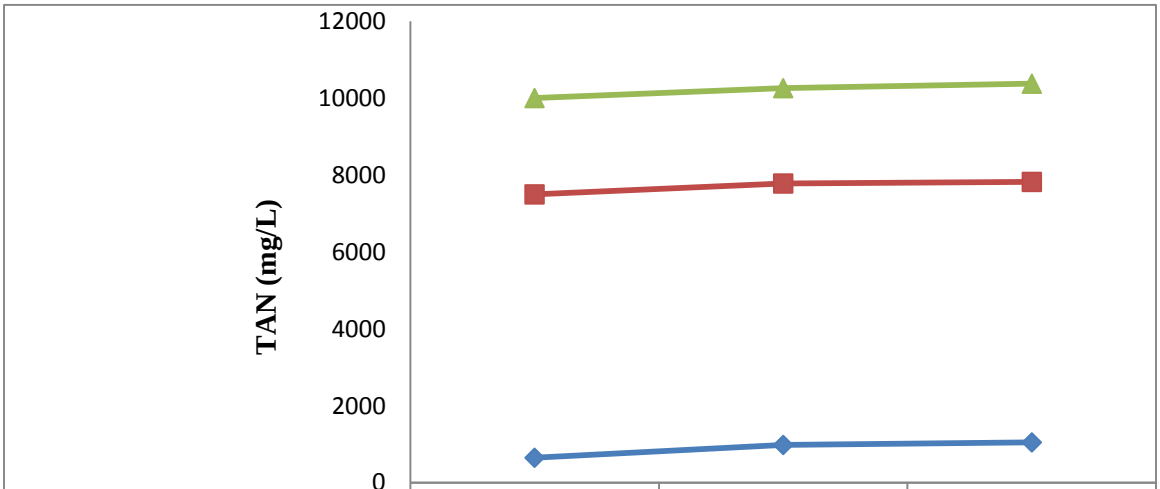
The biogas composition analysis at pH 8.5 is given in Figure 5.8 below. Similar to what obtained at pH 7.5, methane percentage reduced as the concentration of TAN increased. Methane percentages in the biogas produced by reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L were 12 % and 1.9 %, respectively. However, CB-reactors had 50.6 % of biogas as methane. Towards the end of the test, methane percentages in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L reached as low as 0 %.



	Nirogen	Methane	Carbon-di-oxide
Control	2.2	50.6	47.2
Control + 7500 mg/L TAN	13.5	12	74.5
Control + 10000 mg/L TAN	22.8	1.9	75.3

Figure 5.8: Biogas Composition under pH 8.5 (phase 1)

The analysis of the TAN concentrations in the reactors at pH 8.5 is presented in Figure 5.9.

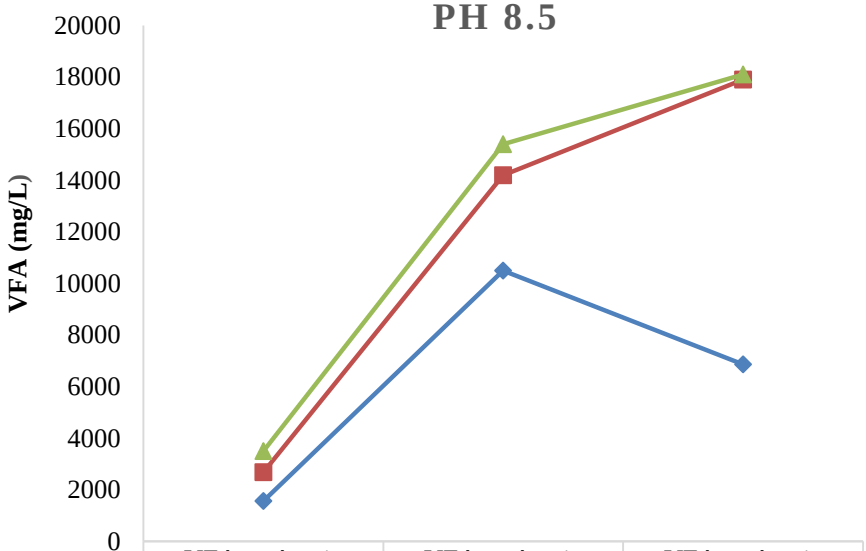


	TAN (mg/L) at Start	TAN (mg/L) at day 21	TAN (mg/L) at day 60
Control	650	986	1050
Control + 7500 mg/L TAN	7500	7780	7820
Control + 10000 mg/L TAN	10000	10260	10380

Figure 5.9: TAN Concentration under pH 8.5 (Phase 1)

The analysis showed that TAN concentrations increased in the reactors, as observed at pH 7.5 due to the hydrolysis of the protein in the waste mixture. At the end of the test, TAN concentration increased from 650 mg/L to 1050 mg/L in CB-reactors while TAN increased from 7500 mg/L to 7820 mg/L and from 10,000 mg/L to 10,380 mg/L in reactors containing initial TAN concentrations of 7,500 mg/L and 10,000 mg/L respectively.

Figure 5.10 shows the analysis of the VFA carried out during the experiment. At the beginning of the experiment, VFA concentration was 1560 mg/L in CB-reactors while containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L had initial VFA concentrations of 2678 mg/L and 3500 mg/L respectively.



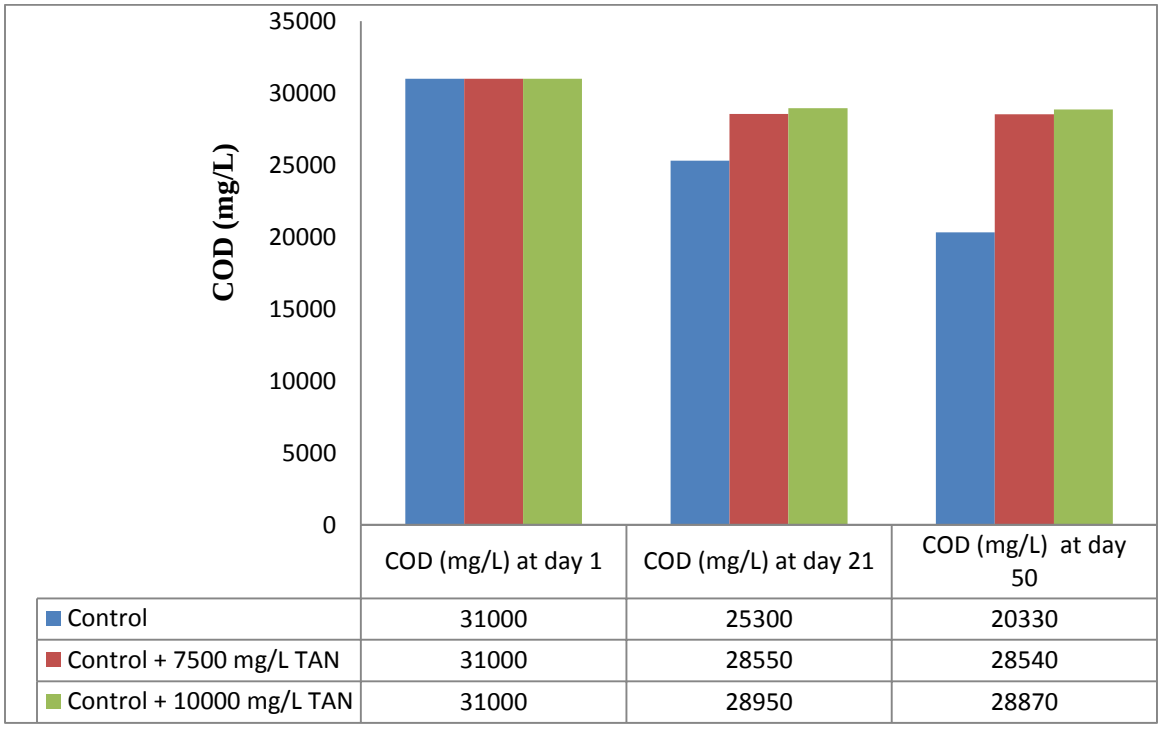
	VFA as Acetic Acid (mg/L) at Start	VFA as Acetic Acid (mg/L) at day 21	VFA as Acetic Acid (mg/L) at day 60
Control	1560	10500	6860
Control + 7500 mg/L TAN	2678	14200	17900
Control + 10000 mg/L TAN	3500	15400	18100

Figure 5.10: VFA Concentration under pH 8.5 (Phase 1)

On day 21, CB-reactors had VFA concentration of 10,500 mg/L while reactors containing TAN concentrations of 7,500 mg/L and 10,000 mg/L had VFA concentrations of 14,200 mg/L, and 15,400 mg/L respectively. This can be attributed to the breakdown of the

volatile acids, sugars and amino acids in the waste mixture. At the end of the experiment, VFA concentration had dropped to 6860 mg/L in CB-reactors while in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L, VFA increased to 17,900 mg/L and 18,100 mg/L respectively. The low concentration of VFA in CB-reactors indicates that the most of the VFA produced have been used up for biogas production, as reflected in the CBP of the CB-reactors. However, VFA accumulated in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L, due to the toxicity of ammonia on methanogenic bacteria, preventing the uptake of VFA for biogas production.

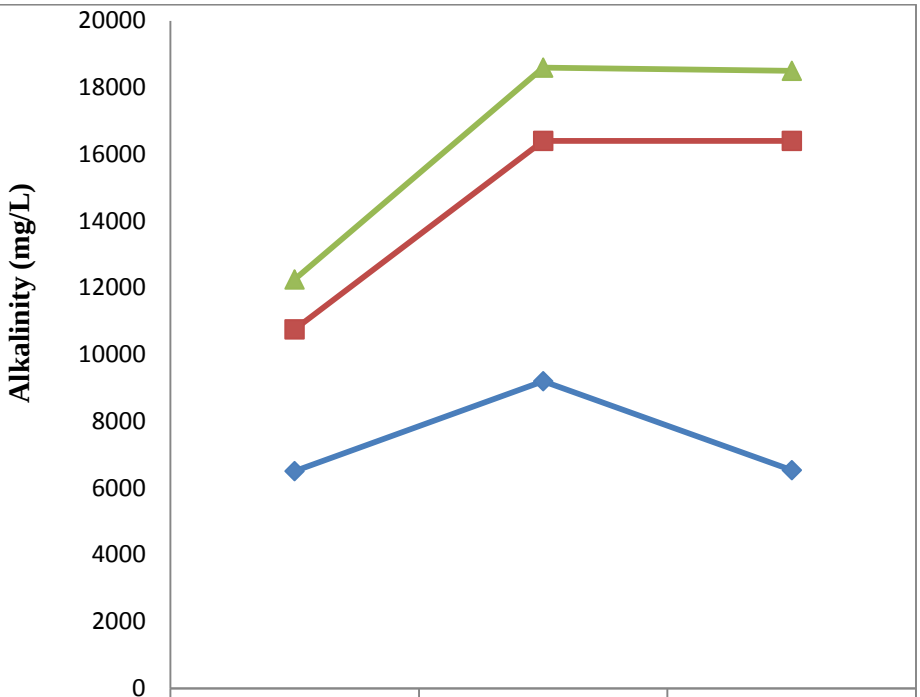
Figure 5.11 shows the COD analysis at pH 8.5. At the conclusion of the test, the COD concentration in CB-reactors was 23,330 mg/L while reactors containing 7500 mg/L TAN and 10,000 mg/L TAN had a COD of 28,540 mg/L and 28,870 mg/L respectively. As earlier discussed, ammonia toxicity reduced the utilization of the COD by bacteria in reactors containing TAN concentrations of 7,500 mg/L, and 10,000 mg/L.



	COD (mg/L) at day 1	COD (mg/L) at day 21	COD (mg/L) at day 50
Control	31000	25300	20330
Control + 7500 mg/L TAN	31000	28550	28540
Control + 10000 mg/L TAN	31000	28950	28870

Figure 5.11: COD Concentration under pH 8.5 (Phase 1)

The alkalinity analysis at operating pH of 8.5 is presented in Figure 5.12. Similar to the results obtained at pH 7.5, alkalinity increased as the concentration of TAN in the reactors increased. At the end of the experiment, alkalinity reached as high as 16,400 mg/L and 18,500 mg/L in reactors containing 7,500 mg/L and 10,000 mg/L of TAN respectively. The high alkalinity caused by high TAN concentrations in the reactors resulted in toxicity to the methanogenic bacteria as reflected in the low CBP in these reactors.



	Alkalinity (mg/L) at day 1	Alkalinity (mg/L) at day 21	Alkalinity (mg/L) at day 50
Control	6510	9200	6538
Control + 7500 mg/L TAN	10750	16400	16400
Control + 10000 mg/L TAN	12250	18600	18500

Figure 5.12: Alkalinity concentration under pH 8.5 (Phase 1)

5.3.2 The effect of pH

The effect of the operating pH on methanogenic activity was very significant throughout this study. A comparison of the CBP from reactors having similar initial TAN

concentrations at different pH values of 7.5, and 8.5 is shown in Figure 5.13 and Figure 5.14. For CB-reactors (Figure 5.13) CBP of 2854 mL was produced at pH 7.5, compared with CBP of 1624 mL produced at pH 8.5. This implies that CB-reactors operating at pH 8.5 produced 43% lesser biogas compared with CB-reactors operating at pH 7.5. This significant difference in biogas production may be attributed to the fact that the pH of 8.5 is not in the favourable pH range of 6.5-7.5 for methanogenic bacteria (Mohan et al., 2008)

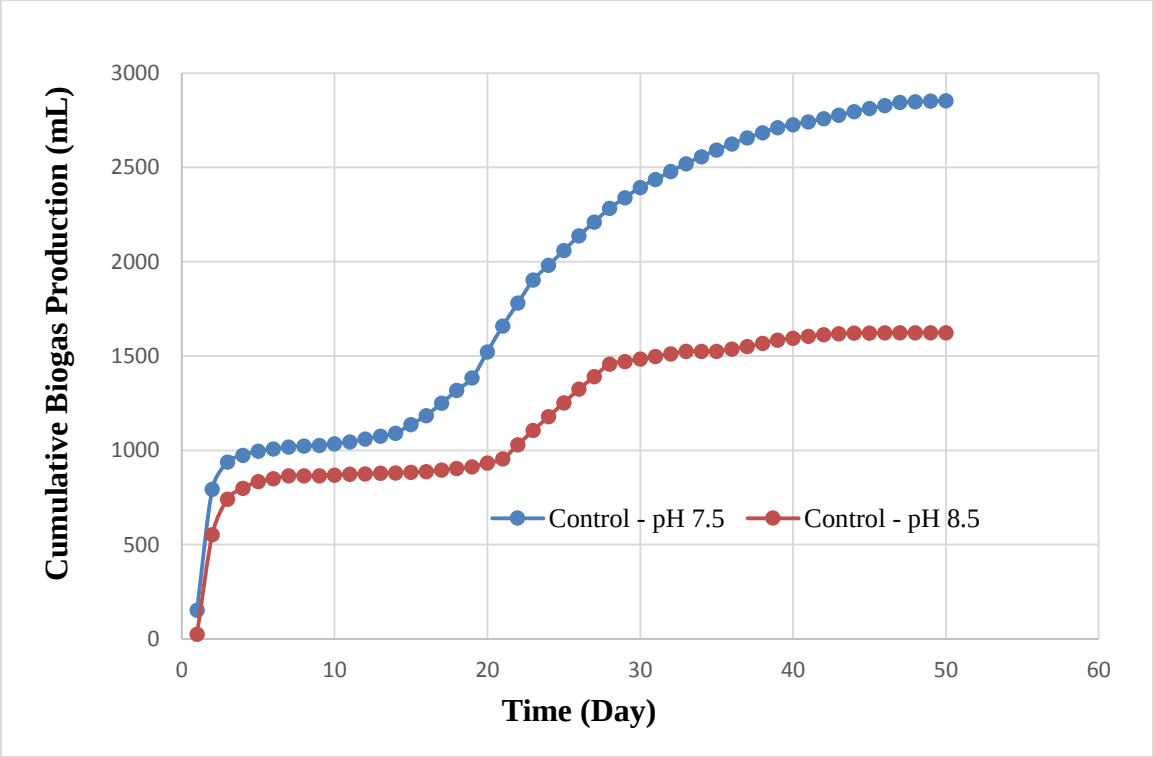


Figure 5.13: Cumulative Biogas Production under pH 7.5 vs pH 8.5 (Phase 1)

Also, the significant difference in CBP may be attributed to the difference in composition of the FAN in TAN concentrations at the different operating pH values of 7.5 and 8.5 as shown in Figure 5.15 and Table 5.3.

Throughout the study TAN concentrations in all the reactors were approximately equal but the FAN concentrations varied and increased with increasing pH and TAN concentration. FAN concentration reached as high as 278 mg/L at pH 8.5, while FAN concentration was just 34 mg/L at pH 7.5 at the end of the test. This shows that FAN

concentration of 278 mg/L is significantly inhibitory to methanogenic bacteria resulting in the low biogas production observed in CB-reactors at pH 8.5.

Similar results were observed in other reactors containing initial TAN concentrations of 7,500 mg/L and 10,000 mg/L at pH 7.5 and pH 8.5 as shown in Figure 5.13. CBP in reactors containing initial TAN concentration of 7,500 mg/L at pH 8.5 was 71 % lesser than the CBP at pH 7.5, while a 73 % lesser CBP at pH 8.5 was observed in reactors containing initial TAN concentration 10,000 mg/L operating at pH 7.5.

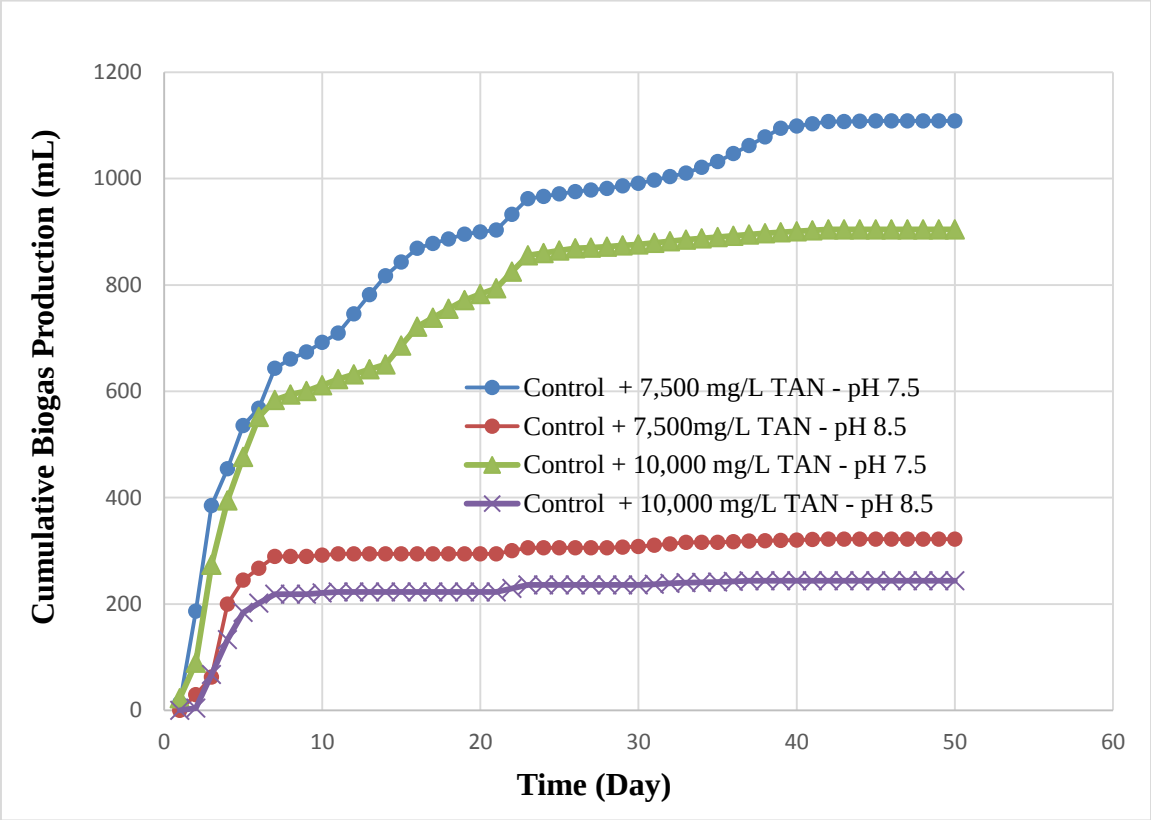


Figure 5.14: Biogas production at different pH values and similar TAN concentration of 7,500 mg/L and 10,000 mg/L (Phase 1)

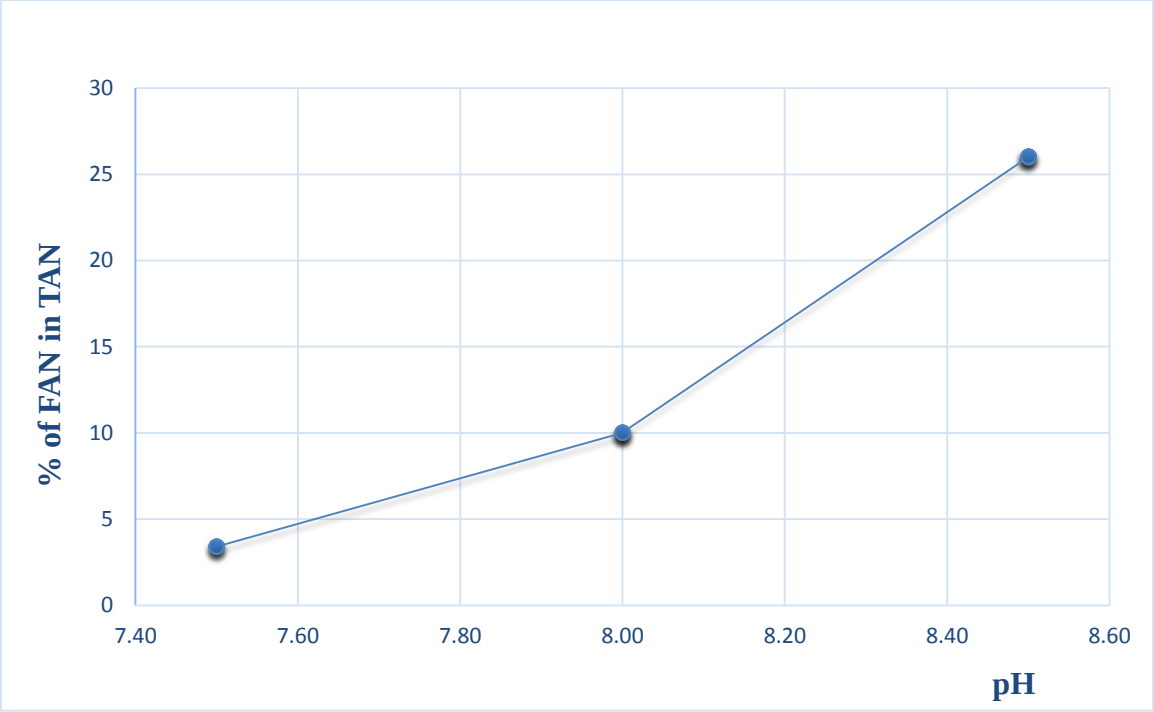


Figure 5.15: % of FAN in TAN as a function of pH and temperature.

Table 5.3: Percentage of FAN in TAN as a function of pH and temperature.

Initial TAN (mg/L)	pH	FAN (mg/L)	% FAN	Final TAN (mg/L)	pH	FAN (mg/L)	% FAN
650	7.50	22	3	1010	7.48	34	3
7500	7.50	255	3	7870	7.51	274	3
10000	7.50	340	3	10450	7.53	380	4
650	8.50	169	26	1050	8.51	278	26
7500	8.50	1952	26	8100	8.49	2073	26
10000	8.50	2603	26	10420	8.52	2806	27

The volume of methane produced per gram of COD degraded is presented in Table 5.4. Across all the operating pH levels examined, the volume of methane produced per gram of COD degraded (COD_d) reduced as the TAN concentrations in the systems increased. CB-reactors produced 278 mL CH_4 /g COD_d and 256 mL CH_4 /g COD_d at pH 7.5 and pH 8.5 respectively. However, reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L had 240 mL CH_4 /g COD_d, and 32 mL CH_4 /g COD_d at pH 7.5. Similar trends were observed in other pH levels as shown in Table 5.4. Across all pH levels, reactors containing TAN concentrations of 7500 mg/L and 10,000 mg/L had the least methane gas produced per gram of COD_d. Although the volumes of methane produced per of COD obtained are lesser than the standard 350 mL CH4 /g COD, The volume of methane produced per gram of COD_d by CB-reactors fall within the ranges reported in the literature (Alqaralleh et al., 2015)

Table 5.4: Methane produced per gram of COD degraded

BMP	pH 7.5	pH 8.5
	mL CH_4 / g COD_d	**mL CH_4 / g COD_d**
Control	278	256
Control + 7500 mg/L TAN	240	52
Control + 10000 mg/L TAN	32	7

5.3.3 Phase 2 - Gradual TAN Loading - (SW+L)

In order to examine the possibility of adapting the mesophilic bacteria, TAN concentration was increased gradually at 1000 mg/L-TAN weekly, at operating pH values of 7.5 and 8.5. Unlike reactors operating at pH 8.5, the inhibitory effect of TAN on biogas production was insignificant in at pH 7.5 in the first week.

The average DBP from the reactors when TAN loading was 1000 mg/L in the first week was 138 mL/d, at pH 7.5, about 7 ml/d lesser than the average DBP from CB-reactors at operating at pH 7.5. However, at pH 8.5, the reactors under gradual TAN loading had average DBP of 84 mL/d, 32% lesser than the average DBP from CB-reactors operating at pH 8.5. In the second week, with TAN concentration reaching 2,350

mg/L, the average DBP was approximately similar to the average DBP from CB-reactors operating at pH 7.5. In the 3rd week when TAN reached 3,420 mg/L, the reactors under gradual loading (GTL) produced 24 mL/day, 60% lower than the DBP from CB-reactors. By the start of the fifth week with TAN reaching 5,340 mg/L (182 mg/L-FAN), the reactors under GTL produced 11 mL/day of biogas, about 87% lesser than that of the CB-reactors. By the start of the 7th week to the start of the 8th week (day 49-50) with TAN reaching 8,430 mg/L TAN, GTL reactors had 0 mL/d of DBP while CB-reactor had 13 mL/d of DBP. The gradual TAN loading had more negative effect at pH 8.5. DBP from GTL reactors remained as low as 1.7 mL/d from the 3rd week to the 6th week and biogas production stopped completely from the 7th week. Figure 5.16 shows the CBP under gradual TAN loading up to 8,000 mg/L versus abrupt TAN loading of 7,500 mg/L at pH 7.5 and 8.5. Under GTL at pH 7.5, CBP reached as much as 2117 mL, compared with the 1108 mL produced under abrupt TAN loading. Likewise, under GTL at pH 8.5, CBP reached 1625 mL compared with the 322 mL produced under abrupt loading.

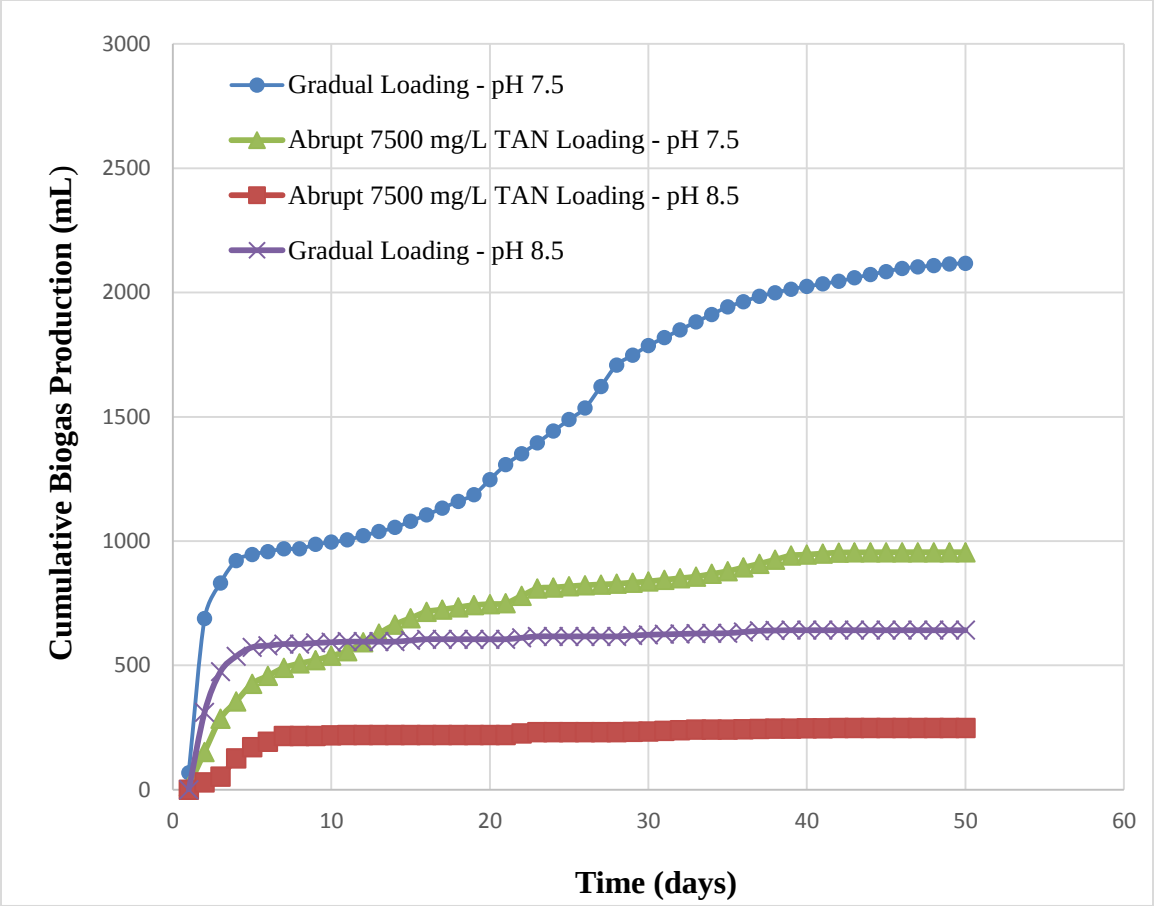
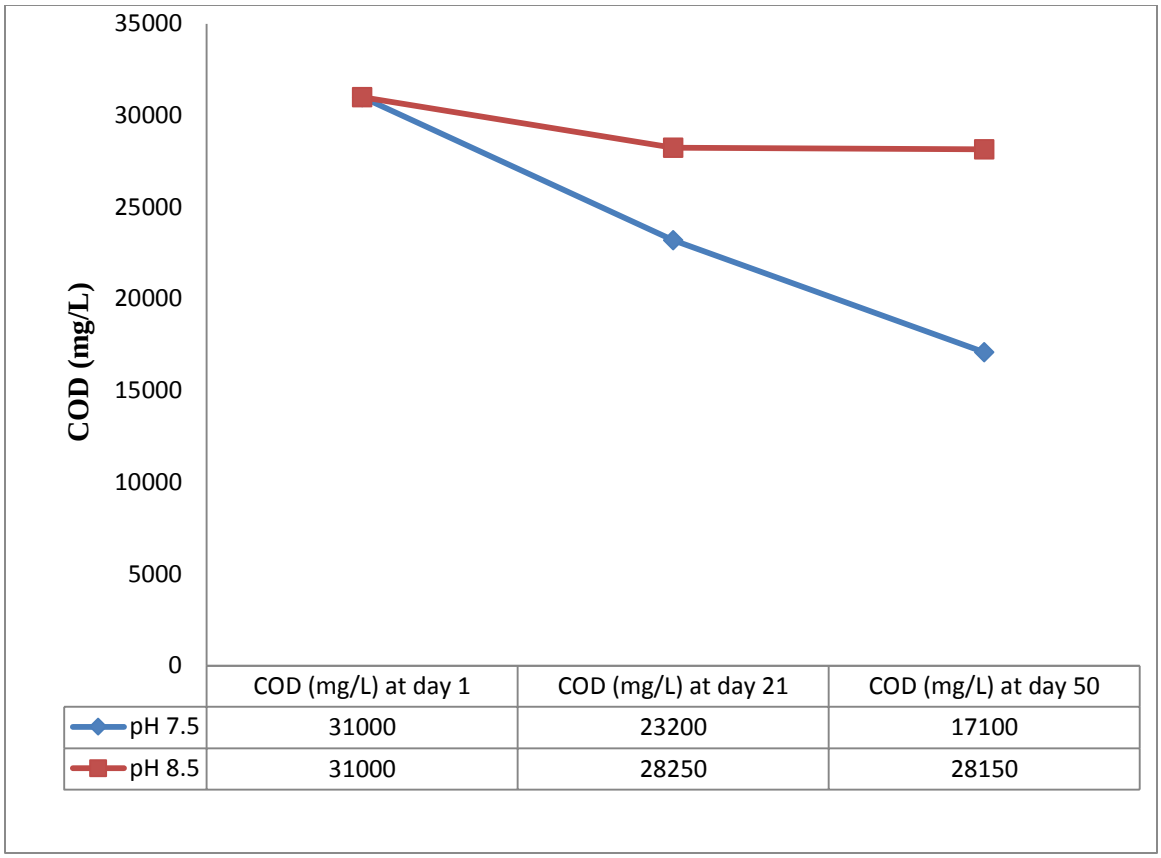


Figure 5.16: Biogas production under gradual and abrupt TAN loadings at pH 7.5 and pH 8.5 (Phase 2)

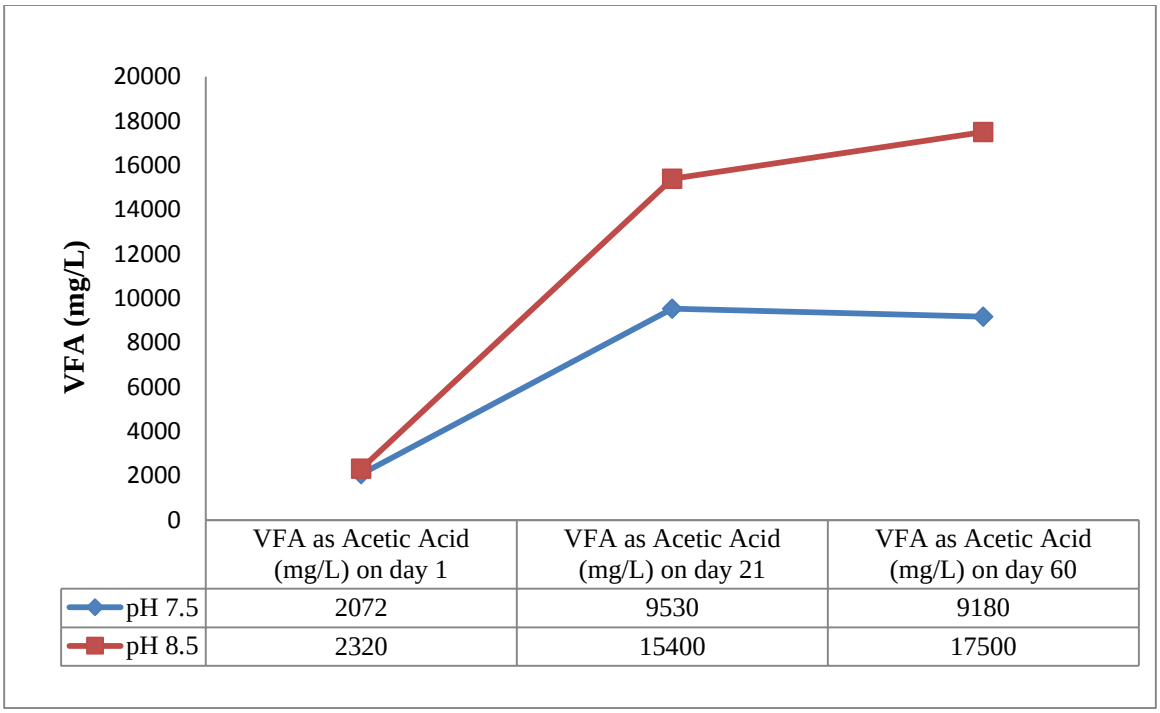
The COD analysis under gradual TAN loading is presented in Figure 5.17. The study shows that the COD consumption rate decreased as the concentration of TAN added increased. At the end of the study, COD concentrations in the reactors reduced to 17,100 mg/L, and 28,150 mg/L at pH 7.5 and 8.5 respectively. The observed difference in the effluent COD concentrations can be attributed to the fact FAN concentration inhibited the utilization of the COD for biogas production by the methanogens, especially at 8.5. As discussed earlier, FAN accumulation and inhibition increase with increasing pH and temperature.



	COD (mg/L) at day 1	COD (mg/L) at day 21	COD (mg/L) at day 50
pH 7.5	31000	23200	17100
pH 8.5	31000	28250	28150

Figure 5.17: COD concentration under gradual TAN loadings at pH 7.5 vs 8.5 (Phase 2)

Figure 5.18 shows the analysis of the VFA under gradual TAN loading. At the beginning of the experiment, VFA concentr Table 3.2ations in the reactors were 2072 mg/L, 2320 mg/L at pH of 7.5, and 8.5 respectively. At the end of 21 days, VFA concentrations in the reactors had increased to 9530 mg/L, and 15400 mg/L at pH of 7.5, and 8.5 respectively. With gradual TAN loading up to 8430 mg/L at the end of the test, VFA concentration reduced to 9180 mg/L at pH 7.5, 8.0 and increased to 17,500 mg/L at 8.5. This implies that at the pH of 7.5, the methanogens were able to utilize the bulk of the VFA for biogas production than at pH 8.5.



	VFA as Acetic Acid (mg/L) on day 1	VFA as Acetic Acid (mg/L) on day 21	VFA as Acetic Acid (mg/L) on day 60
pH 7.5	2072	9530	9180
pH 8.5	2320	15400	17500

Figure 5.18: VFA concentration under gradual TAN loadings at pH 7.5 vs pH 8.5 (Phase 2)

5.4 Conclusion

The effect of ammonia AD of MSW mixed with real landfill leachate to simulate an anaerobic bioreactor landfill was examined. The ammonia concentrations examined were 7500 mg/L and 10,000 mg/L under pH 7.5 and 8.5. The outcome of the study showed that ammonia is inhibitory to methanogenic activity. CBP reduced as the TAN concentration increased as shown in Figure 5.20. Compared with control reactors, reactors containing 7500 mg/L TAN at pH 8.0 and pH 8.5 had 61 % and 80 % reduction in CBP. Likewise, reactors containing 10,000 mg/L TAN at pH 8.0 and pH 8.5 had 68 % and 85 % reduction in CBP, compared with control reactors as shown in Table 5.5.

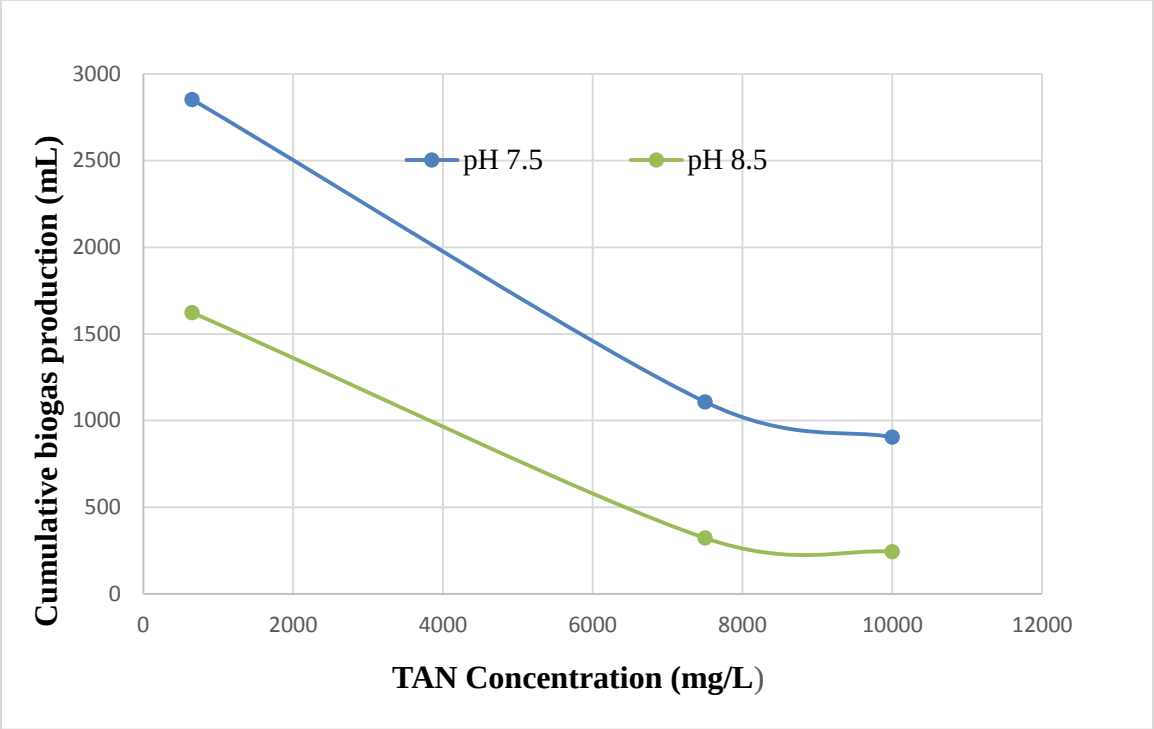


Figure 5.19: Cumulative biogas production versus TAN concentration

Table 5.5: Percentage reduction in biogas compared with control reactors

% Reduction in Biogas Production		
BMP	**pH 7.5**	**pH 8.5**
Control + 7500 mg/L TAN	61	80
Control + 10,000 mg/L TAN	68	85

The study also showed that pH influenced the inhibitory capacity and the FAN component of TAN. At high pH (i.e. 8.5), FAN component of TAN was about 26 % and was inhibitory to the methanogens. Results also showed that mesophilic bacteria could be adapted to a TAN concentration of about 5000 mg/L at pH 7.5 through gradual TAN loading.

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Chapter 6

Conclusions and Recommendations

6.1 Conclusions

Results of this research confirmed that ammonia is inhibitory to methanogenic activity, especially at high TAN concentrations (i.e. 7500 mg/L and 10,000 mg/L) and pH 8.5. Reactors containing 7500 mg/L and 10,000 mg/L had as much as 80-85 % reduction in CBP, compared with control reactors.

The operating pH had a significant influence on the inhibitory capacity and the FAN component of TAN. At operating pH of 8.5, FAN made up about 27 % of TAN, causing extreme toxicity. Results also confirmed that through gradual ammonia loading, bacteria may become adapted to high ammonia concentrations. Gradual ammonia loading enhanced the ability of mesophilic bacteria to adapt to as much as 5000 mg/L TAN (at pH 7.5). Results also show that operating the reactors in the semi-continuous mode by replacing 3g of digestate containing high TAN concentrations of 7500 mg/L and 10,000 mg/L with 3 g fresh substrate improved the activity of the mesophilic bacteria.

The effect ammonia on AD of OFMSW with real landfill leachate with anaerobic inoculums leachate to simulate an anaerobic landfill was also studied. Similar results were obtained as in OFMSW digested using only mesophilic anaerobic inoculums. The inhibitory effect of TAN also increased with pH and FAN. Compared with control reactors, reactors containing 7500 mg/L TAN and 10,000 mg/L TAN had about 80-85 % reduction in CBP.

6.2 Future Work

This study confirms the toxicity of ammonia on methanogenic activity during anaerobic digestion. The possibility of adapting bacteria to high ammonia concentrations needs to be studied on a larger scale and possibly on a field scale, instead of batch reactors. The

possibility of operating digesters treating waste containing high ammonia concentrations like piggery and poultry wastes should be studied as a larger scale. Also, the toxicity of ammonia may be reduced by adding low cost absorbent materials such as zeolite to the feed.

Appendix

Appendix A

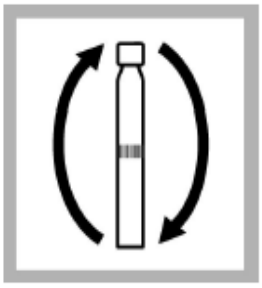
A. 1a. Procedure for measuring COD (Method 8000, Hach USA)

Test procedure

1. Set the DRB200 reactor power to on. Set the temperature to 150 °C.

2. Measure 100 mL of sample in a blender. Blend for 30 seconds or until homogenized.
If the sample does not have suspended solids, ignore this step.

3. Pour the homogenized sample into a 250-mL beaker and stir slowly with a magnetic stir plate.
If the sample does not have suspended solids, ignore this step.



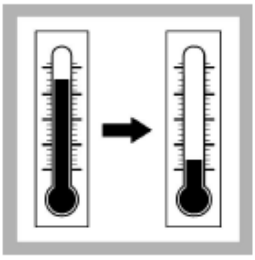
4. Invert a test vial several times to mix.

5. Use a pipet to add 2,0 mL of sample to the test vial.

6. Hold the vial by the cap, over a sink. Invert gently several times to mix. **The vial gets very hot during mixing.**

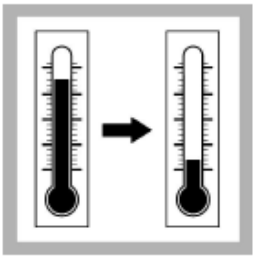
7. Insert the vial in the preheated DRB200 reactor. Close the lid.

8. Keep the vial in the reactor for 2 hours.

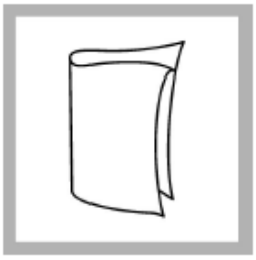


9. When the timer expires, set the reactor power to off. Let the temperature decrease for about 20 minutes to 120 °C or less.

10. Hold the vial by the cap and invert gently several times while the vial is still hot.



11. Put the vial in a test tube rack. Let the temperature of the vial decrease to room temperature.



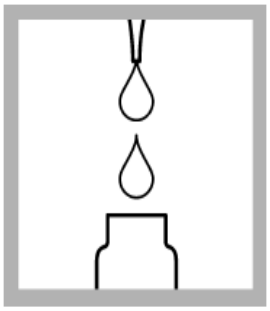
12. Clean the vial.

A. 1b. Procedure for measuring COD continued – Method 8000 (Hach USA)

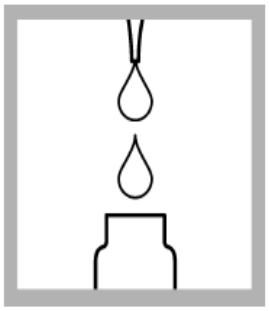
13. DR 1900 only: Select program 821 (LR) or 822 (HR). Refer to Before starting on page 1.

14. Insert the vial into the cell holder. DR 1900 only: Push **READ**. Results show in mg/L COD.

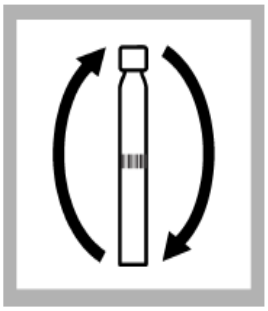
A. 2 Procedure for measuring Total Alkalinity (Method 10238, Hach USA)



1. Use a pipet to add 2.0 mL of Solution A to the test vial.

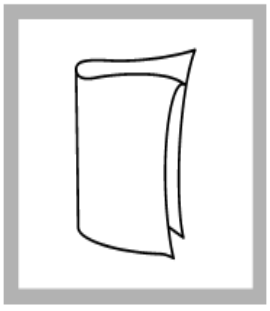


2. Use a pipet to add 0.5 mL of sample to the test vial.

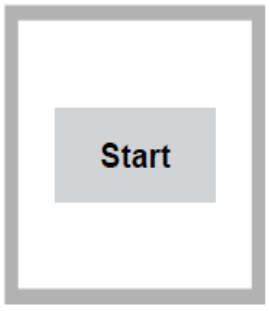


3. Tighten the cap on the vial and invert until completely mixed. Make sure that the contents are well mixed.

4. Start the reaction time of 5 minutes.



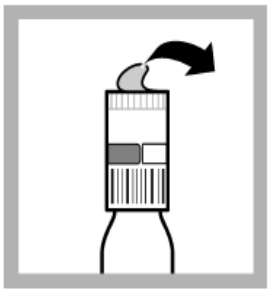
5. When the timer expires, clean the vial.



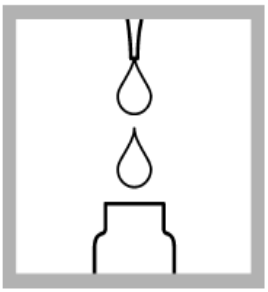
6. DR 1900 only: Select program 870. Refer to Before starting on page 1.

7. Insert the vial into the cell holder. DR 1900 only: Push **READ**. Results show in mg/L $CaCO_3$.

A. 3 Procedure for measuring Total Ammonia Nitrogen (TAN), (Method 10205 Hach USA)

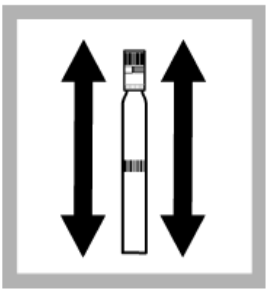


1. Carefully remove the lid from the DosiCap™ Zip cap. Remove the cap from the test vial.



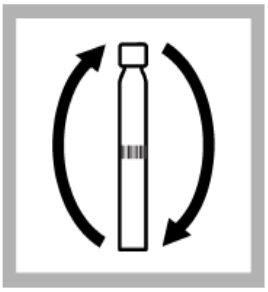
2. Use a pipet to add 0.2 mL of sample to the test vial. Immediately continue to the next step.

3. Turn the DosiCap Zip over the test vial so that the reagent side goes on the vial. Tighten the cap on the vial.

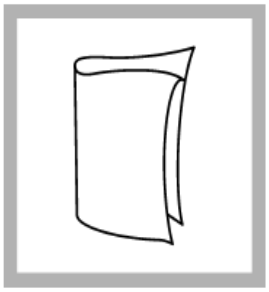


4. Shake the vial 2–3 times to dissolve the reagent in the cap.
Look through the open end of the DosiCap to make sure that the reagent has dissolved.

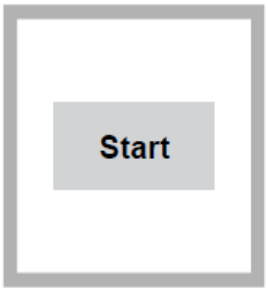
5. Start the reaction time of 15 minutes.



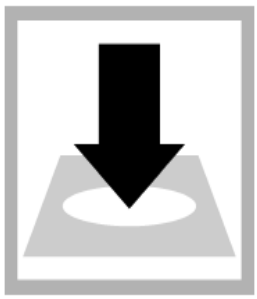
6. When the timer expires, invert the vial 2–3 times. The color is stable for an additional 15 minutes after the timer expires.



7. Clean the vial.

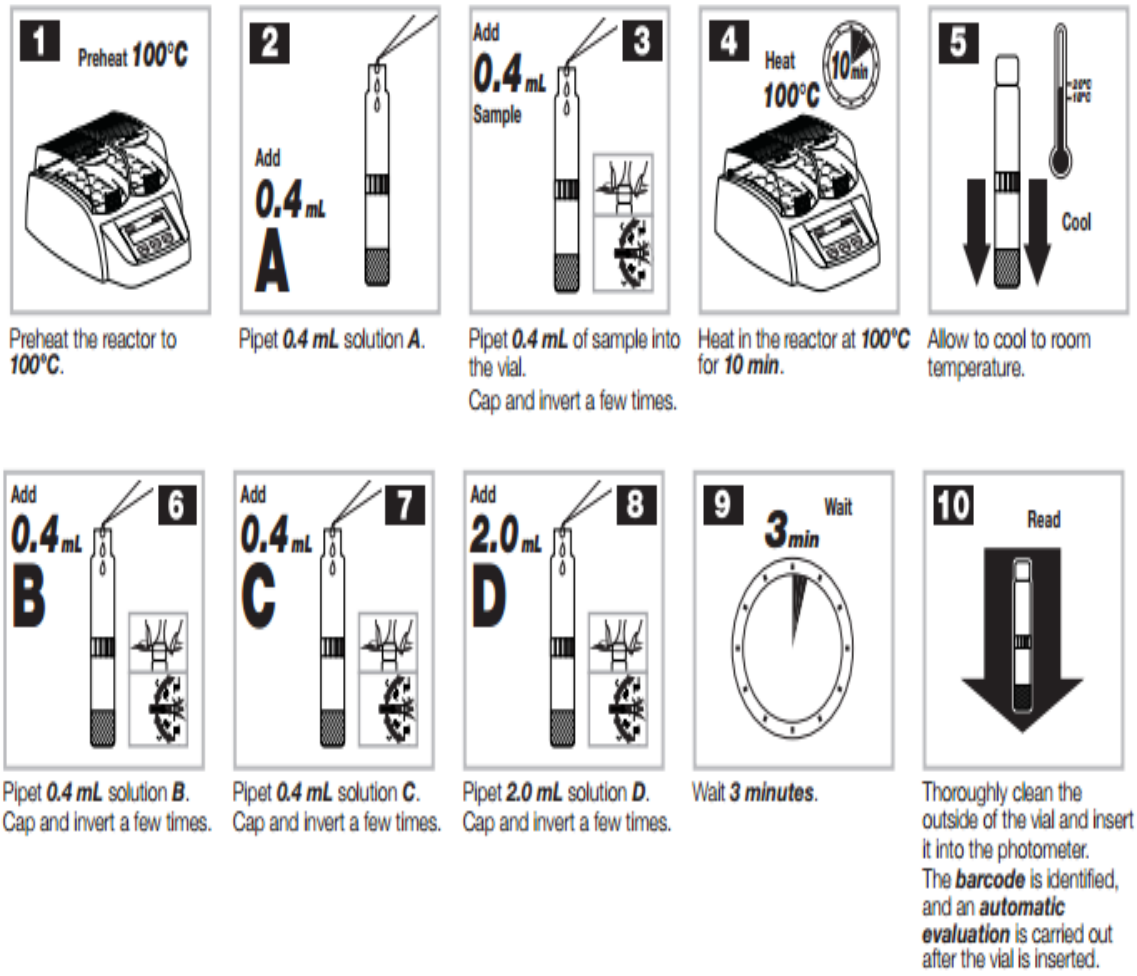


8. DR 1900 only: Select program 832. Refer to Before starting on page 1.

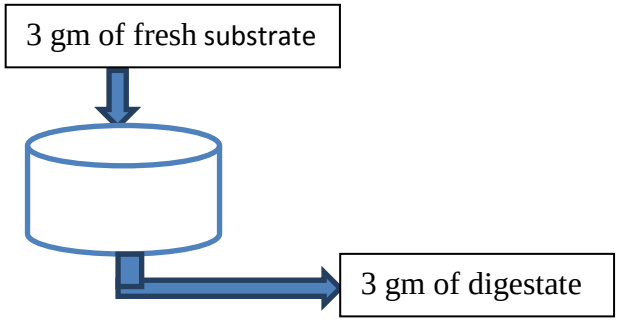
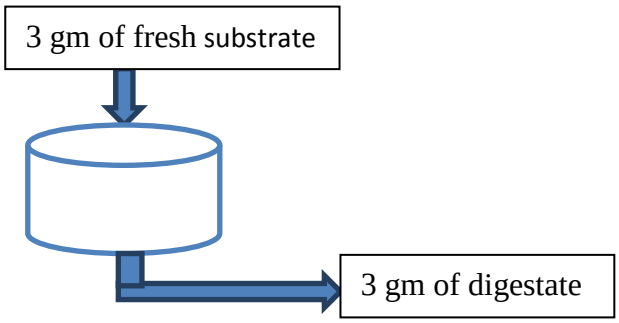
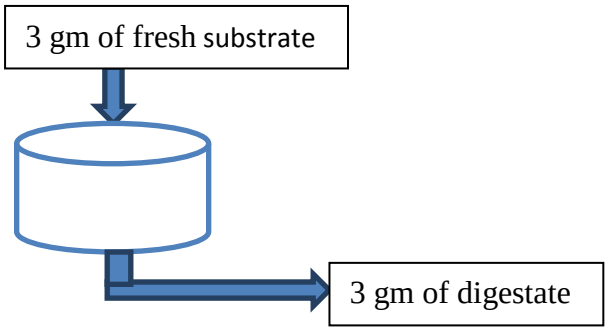


9. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in NH_3–N.

A. 4 Procedure for measuring Volatile Acids as Acetate (Method 10240, Hach USA).



Appendix B

HRT (days)	Digestate Removed (g)	fresh substrate added (g)	Schematic diagram
4	3	3	
7	3	3	
15	3	3	

Appendix C

U-tube Manometer