THE EFFECT OF FREE NITROUS ACID PRETREATMENT ON THE ANAEROBIC DIGESTIBILITY OF THICKENED WASTE ACTIVATED SLUDGE

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The Effect of Free Nitrous Acid Pretreatment on the Anaerobic Digestibility of Thickened Waste Activated Sludge

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Abstract

Sludge pretreatment technologies as an avenue to improve solids handling in a WWTP has gained attention and significant research efforts are being directed towards studying several available techniques. The use of FNA as a chemical pretreatment for the AD has shown the potential to enhance the hydrolysis stage by releasing the internal organic matter of TWAS via its biocidal action. The effect of FNA on improving the biodegradability of TWAS was investigated in this thesis. The effect of the FNA on the TWAS characteristics and the methane production in batch tests was first studied. The optimum FNA dose was determined from the batch tests based on both solubilization and methane yields and then tested in a semi-continuous flow system. As the semi-continuous flow system failed when the optimum FNA dose obtained from the batch study was used, another set of semi-continuous flow experiments were conducted using different FNA doses.

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List of Abbreviations

AD	Anaerobic Digestion
AOB	Ammonia Oxidizing Bacteria
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
DDW	Deionized Distilled Water
DMDO	Dimethyldioxirane
EPS	Extracellular Polymeric Substances
F/M	Food to Microorganism
F/T	Freeze-Thaw
FID	Flame Ionization Detector
FNA	Free Nitrous Acid
GC	Gas Chromatograph
HRT	Hydraulic Retention Time
MSW	Municipal Solid Waste
NOB	Nitrite Oxidizing Bacteria
POMS	Peroxymonosulphate
SBR	Sequencing Batch Reactor
SRB	Sulfate Reducing Bacteria
SRT	Solids Retention Time
TCD	Thermal Conductivity Detector
VFAs	Volatile Fatty Acids
WAS	Waste Activated Sludge
WWTPs	Wastewater Treatment Plants

1.0 Introduction

As the amount of sludge that is produced from wastewater treatment plants (WWTPs) continues to rise, the cost of handling solids also increases contributing to a significant portion of the total operating cost of the facility. In a typical metropolitan area such as Toronto, its largest WWTPs, Ashbridges Bay, has seen an 8% rise in solids production over the past 6 years [1, 2]. This increase in sludge production is due to more stringent environmental regulations that govern the quality of wastewater treatment. This means that WWTPs which are already experiencing larger influent volumes produce even higher quantities of solids [3]. Additionally, the steady growth of population (about 8% growth in Toronto over the last 5 years) with more people migrating towards urban centers, increases the burden on the servicing WWTP [4]. The increase in sludge production can pose a problem for the development of growing and established urban areas since more funds are required for proper handling and disposal of sludge that could have been directed towards urban improvement strategies [5]. Sludge minimization techniques are important not only for solids reduction to lower operating costs, but to improve its characteristics for aesthetic and health reasons. These techniques include alkaline stabilization with lime, aerobic digestion, composting, pelletization and anaerobic digestion. These methods stabilize biosolids to minimize odour, reduce pathogens and transform degradable organic matter into inert material such that the solids meet the regulations for disposal [6-8].

Of the techniques for sludge stabilization, anaerobic digestion (AD) is one of the oldest and most commonly applied particularly in municipal and industrial wastewater treatment because of its potential for bioenergy recovery and the reduction of environmental footprint [3, 9]. However, low digestion efficiencies characterize the digestion of waste activated sludge (WAS) even when long retention times are allowed. To improve the process, physical, chemical, or biological treatments are applied to the substrate prior to AD to speed up the process and improve the efficiency.

Many of these techniques are energy intensive and often have adverse environmental impacts. This results in higher operational costs to run the pretreatment and/or ensure that environmental impacts are mitigated before the wastewater stream is released [5]. Free nitrous

acid (FNA) is a novel chemical technique for WAS pretreatment. Owing to the fact that it degrades naturally in the wastewater stream, environmental impacts that characterize other techniques are absent in this case. Some research has gone into the batch studies of AD conducted after the use FNA for pretreatment [8]. However, nothing has been done for a semi-continuous process. This is deemed important since semi-continuous AD is popular for large scale waste management. Therefore, the objective of this study is to investigate the effect of FNA pretreatment during semi-continuous AD.

The following chapter of this report covers a literature review on anaerobic digestion in the wastewater treatment process, pretreatment techniques that have been studied and are gaining popularity and the mechanisms of FNA in enhancing AD. Chapter 3 outlines the procedure of the experiments that were undertaken in terms of the pretreatment, batch, and semi-continuous tests as well as the analysis that were done. In chapter 4, the results that were obtained are presented and discussed in relation to previous studies and their relevance. The report concludes with chapter 5 in which the report is summarized with the findings of the investigation and suggestions for future research.

2.0 Literature Review

The proper treatment of wastewater has always been critical to public health and the social development of a community. However, progress towards proper waste handling on a global scale has been slow and tends to be reactive. From the historical times, 800 BC, when wastewater was merely conveyed away from residential settlements without treatment, until now when a fairly advanced wastewater treatment is in place, developments to the process occur in response to unsatisfactory conditions related to human health. These have ranged from the presence of malodors to water-logged lands, eutrophication, and the degrading quality of receiving water bodies. Even now, emerging micropollutants from industry and consumer goods continue to put a strain on the complex treatment system [9].

The objective of WWTPs is to separate as much contaminants as possible from the liquid stream (based on regulatory requirements but taking economic feasibility into consideration) using different physicochemical and biological processes before it is released into the receiving rivers or streams. Management of the solids that are produced from these facilities has become a challenge since traditional disposal methods such as land application for agriculture, landfilling, incineration etc. are no longer attractive options due to space constraints and the growing global concern about the deterioration of environment quality [10]. The introduction of sludge stabilization was to reduce the environmental impacts of sludge disposal by reducing pathogen contents and degrading the putrescible fraction of the solids. Some of these methods include aerobic digestion, anaerobic digestion, alkaline stabilization, composting and pelletization [11].

Aerobic digestion allows microbes in the sludge to undergo endogenous digestion since there is no supply of substrate. However, oxygen needs to be supplied and sufficient mixing is required [11]. Alkaline stabilization is the application of lime or alkaline reagents to raise the pH of sludge. While it can be cost effective, the basic conditions can be detrimental to equipment [12]. Composting is more common for small WWTPs but land availability becomes a challenge in order to be applied to larger scale plants [11]. Pelletization is the heating of sludge to eliminate moisture. While the residue is usually free of pathogens, the process emits gases that can be hazardous [13].

AD is a sludge stabilization technique that occurs in the absence of oxygen [11]. It is an old and well established biological approach to sludge management [12]. Recently growing concerns about climate change, energy demand and other environmental quality issues have made AD the focus of advanced research for process improvement. Some of the benefits of AD are discussed below [1, 3, 6, 8, 9].

- Less energy intensive – when treatment of high strength solids that is, solids with high chemical oxygen demand (COD) concentration, is required, the energy that is necessary to maintain the AD reactor temperature can be as low as one-fifth of that required to treat the same waste aerobically.
- Production of residual solids with high stability – the solids that are separated from the liquid wastewater stream in the primary and secondary clarifiers in a treatment facility have a large organic fraction which is highly subject to putrefaction. During the AD process, most of the organic matter is converted to biogas by microbial action leaving a residue that is less prone to degradation.
- Energy generation – The methane content of biogas that is produced from AD is widely used as energy and recognized as a clean source of energy. As a result, it is preferred for biogas production to be improved. WWTPs often use the biogas that is produced from the digesters to supplement their fuel requirements.
- Lower environmental impact – Some the pathogens and putrescible matter in the sludge are destroyed during AD which would otherwise give off unpleasant odors. Low biomass yields translate to a reduction in the volume of sludge that needs to be disposed. Also, the substitution of energy from non-renewable sources with biogas that is produced reduces green-house gas emissions.

Therefore, AD lowers disposal costs, the need for available land, emissions, and malodors. However, AD is hindered by the following factors [6, 7].

- Slow degradation - the slow start up and reaction rate of microbes that are involved in the AD process often lead to large digester tanks or long retention times required for only partial decomposition or organics.

- Inhibitors - the anaerobic bacteria that are required are sensitive to multiple inhibitors such as ammonia, sulphide, heavy metals, sodium, potassium, hydrogen, volatile fatty acids (VFA) and long chain fatty acids (LCFA), some of which are produced during the AD process itself.
- Low quality supernatant – the liquid effluent from this process still needs to undergo further treatment before being released into the environment. This may be in form of an aerobic system in series with the digester or the supernatant may be sent to the beginning of the WWTP process.
- Damage to equipment – some of the other compounds in the biogas such as carbon dioxide, hydrogen sulphide, moisture, volatile siloxanes can cause corrosion of the equipment and lead to odour problems.
- Cost of alkalinity – The AD process requires about 3000 mg/L $CaCO_3$ to keep the pH at optimum level. Depending on the characteristics of the wastewater to be treated, alkalinity may need to be added to enhance AD, increasing the operation costs.

The activities of distinct groups of microorganisms during AD process have been divided into four stages by researchers – hydrolysis, acidogenesis, acetogenesis and methanogenesis [7, 16]. A summary of the progression of these stages is presented in Figure 2-1. Particulate organic matter and compounds with high molecular weight such as proteins, lipids etc. are broken down into soluble material e.g. sugars and amino acid that can be further degraded by bacteria in the acidogenesis stage. Monomers such as amino and fatty acids are utilized during acidogenesis to form volatile fatty acids (VFAs) including propionate and butyrate as well as ammonia (NH_3), carbon dioxide (CO_2) and other by products. During acetogenesis, the organic acids and alcohols produced during the preceding stage are converted to acetate, CO_2 and hydrogen (H_2) at low concentrations of H_2 in the system. Acidogenesis and acetogenesis stages are often combined in a stage referred to as fermentation. The final products from the fermentation process are used by diverse groups of methanogens to produce methane in the methanogenesis stage. *Acetoclastic methanogens* break down acetate to produce methane (CH_4) and CO_2 while the hydrogen-utilizing methanogens use H_2 and CO_2 to produce CH_4 [6, 7] .

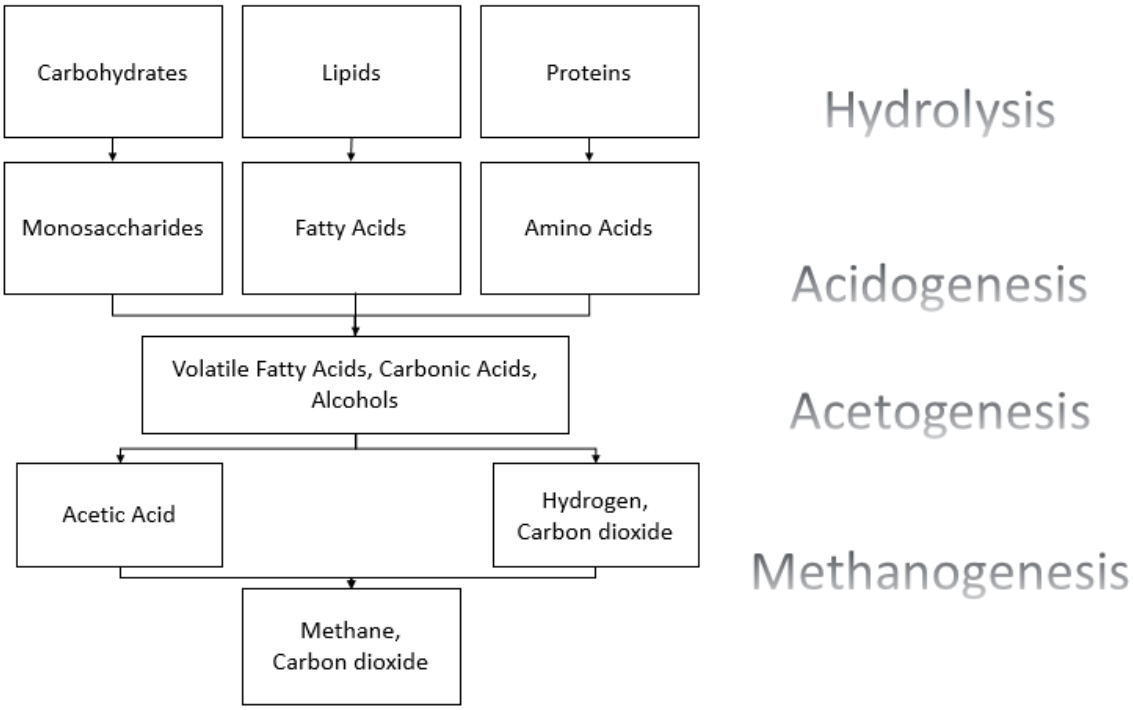

Figure 2-1. Flowchart showing stages of Anaerobic digestion, adapted from [7, 17]

Designing and maintaining an AD process that is well functioning requires that certain environmental factors be kept constant. Failure of the process can be identified by the level of some of those factors. They include solids retention time (SRT), hydraulic retention time (HRT), temperature, alkalinity, pH, presence of inhibitory substances, and the accessibility of nutrients for the microorganisms [6]. SRT plays a vital role in the digestion efficiency of the process. Less than 5 day SRT leads to washout of methanogens but more stabilized digestion is observed when SRT is over 10 days [7]. Alkalinity and pH can be key indicators of a process imbalance due to the presence of toxins, over loading etc. Well-functioning digesters have alkalinity concentrations from 2000 – 5000 mg/L $CaCO_3$. Temperature fluctuation of over 1°C per day can be inhibitory to organisms in AD process. The digester tanks should be kept at mesophilic temperature which is from 30 to 38 °C or thermophilic temperature ranges from 50 to 57°C [6, 7] .

2.1 Pretreatments for Anaerobic Digestion

When the substrate is readily degradable, methanogenesis is the process bottle-neck. On the other hand, when digesting complex substrates, such as biosolids, in which organic material are not easily accessible to the bacteria, hydrolysis has been reported to be the rate limiting step [15]. This often results in long retention times of 20 to 30 days and large digester tanks being required to achieve low digestion efficiencies of 30 to 50% [7]. Cells are the major constituents of the organic matter in biosolids. The cell walls are made of linked glycan and peptide chains which provide strength for protection to the cells and are recalcitrant to microbial degradation. In addition, flocs are held together by extracellular polymeric substances (EPS) which also resist degradation [3, 7] . EPS and cell walls are disintegrated in the hydrolysis stage to release soluble and readily degradable material. As such by disintegrating the substrate prior to AD, the hydrolysis stage can be accelerated [3, 7] . Disintegration techniques can be physical, biological, chemical or a combination of methods and have been implemented as pretreatments to improve the AD process [7, 8, 18] .

2.1.1 Physical Pretreatment

Physical techniques can include the use of thermal, mechanical and freeze-thawing action on substrates to enhance hydrolysis [3]. Thermal pretreatment techniques have been extensively studied and are used in the field not only for disintegration but to improve dewaterability and remove pathogens from substrates [15]. The optimal temperature and time required for pretreatment depends on the substrate but temperature ranges from 70 to 200°C have been used. The temperature applied and efficiency of the pretreatment process are positively correlated as an increase in solubilization of organic material has been observed at higher temperatures. However, it has been reported that at over 170 °C, compounds which are inhibitory to microbial communities are released and complex substrates that are difficult to degrade are formed thus reducing the efficiency of AD [8, 16] . When used to treat readily available substrates, thermal pretreatment can lead to the destruction of volatile organic matter resulting in reduced methane production. In recalcitrant substrates, thermal pretreatment has resulted in increased methane production of up to 78%. While mesophilic AD

is usually enhanced by this technique, the improved effect is less in thermophilic AD [7, 16] . Microwave irradiation is a form of thermal pretreatment that has been studied more recently. Separate from its thermal effect, this technique can polarize macromolecules to split hydrogen bonds [18].

Mechanical pretreatment methods improve AD efficiency mainly by reducing the particle size of substrates and partial solubilization through shear stress [7]. This is because a particle with smaller radius has larger specific surface area which makes it more accessible to bacteria. Techniques incorporating mechanical action include lyse-centrifuge, liquid shear, high pressure homogenizer, sonication, maceration, liquefaction etc. Effects other than radius size reduction are observed in mechanical methods such as high frequency ultrasonication in which substrates are oxidized. Mechanical pretreatments are easier to apply, have low odor emissions, improve dewaterability of AD residue and are fairly energy intensive. However, they have no impact on pathogen removal and are less efficient in AD improvement [7, 16] .

In areas with cold climate conditions, freeze-thaw (F/T) pretreatment can be a cost-effective option to enhance AD. Ice crystals that form during the freezing stage compress and compromise the cell wall. When F/T is used to treat wastewater sludge, the release of EPS and solubilization of organic matter has been observed, leading to up to 36% improvement in methane yield. In addition, sludge dewatering and settling is enhanced once the thaw cycle is completed. Efficiency of this pretreatment is affected by freezing temperature and curing time [20 - 22].

2.1.2 Biological Pretreatment

These techniques can take aerobic or anaerobic form and may include the supplementation of bacterial strains or enzymes prior to or during AD [16, 23] . In whichever form the biological treatment takes, the breakdown of cell walls is because of reactions that are catalyzed by enzymes [7]. Aerobic methods such as composting and micro-aeration capitalize on the enhanced growth of microorganisms and by extension increased production of enzymes that catalyze hydrolysis [15].

Inoculation of substrate with aerobic bacteria has shown success in enhancing hydrolysis. An aerobic thermophilic bacterium strain with 100% sequence similarity to

Geobacillus thermodenitrificans was determined to be responsible for biogas production over twice the volume produced by untreated sewage sludge in one study [21]. Digestion of municipal solid waste (MSW) was also enhanced by a constructed thermophilic microbial consortium after four days of pretreatment [18]. The additions of mature compost and mushroom compost extracts have shown significant improvement in methane yield in MSW and paper and pulp sludge respectively [19, 27].

2.1.3 Chemical Pretreatment

Chemical pretreatment enhancing AD usually involves the addition of chemicals to the substrate of interest. These chemicals can be grouped into three based on their reactions with the cell wall and membrane to release organic matter from the cells – acids, alkalis and oxidants [15].

2.1.3.1 Acid Pretreatment

This technique is usually performed with dilute acid due to the production of toxic by-products such as furfural and hydroxymethylfurfural by strong acids. The main mode of acid pretreatment is the solubilization of hemicellulose which are complex carbohydrate structures to release monomers and oligomers that may have been unavailable to microbes [24]. The use and research of purely acid pretreatment is not popular; however, the addition of acid can make other methods such as thermal pretreatment more efficient and cost effective [16, 29].

Hydrochloric Acid

Hydrochloric acid (HCl) has been more extensively studied than any other acid reagent for treatment of sludge. Apul et al. [26] studied the effect of pH values of 1.5,2.5 and 4.5 using 1N of HCl on sludge that was treated for 30 minutes. They observed 10 times more SCOD in the sample that was pretreated to pH of 1.5 when compared to the control [26]. This is closely mirrored by another study that reported 900% increase in SCOD using 1 or 2N HCl for 20 minutes of pretreatment [27]. A 22% increase in SCOD to TCOD ratio as well as an increase in soluble proteins and carbohydrates was reported in a different study after a period of 20 days

where pretreatment was carried out with 2M HCl to adjust sludge to pH 4 compared with the control which had 13.8% SCOD to TCOD ratio [28]. Devlin et al. [25] used 37% HCl and observed a rising trend of SCOD, soluble proteins and carbohydrates with a decrease in pH value such that the highest soluble concentrations which was at least 4 times the control, were obtained in the sample that was treated to pH 1 for 24 hours.

An increase in the concentration of soluble material means more material is available for digestion by bacteria and this is supported by the results from Devlin et al. [25], as all of the pretreated samples produced greater quantities of biogas than the control. The sample that was treated to pH 1 yielded the highest volume of biogas compared to other pretreated samples at 32% more than the control after 21 days of batch AD. In addition to quantity, the rate of biogas production was also seen to improve with acid pretreatment as after 7 days, the sample at pH 1 had yielded more biogas than the control sample did after 21 days [25]. Contrarily, other studies have reported lower methane yields after acid pretreatment in comparison to control samples. Contact time could be a factor in these results, some studies used a contact time of only 20 minutes [30].

Sulfuric Acid

Sulfuric acid (H_2SO_4) has been laboratory tested for its effectiveness in treating sludge. In Hidalgo et al. [29], 30 mg/l H_2SO_4 was used to adjust the pH of the sludge to 2. However, only the quantity of biogas produced after batch digestion was considered for this study. An increase in 79% compared with the control test was observed in the sludge sample that was subject to acid hydrolysis [29].

Positive and Negative Effects

In several studies, acid pretreatment has been observed to improve the dewaterability characteristics of sludge. When the pH of the sludge is between 2.5 and 3.5, the negative charges between the particles are neutralized such that the surface inter-particle repulsive forces are reduced. This leads to a minimum amount of polymer being required for flocculation and greater dewatering efficiency after AD [29, 31, 34] .

Chen et al. [31], discovered that the optimum pH that is required for sludge filtration dewatering was 2.5 and 1.5 for centrifugal dewatering after using H_2SO_4, a maximum of 46% reduction in sludge volume was recorded. However, AD was not conducted prior to this observation [31]. By using HCl pretreatment to pH 2, Devlin et al. [25] recorded a 40% reduction in the amount of polymer that was utilized in the dewatering process compared with the untreated sludge after AD. Similarly, Apul O. [27] noted that sludge settling characteristics improved when sludge was treated to pH 2.5 as the turbidity of the supernatant was about 25% less than the control.

Other studies have found an opposing effect before and after AD. At pH 2.5 before AD, the capillary suction time (CST), a measure of filtration characteristics and turbidity of supernatant was lower. However, after AD, the CST was not significantly different between the control and acid treated sludge. Also, the optimum turbidity of supernatant was found at pH 1.5; at pH 2.5 the turbidity was even more than the control [26].

Acid pretreatment has also been shown to reduce pathogen content in sludge. Salmonella was observed to be completely eradicated in sludge samples treated to pH 2 compared with the control after digestion. E coli, on the other hand, was reduced by 3 log after treatment but increased again in digested samples [25].

Chen et al. [28] observed that acid treated sludge released around 3 times more soluble phosphorous and ammonia than the control samples during AD. This increase in concentrations will result in greater nutrient loading unto the treatment facilities for the digester supernatant. The study was conducted for samples treated to pH 4, 5 and higher, so the effect at lower pH values was not considered and should be investigated. In addition, the sludge samples were not neutralized after acid treatment and so AD was not optimized.

To optimize the AD process, the pH should be between 6.5 – 7.2 which is the optimum pH for the activities of the most sensitive bacteria that operates in the process [7, 32] . This means that after sludge pretreatment using acids, it is important to neutralize. This requires the addition of alkali before digestion and leads to higher costs and concern about chemical addition into the process [16, 33].

2.1.3.2 Alkaline Pretreatment

Alkaline reagents are more commonly researched and used for AD pretreatment. The mechanism of enhancing hydrolysis is by increasing bio accessibility of substrates through expanding their specific surface area. This expansion occurs because of saponification and solvation processes [15]. Sodium hydroxide (NaOH), potassium hydroxide (KOH), magnesium hydroxide ($MgOH_2$) and calcium hydroxide ($CaOH_2$) are alkalis in decreasing order of effectiveness that have been shown to enhance sludge solubilization. However, as a pretreatment for AD, high alkali dosages leading to high concentrations of sodium (Na^+) and potassium (K^+) can be a hindrance to the AD process [23, 33] . When sludge is the substrate of interest, NaOH is the preferred chemical compared with other alkalis as it is more efficient, requiring lower dosage [32].

Kim et al. [33] examined the effectiveness of various alkalis in solubilization by treating WAS to pH 12 for 30 minutes using NaOH, KOH, $MgOH_2$ and $CaOH_2$ individually. The findings were that compared to the control, COD solubilization was improved by 39.8%, 36.6%, 10.8% and 15.3% when NaOH, KOH, $MgOH_2$ and $CaOH_2$ were applied respectively [33]. These results were similar albeit lower than those obtained by Penuad et al. [34], with the same order of effectiveness in COD solubilization of 60.4%, 58.2%, 29.1% and 30.7% respectively. The lower COD solubilization which was observed in the dibasic alkalis ($MgOH_2$ and $CaOH_2$) was attributed to their partial dissolution compared with the monobasic alkalis (NaOH and KOH) [37, 38].

Sodium Hydro-oxide

NaOH pretreatment of paper and pulp sludge for 6 hours at 1.2% concentration was shown to increased COD solubilization by 12 times, and increase biogas generation by 88% [23]. Many studies have observed that increasing the concentrations of sodium hydroxide for WAS pretreatment results in increasing COD solubilization. Kim et al. [33] reported 44% increase when 7g/L NaOH was used in the pretreatment. Higher concentrations than this yielded lower solubilization. Removal efficiency of SCOD after digestion improved with the use of NaOH. Compared with the control, the study recorded 4 times the removal SCOD and over 9% VS

reduction. In addition, methane generation after treatment to pH 12 was improved by 12% [33].

Other studies have incorporated the addition of an acid to neutralize WAS after NaOH treatment. Li et al. [35] treated WAS to 0.1 g NaOH/g VS and 0.3 g NaOH/g VS for 24 hours and then added 1 mol/L of HCL to neutralize the samples to pH 7. Concentrations of soluble protein and carbohydrates increased from 0 – 0.83g/L and 0.07 – 0.47g/L respectively. An increase in methane generation of 57.6% and 88% for the 0.1 g NaOH/g VS and 0.3 g NaOH/g VS respectively was also observed [35]. Xu et al. [3] pretreated WAS to pH 10 with 5N NaOH for 8 days after which the sample was neutralized to pH 7 for AD. They observed that the degree of solubilization increased by 44% and concentrations of soluble protein and carbohydrates increased to 3 – 4 times the control. Also, the generation of biogas improved by 41.41% and VS removal was improved by 5.3%.

Shao et al. [36] determined the optimum pH for 4mol/L NaOH treatment of WAS by adjusting the pH of samples to 8, 9, 10, 11, and 12 for 24 hours and readjusting the pH to the initial value using HCl. VS reduction was observed to improve by 7% with increasing pH until 11. At pH 12, VS reduction was even less than the control. Similarly, biogas production increased with pH until 10 by 15.4% after which it decreased. The sample that was treated to pH 12 showed a decrease in biogas production by 18% compared with the control [36]. The deteriorating effectiveness of NaOH on biodegradability at pH 12 has been attributed to the inhibition of methanogens caused by high concentrations of sodium. A biodegradability factor of 0.97 was observed when 5g/L NaOH was used to treat WAS to pH 12 for 1 hour without neutralization [29].

Potassium Hydro-oxide

Valo et al. [37] observed that treatment with KOH for 1 hour at pH 10 and 12 yield 9.3% and 30.7% increase in COD solubilization. However, further study showed that at pH 10, the increase in soluble solids were not due to a change in VS but corresponded to the addition of KOH. While majority of the solubilized solids was from an increase of VS at pH 12, a significant amount was also due to KOH. No significant improvement to biogas generation was recorded

using KOH. This was attributed to either the inhibition of methanogens by refractory molecules that were solubilized by the alkali or the limited amount of solubilized organic matter [37].

2.1.3.3 Oxidation Pretreatment

The traditional oxidation technique utilized air or oxygen at elevated temperatures or pressures for sludge disintegration. The odor problems associated with this method were addressed in the Cambi process in which thermal pretreatment was incorporated with the oxidative process. Of recent, advanced oxidation which is the use of strong oxidants such as ozone (O_3) and hydrogen peroxide (H_2O_2), originally used as disinfectants have been of more research interest and field application as pretreatments to AD. These are particularly favored since they do not produce salt or chemical residue during the pretreatment process [7, 16] .

The hydroxyl ions produced during advanced oxidation have high oxidation potential and contribute to hydrolysis through reaction with and destruction of organic cellular material. Peracetic acid is another oxidant used for water purification in the industry that has been recently studied because it is readily biodegradable. Appels et al. [38] observed 21% improvement in biogas yield during AD of WWTP sludge.

Fenton Process

When iron ions (Fe^{2+}) and H_2O_2 are combined, the oxidation pretreatment is referred to as the Fenton process. Ferrous iron is used to catalyze the splitting reaction of H_2O_2 to form hydroxyl radical [39]. This process requires a low pH for optimum treatment however other peroxidants such as peroxymonosulphate (POMS) and dimethyldioxirane (DMDO) that do not require extreme conditions are being studied [7].

Ozone

Studies have gone into optimizing ozone oxidation pretreatment. O_3 promotes osmosis through cell walls which compromises its integrity and releases intracellular material. Microbubble systems are one such way in which sludge solubilization by ozonation has been enhanced though the effect on anaerobic digestibility is yet to be seen [40]. Erden & Filibeli [40] and

Bougrier et al. [41] showed that cell lysis is a predominant mechanism in enhancing hydrolysis with O_3 pretreatment. O_3 generates radicals that oxidize organic matter. Higher concentrations of oxidants appear to increase VFA concentrations which is toxic in excessive amounts to methanogens. The ability for strong oxidants to react with sludge enhances mineralization of the solids thus increasing sludge volume.

The combination of ultrasound and O_3 is another type of advanced oxidation process that is fairly new. Ultrasound radiation makes ozonation more efficient by improving the mass transfer rate of ozone into the substrate of interest. Studies have shown up to two times the methane production from using this method but more research is required to optimize the process in terms of O_3 concentration [39].

2.1.3.4 Limitations of Chemical Pretreatment

Some of the limitations that are associated with the use of chemical reagents in treating material prior to undergoing AD are outlined below [15].

- Cost of reagents – the operating costs for purchasing chemicals that are required for the pretreatment process can be significant. This can be due to consumption of the reagent by the biomass such that higher chemical doses are required to sufficiently enhance AD. In addition, extreme pH conditions that characterize chemical pretreatment methods need to be re-neutralized prior to AD with other chemicals thus contributing to higher costs.
- Equipment damage – extreme pH conditions can be problematic for equipment maintenance because of scaling and corrosion.
- Loss of fermentable sugar – during pretreatment, the breakdown of complex substrates can reduce the available material for methane production during AD due to the long contact time required.
- Environmental impacts at the end of sludge treatment process
- Adverse effects on agricultural application
- Effect of ions – some of the cations that are present in chemicals can be inhibitory to biogas production in certain concentrations.

2.2 Free Nitrous Acid

FNA is the unstable, protonated form of nitrite [42]. Due to the likelihood for degradation of FNA in any form other than in very dilute and cold solutions, FNA cannot be stored much less sold as is. However, it can be produced during the reaction of strong acids with inorganic nitrites. For industrial or commercial purposes, sodium nitrite is a major source. For example, in a reaction with hydrochloric acid, FNA can be formed thus:

$$NaNO_2 + HCl \rightarrow HNO_2 + NaCl$$

To determine the concentration of FNA, the relationship between pH and nitrite concentrations is generally used along with the equilibrium acid dissociation constant which varies with temperature. These are given by Equation (1) and Equation (2). Where Ka is the ionization constant which is dependent on temperature T in °C and S[- N] is the concentration expressed as nitrogen [43].

$$Ka = \frac{e^{-2300}}{273 + T} \qquad \text{Equation (1)}$$

$$S[HNO_2 - N] = \frac{S[NO_2 - N]}{(Ka * 10^{pH})} \qquad \text{Equation (2)}$$

Significant emission of nitrous oxide has been observed in the use of sodium nitrite to produce FNA. Nitrous oxide, commonly known as laughing gas, is of environmental concern because it is a greenhouse gas with almost 300 times the ability to trap heat within the atmosphere. FNA decomposes to form nitrous oxide through the following reaction [44]:

$$4HNO_2 \rightarrow 2HNO_3 + N_2O + H_2O$$

In the field of chemistry, FNA is used to test for the presence of primary, secondary and tertiary amines based on the products that form during its reaction with amines. In addition, its

reaction with primary aromatic amines produces intermediate diamine salts that in turn combine with other compounds to form synthetic dyes. These dyes can be used in the production of consumer goods such as food, cosmetics, clothes etc.

The biocidal effect of FNA has been exploited in field scale tests to inhibit the activity of sulfate reducing bacteria (SRB) in sewer networks. SRB action in sewer pipes leads to the production of hydrogen sulfide that emits strong offensive odors through manholes or pumping stations and cause corrosion of pipes. Through intermittent dosing, concentration of hydrogen sulfide was reduced by over 95% at about 830 meters downstream of the dosing point. In addition, the long-term use of FNA resulted in slower recovery of the SRB within the sewer network. Cost analysis showed that incorporating an FNA dosing regime is much cheaper than other chemicals that have been used for similar purpose [43].

Another recently developed use of FNA is as a pretreatment of algal cells that are essential to the production of biodiesel. In large-scale production, the extraction of lipids from these algal cells is a key limiting process. The ability of FNA to disrupt algal cell envelopes and increase the yield of lipid extraction was examined in a study. After 48 hours of pretreatment with FNA, experiments showed an 89% increase in lipid yields over untreated samples and a 4% increase over microwave pretreatment which is known to be an efficient pretreatment method [42].

The addition of FNA to sludge has been observed to improve its biodegradability which in turn reduces the volume of sludge. A study was conducted on the biodegradation of sludge from a denitrifying sequencing batch reactor (SBR). After 6 days of aerobic digestion, the sludge that had been treated with FNA showed 50% degradation as opposed to the untreated sludge in which degradation was almost undetected. The pretreatment of WAS with FNA has also been seen to improve methane generation from the sludge via anaerobic process.

Methane generation from anaerobic processes has gained popularity as a source of renewable bioenergy. A lab scale study showed that by increasing the concentration of FNA that is used to treat WAS through contact for 24 hours, the methane potential of the sludge also increases. This was confirmed in a full-scale study that was carried out on a wastewater treatment plant where 30% increase in methane production was observed when the sludge was treated with

FNA at 2.13 mg N/L. This method of pretreatment is environmentally friendly as it boasts a net reduction in CO_2 emission [8].

2.2.1 Production of FNA in WWTP

Law et al. [42] showed that FNA could be produced sustainably from wastewater treatment process. The current treatment of nitrogen rich supernatant from anaerobic digestion in a wastewater treatment plant can be modified to support complete rather than partial conversion of ammonium to nitrite by ammonia oxidizing bacteria (AOB). This process consumes a considerable amount of alkalinity such that the effluent stream has low buffering capacity. Thus, only minimal acid addition is required to convert nitrite to FNA. The process through which FNA can be produced in a WWTP is shown in Figure 2-2.

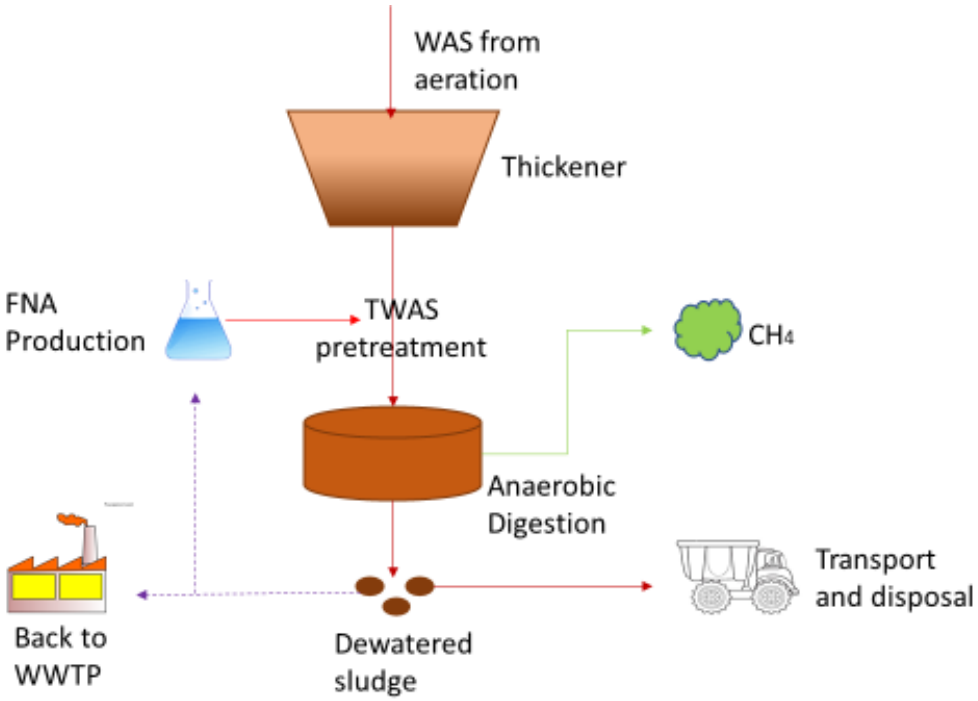

Figure 2-2. Schematic of WWTP showing the process of FNA production, adapted from [8]

In the study, a short SRT was used to washout nitrite oxidizing bacteria (NOB) to prevent further conversion to nitrate. Towards the later stages of the process when the SRT was

increased for higher AOB accumulation, low pH resulting in increasing FNA concentrations was proposed to have kept NOB activity at low levels. Possibly because of the high concentrations of nitrite in the reactor, the study reported less 0.1% nitrogen conversion to nitrous oxide [42].

The application of FNA that has been produced from a WWTP to pretreatment of sludge has a number of benefits over other chemical methods and pretreatment techniques in general. When FNA is produced from the anaerobic digestion liquor, the nutrient loading to the anammox process is relieved since the ammonia in the liquid is converted to nitrite which is converted to FNA that is easily degraded. Also, the nitrite rich liquor has a low buffering capacity so that only a small amount of acid is required to drop its pH to generate FNA. This means that fewer chemicals need to be removed later in the WWTP process or treated in the effluent stream. In addition, the facility equipment are not subject to the type of damage that is typically observed when chemicals are used. Finally, the energy requirement for this pretreatment technique is low and in combination with the internal production of the key chemical, FNA presents an economically attractive alternative [49, 50] .

The studies using FNA as a pretreatment technique have investigated its effect on aerobic, alternating anoxic-aerobic, and anaerobic batch digestion [51, 52] . However, no studies have evaluated the effect of FNA pretreatment on a semi continuous anaerobic digestion process. As this is more commonly used in large scale operations, it is crucial to evaluate the performance of FNA under this condition before it can be adopted at a full scale level [14].

3.0 Materials and Methods

3.1 Materials

TWAS and inoculum samples were collected from three WWTPs in Ontario, Canada. The batch test was conducted using TWAS from Adelaide WWTP in London, ON and inoculum from St Marys WWTP, St Marys, ON. Adelaide WWTP is one of six treatment facilities in London receiving 27,455 m^3/day of raw wastewater. The WAS from the aeration tank is thickened with polymer and air flotation tanks before being sent to Greenway WWTP for incineration. TWAS volume of 63,084 m^3 containing 4.4% total solids (TS) content was sent from Adelaide to Greenway in 2016 [49]. In St Marys WWTP, the WAS is thickened with a rotary drum thickener and flocculant before being sent to the AD tank where the inoculum was collected [50].

The semi-continuous experiment was conducted using TWAS and inoculum from Ashbridges Bay WWTP. Ashbridges Bay WWTP is one of the largest treatment facilities in Canada serving over 1.5 million people in Toronto, ON. WAS from the final clarifier is thickened with air flotation tanks and thickening polymer such that the resulting TWAS has average of 3.4% TS content and VS content of 71% of TS. TWAS samples for the experiment were collected at the outflow from the flotation tanks. Ashbridges Bay WWTP has 20 digesters that receive a total of 6,530 m^3/day of sludge. This sludge is comprised of one-third TWAS and two-thirds primary sludge. The digester tanks are 30 to 33 m in diameter, operating at a mesophilic temperature range between 34 and 38°C. The average SRT is 23 days, resulting in biogas production of 64,560 m^3/day that is repurposed within the plant as fuel and flared when in excess. The resulting biosolids from the AD tanks have total solids (TS) content of 1.8% before undergoing dewatering with polymer and centrifugation. The inoculum was collected at the effluent point of the digester tanks, at a depth of 5 meters, before the dewatering process [1]. The summary of sample characteristics is presented in Table 3-1.

Table 3-1. Characteristics of TWAS and inoculum

Material	TS (g/L)	VS (g/L)	TCOD (g/L)	pH
Adelaide's TWAS	36.2	33.2	34.4	7.2
St Mary's Inoculum	19.4	14.9	22.4	7.1
Ashbridges' TWAS	49.9	34.8	47.2	7.4
Ashbridges' Inoculum	20.5	6.4	17.0	7.3

3.2 TWAS Pretreatment with Free Nitrous Acid (FNA)- Experimental Setup

After sample pickup, the TWAS was stored in the refrigerator below 4°C until it was needed. Other studies reported that beyond 2.13 mg N/L FNA pretreatment had no positive effect. Therefore, concentrations between 0 and 2.8 mg N/L FNA were selected to be studied at a contact temperature of 25°C and pH of 5.5 ± 0.2 for contact time of 24 hours which had been determined to be optimum levels for pretreatment [47, 51]. Using the NO_2 - FNA equilibrium equation, Equation (1), the corresponding nitrite (NO_2) concentrations were used to apply the required FNA concentrations to the TWAS. These values are presented in Table 3-2. Bottle 2 was used to ensure that the effect of lowering the pH to 5.5 was accounted for while Bottle 1 represented the true raw sample without any pretreatment.

The pH of the TWAS was adjusted using 3M HCl and 1M NaOH was kept on hand for readjusting the pH if needed. 5g NO_2-N/L stock solution was prepared by dissolving 12.3g of sodium nitrite ($NaNO_2$) salt in 500 mL deionized distilled water (DDW). Once the required concentrations of NO_2 were determined for the desired FNA, the corresponding volumes of the stock solution were calculated using Equation (3) and added to the bottles of TWAS.

$$V_{sol} = S[NO_2 - N] * \frac{V_{TWAS}}{\frac{5g}{L}}$$ Equation (3)

Where V_{sol} is the volume of stock solution required, V_{TWAS} is the volume of TWAS to be treated (200 mL) and S[NO_2-N] is the selected NO_2 concentration. Once the stock solution was added and well mixed, the test bottles were placed in Grant Combined Orbital/Linear Shaking Water Bath, Model OLS200, gently mixing at 100-150 rpm to prevent any mechanical breakdown of particles for 24 hours. The pH was monitored for 24 hours and adjusted to 5.5 with HCl as required. The volume of acid required and the pH trend over the 24 hours is shown in Figure 3-1.

Table 3-2. Pretreatment conditions for batch and semi-continuous systems

Bottle #	Nitrite conc. (mg N/L)	pH	Temp °C	FNA* (mg N/L)
1 (raw)	0	7.2	25	0.0
2 (control)	0	5.5	25	0.0
3	50	5.5	25	0.35
4	100	5.5	25	0.7
5	200	5.5	25	1.4
6	400	5.5	25	2.8

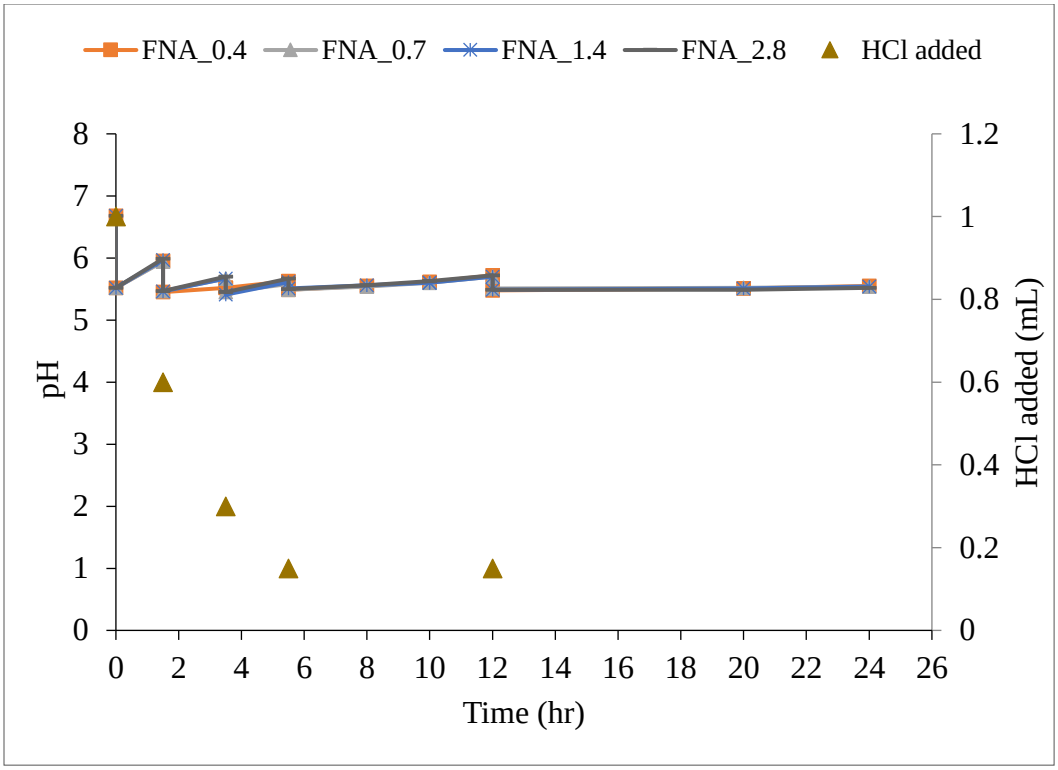

Figure 3-1. Amount of HCl added to maintain pH at 5.5 and pH profile during pretreatment

In order to adjust the pH during the pretreatment experiment, a total of 2 mL of HCl was required to maintain the pH for all samples. As a result, during pretreatments for the semi continuous system, pH was not adjusted but monitored at the beginning and the end of pretreatment, the different between the initial and final pH was 0.1 ± 0.2. The summary of the pretreatment procedure is shown in Figure 3-2. After the 24-hour period, the shaker was turned off and samples were collected for wet chemistry analysis and to feed the batch or semi-continuous system without further modifications (i.e. pH adjustment). The pretreatment was carried out once for the batch test and every day (or every other day occasionally) for 72 days for the semi-continuous test.

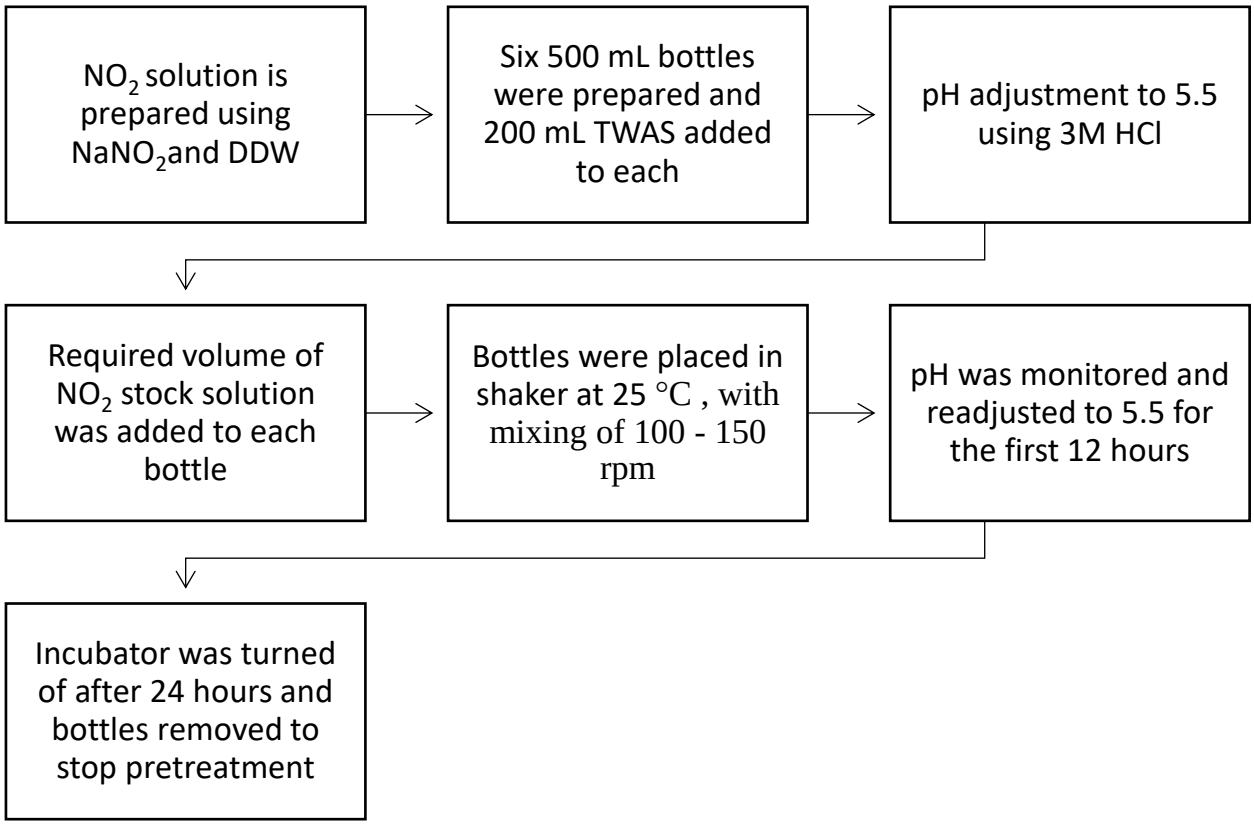

Figure 3-2. Flowchart showing summarized procedure for pretreatment

3.3 Biochemical Methane Potential (BMP) Test for Batch Procedure

The BMP test was conducted using 25 mL of TWAS from Adelaide WWTP and 225 mL of inoculum from St Mary's WWTP for each pretreatment level (using 0, 0.35, 0.7, 1.4 and 2.8 mg N/L FNA). This combination resulted in a food to microorganism (F/M) ratio of 0.4g COD/g VSS. Each BMP test was conducted in triplicates. Three blank bottles containing only 225 mL of inoculum were also assessed to account for the methane contribution from the inoculum. The headspaces in the bottles were flushed with nitrogen gas for 3 to 5 minutes at 5 to 10 psi in order to maintain the anaerobic conditions. After the flushing, the bottles were sealed and placed in a Thermo Scientific Benchtop Orbital shaker, Model MaxQ, and kept at a temperature of 37°C. The shaker was continually mixed at 150 rpm. Figure 3-3 shows the set-up that was used for the BMP test after following the guidelines in Angelidaki et al. [52].

Figure 3-3. Benchtop shaker used for BMP test - Thermo Scientific Benchtop Orbital

The biogas that was produced during the BMP test was filtered through NaOH salt in order to strip the CO_2 component from the biogas leaving only methane. The volume of methane was determined by measuring the headspace pressure of the BMP bottles with a manometer and the ideal gas law equation given in Equation (4).

$$V_a = \frac{P_h V_h}{P_a} \qquad \text{Equation (4)}$$

Where V represents the volume of biogas at 37°C, P is the pressure, while a and h subscripts are atmosphere and headspace, respectively [25]. The test was run for 25 days with methane production measured every day for the first three days of the test and then every two days until the end.

3.4 Semi Continuous Experiment Setup and Procedure

This stage of the experiment was set up using six glass reactors (2 L each) with two spouts and airtight caps fitted with stirrers. These reactors were placed in an uncovered 28 L PolyScience General Purpose water bath, Model WB28 filled with water. The water bath was set up to heat the containing water to 50°C to ensure that the reactor internal temperature was 35 ± 2°C. The internal temperature of the reactor was measured by immersing an analog thermometer into the reactor content each day. Since the water bath was open and subject to evaporation, the water level had to be maintained twice a day. The caps of each of the six reactors had tube spacing to facilitate gas flow to six wet-tip gas meters via Tygon-S3^{TM} B-44-3 tubing. The gas meters measured the volume of gas produced from the bioreactors at 15 to 18 mL gas per tip. The arrangement of this equipment is shown in Figure 3-4.

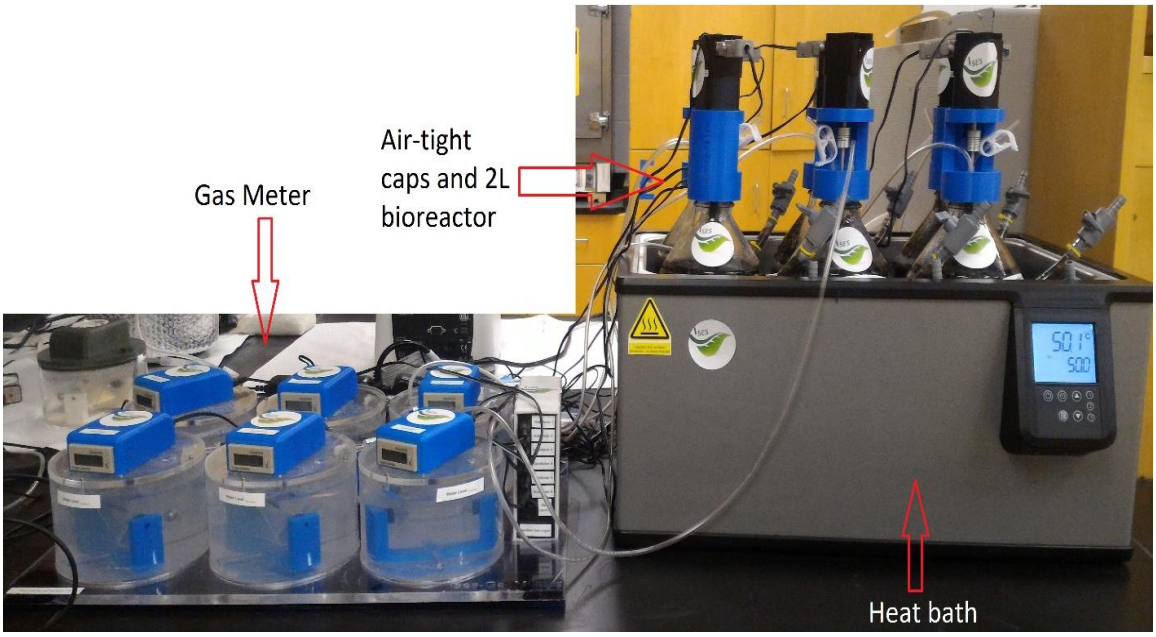

Figure 3-4. Equipment set up for semi-continuous anaerobic digestion process

Each reactor was seeded with 1.9 L of digested sludge from working digesters at Ashbridges Bay WWTP. Digested sludge volume of 100 mL was extracted every 24 hours from the lower sprout after which the same volume of raw or pretreated TWAS was subsequently fed into the reactor via the upper sprout using Masterflex L/S digital pump system and Masterflex C-flex tubing. The stirrer was left on during the feeding and extraction process which

took less than 10 minutes per reactor. The semi-continuous process was run for 72 days. The liquid samples were collected twice a week after the 20th day. The pH and temperature of the effluent samples were measured immediately, poured into plastic 250 mL sample bottles, and placed in the refrigerator below 4°C until wet chemistry analysis were completed. The numbers of tips were recorded from each of the gas meters and reset each day.

3.5 Liquid and Gas Sampling Analysis

The analyses that were performed are pH, temperature, TS, volatile solids (VS), total and soluble chemical oxygen demand (TCOD and SCOD), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), VFAs and gas composition (CH_4 and CO_2). With the exception of the solids and total COD tests, the raw, pretreated, and digester effluent samples for all tests were filtered through 0.45 µm VWR Vacuum Filtration Systems, Model 10040-462 in order to perform the soluble analysis. Samples were diluted with DDW in a ratio of 1 to 10 to reduce filtration time. All analyses that were performed were carried out in either duplicates or triplicates. The procedure for each test that was completed is listed below.

- **pH:** The pH for each sample was measured immediately it was collected using VWR Benchtop pH Meter and refillable glass probe, model B10P. This meter was calibrated twice a week with pH reference standards 4, 7 and 10 ± 0.1 (BDH®).
- **Temperature:** Durac Bi-metallic thermometer, a thermal pin, was used to measure the temperature of samples during collection and to ensure that the bioreactors that were used for the semi-continuous study was maintained under mesophilic conditions.
- **Total and Volatile Solids:** 5mL of each sample in aluminum plates were used to measure the solids content by following the standard guidelines provided in Methods 2540B and 2540E for TS and VS respectively [1].
- **Total and soluble COD:** High range (20 – 1500 mg/L) COD reagent vials from HACH were used to follow Method 8000 [2]. This method is based on the reaction digestion method developed by Jirka and Carter [3]. The COD content was then measured using HACH DR3900 spectrophotometer.

- **Ammonia-Nitrogen:** High range (0.4 – 50 mg/L) Amver Nitrogen Ammonia reagent set was used as per Method 10031, the Salicylate method [2]. Concentrations of ammonia-nitrogen were determined using the HACH DR3900 spectrophotometer.
- **Nitrite-nitrogen:** The Ferrous Sulfate procedure, Method 8153 was used in measuring the nitrite content as nitrogen in the samples [2]. This method is based on the work of McAlpine and Soule [4]. The NitriVer® 2 Nitrite reagent Powder pillows were used with HACH sample cells and the HACH DR 3900 for measurement.
- **Volatile Fatty Acids:** The liquid samples were further filtered with 0.2 µm DISMIC Ion Chromatography syringe filter units, Model 25HP. A HP 5890 Series II Gas chromatograph (GC) system was fitted with a Nukol fused silica capillary column and flame ionization detector (FID) to measure acetate, propionate, n-butyrate, n-valerate, iso-butyrate and iso-valerate concentrations. Helium was used as the inert, carrier gas. The initial temperature of the column was 110°C rising at 8°C per minute to 195°C. This final temperature was held in the column for 9 minutes. The injector and detector temperatures were 220°C and 280°C respectively.
- **Gas Composition:** The composition of biogas that was produced from the batch and semi-continuous systems was using the SRI instruments GC, Model 310, which was fitted with a thermal conductivity detector (TCD) and a molecular sieve column, Molesieve 5A [4], mesh 80-100 measuring 6 ft. by 1/8 in. Argon gas flowing at a rate of 30mL/min was used as the carrier gas. The temperature of the TCD and the column was 105°C and 90°C respectively. The nitrogen gas, hydrogen and methane content were determined.

4.0 Results and Discussion

4.1 Effect of FNA on TWAS Characteristics

The effect of FNA pretreatment on the TWAS characteristics was investigated by applying different FNA doses to the TWAS. The FNA doses were; 0.35, 0.7, 1.4 and 2.8 mg N/L. FNA doses were applied to the TWAS at a constant temperature of 25°C and pH of 5.5 for 24 hours. The raw sample refers to the TWAS without any pretreatment, i.e. no FNA addition nor pH adjustment and the sample was stored in the refrigerator at about 4^{o}C. The control sample (FNA = 0 mg N/L) refers to the sample that used to investigate the effect of pH adjustment and differentiate between the effects of FNA and pH adjustment. For the control sample, the pH was adjusted to 5.5 and kept at a constant temperature of 25°C for 24 hours without any FNA additions. This was done in addition to the FNA concentrations mentioned above.

Table 4-1 shows the different water quality characteristics for the raw and treated samples with different FNA doses at the end of the pretreatment procedure. As shown in the table there was no significant differences in TCOD, TS, and VS (less than 5% variation) before and after the pretreatment. This is expected as the pretreatment mainly converts the particulate organic fraction to soluble or colloidal matter. Similar observations were made in the study of other chemical pretreatments [29, 56]. SCOD concentrations increased for all pretreatment levels. The SCOD increased from 2300 mg/L for the raw TWAS to 3500 mg/L for the control sample (i.e. pH was adjusted at 5.5 without adding any FNA). On the other hand, with increasing the FNA dose from 0.35 to 1.4 mg N/L, the SCOD increased accordingly. The highest SCOD of 5590 mg/L was achieved at FNA dose of 1.4 mg N/L. There was no difference in the SCOD for the pretreated samples with 1.4 and 2.8 mg N/L. The highest increase of 140% observed at the second highest concentration FNA, 1.4 mg N/L compared to the raw sample. The aforementioned results indicate that the pretreatment enhances solubilization. This solubilization could be due to the release of soluble organic matter from cells and the breakdown of EPS [7].

Table 4-1. Change in water quality parameters after pretreatment

Batch #	FNA (mg N/L)	TCOD (mg/L)	SCOD (mg/L)	TS (mg/L)	VS (mg/L)	TVFAs (mg COD/L)	NH_4 (mg N/L)
1	Raw	48,500	2,300	36,040	29,460	2,050	87
2	0	47,800	3,510	36,640	29,800	2,564	167
3	0.35	48,100	4,910	36,640	29,420	2,953	90
4	0.7	46,200	5,250	38,140	31,000	3,312	77
5	1.4	47,500	5,590	36,980	29,820	3,586	72
6	2.8	46,900	5,560	38,060	29,920	4,199	62

Figure 4-1 shows the change in SCOD normalized per mass VS with and without FNA pretreatment. As shown in the figure, the SCOD per mg VS increased from 0.08 mg COD/mg VS for the raw sample to a maximum of 0.19 mg COD/mg VS for the FNA dose of 1.4 mg N/L. No further change was observed at 2.8 mg N/L. Wang et al. [8] reported similar trend in SCOD, i.e. with increasing the FNA, the SCOD concentration increased. They applied FNA pretreatment to TWAS in concentrations that ranged from 0 to 2.13 mg N/L. They observed that higher concentrations of FNA yielded higher SCOD concentrations. They reported that FNA pretreatment led to over six times the solubilization. The SCOD of the raw sample (0 mg N/L) was 0.025 mg COD/mg VS compared to 0.16 mg COD/mg VS when the TWAS was pretreated with FNA dose of 2.13 mg N/L [8].

The differences in the results between our study and those that are reported by Wang et al [8] might be due to the nature of the TWAS in each study. The source of the TWAS, mainly the process that produced the TWAS and the sludge age, plays significant role in the response

of the sludge to the pretreatment and its subsequent biodegradability. For example, with increasing the sludge age, digestibility decreases and resistance to pretreatment increases [20].

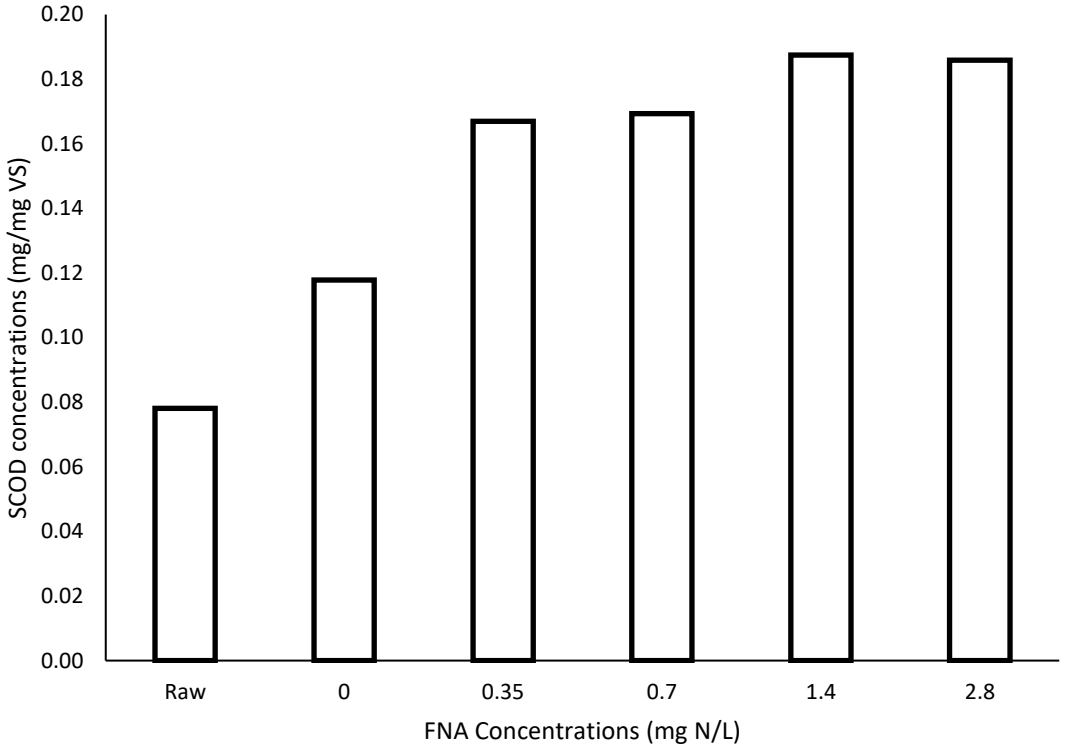

Figure 4-1. Change in SCOD per VS with FNA pretreatment

As shown in Table 4-1, the TVFAs concentrations increased from 2050 mg COD/L for the raw TWAS sample to a maximum of about 4200 mg COD/L at FNA dose of 2.8 mg N/L. The TVFAs normalized per mass of VS is shown in Figure 4-2 for the raw and treated samples. The TVFAs in mg COD per mg VS increased with increasing the FNA concentration. The 2.8 mg N/L FNA treatment yielded twice the amount of TVFAs per mg VS than the untreated sample i.e. 0.07 versus 0.14 mg TVFAs/mg VS.

A substantial portion of the SCOD being present as VFAs means that there was fermentation activity during the pretreatment stage due to the low pH value and the oxygen-free condition that was induced by conducting the pretreatment in closed bottles. 89% of the SCOD was present as TVFAs in the raw sample reducing to 73% in the sample with only pH

adjustment and lowering further in 0.35 mg N/L FNA sample. The TVFAs to SCOD fraction increased with addition of FNA up to 75% in the 2.8 mg N/L sample. Wang et al. [8] observed results that were contrary to the present results in that the highest VFA amount detected was with 0.36 mg N/L FNA pretreatment after which VFA concentration reduced as the amount of FNA increased. This contradiction in the VFAs results is mainly due the difference in the experimental setup. In this study, the pretreatment was conducted in a closed bottle which provided suitable conditions for the fermentation to occur, while in Wang et al. [8] , the pretreatment was conducted in open bottles such that the sludge was exposed to the air inhibiting the fermentation process.

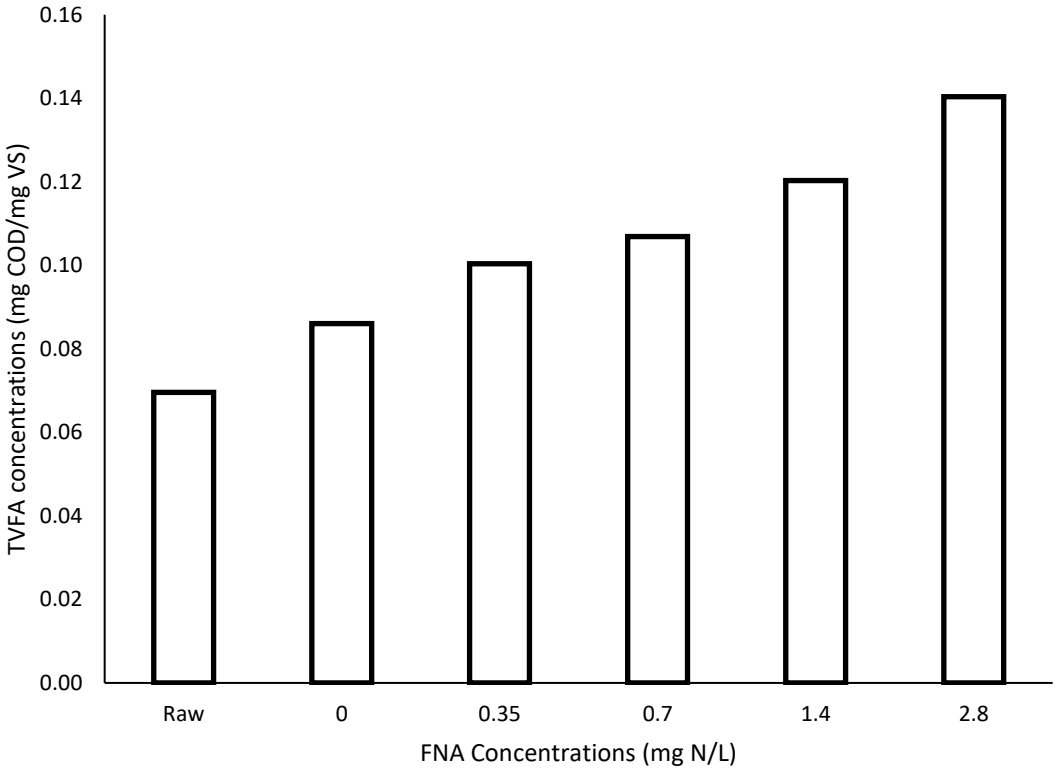

Figure 4-2. Change in total VFA per VS produced with FNA pretreatment

The breakdown of VFAs is shown in Figure 4-3. Acetate and propionate are seen to be the predominant acids in all pretreated samples ranging from 23 to 39% and from 28 to 43% of TVFAs, respectively. However, while propionate increases from 590 mg COD/L in the untreated samples to 1670 mg COD/L when FNA dose is increased to 2.8 mg COD/L, acetate drops from

1010 mg COD/L in the 0 mg N/L FNA sample to 770 mg COD/L in the 0.35 mg N/L FNA sample before subsequently increasing to 990 mg COD/L in the 2.8 mg COD/L sample. The drop in the acetate concentrations in the sample treated with 0.35 mg N/L FNA might be due to some *acetoclastic methanogenesis* activities at no FNA or very low FNA dosage which would have produced methane. However, the biogas produced during the pretreatment stage was not monitored.

Iso-butyrate and n-butyrate concentrations were reduced when pH was dropped to 5.5 without FNA addition from 510 to 360 mg COD/L and from 270 to 150 mg COD/L, respectively. This is may be due to acetogenesis activities where the butyrate would have been converted to acetate followed by methanogenesis activities during which acetate could be converted to methane. However, the concentrations of iso-butyrate and n-butyrate both increase with FNA addition to 680 mg COD/L and 970 mg COD/L, respectively. The maximum iso-butyrate concentration of 680 mg COD/L was observed in the 1.4 mg N/L FNA sample.

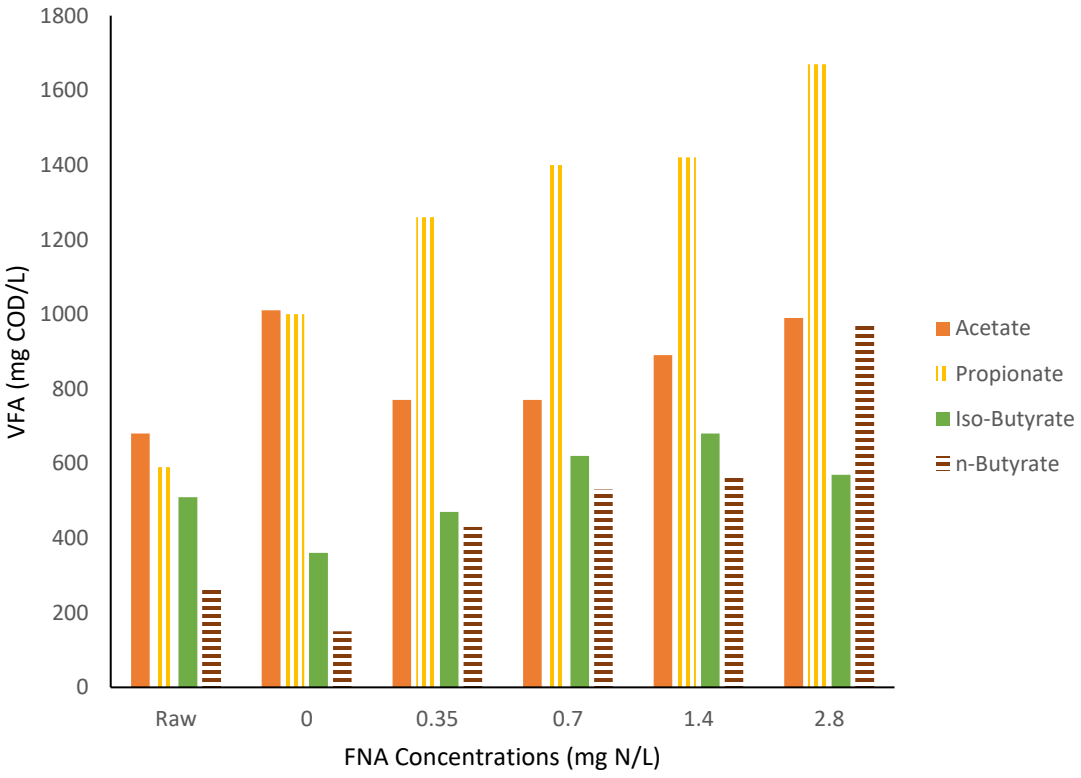

Figure 4-3. VFA breakdown variation with FNA pretreatment

Figure 4-4 shows the ammonia concentrations normalized to per mass of VS for the untreated and the treated samples. As shown in this figure, the ammonia content increased significantly from 3.0 mg NH_4-N/g VS in the untreated sample to 5.6 mg NH_4-N/g VS when the pH was adjusted to 5.5 without FNA addition. However, when FNA was added, the ammonia concentration decreased to 3.1 mg NH_4-N/g VS in the 0.35 mg N/L FNA sample. The decreasing trend continued such that in the 2.8 mg N/L FNA sample, ammonia concentration was 2.1 mg NH_4-N/g VS. This is contrary to the trend that was observed in Wang et al. [8] in which the concentration of ammonia increased from 1.5 mg NH_4-N/g VS in the sample with only pH adjustment, to 4.0 mg NH_4-N/g VS in the sample with 0.36 mg N/L FNA addition. However, similar to this study, further increase in FNA dosage led to a reduction in ammonia up to 2 mg NH_4-N/g VS in the 2.13 mg N/L FNA sample.

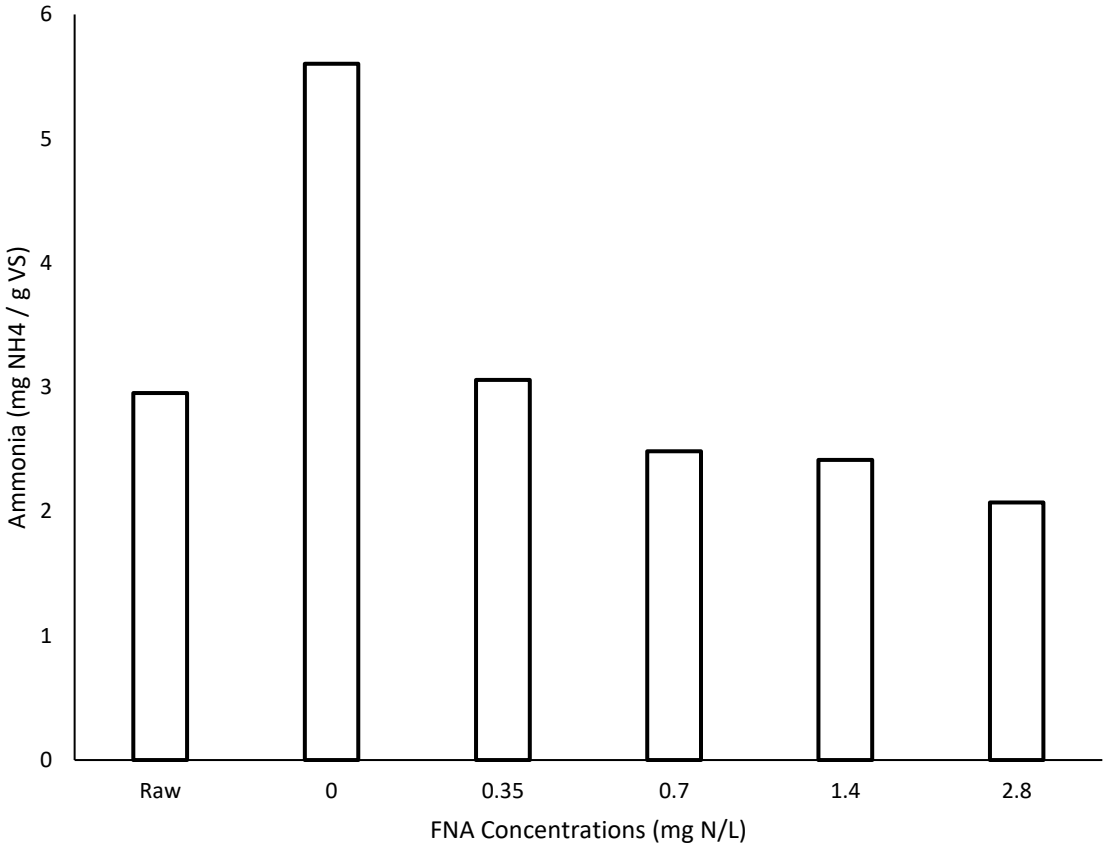

Figure 4-4. Ammonia content with varying pretreatment conditions

4.2 Effect of FNA Pretreatment on Biomethane Production

The production of methane over the BMP test period is shown in Figure 4-5. As shown in the figure, the cumulative methane production increased with time and reached a plateau after about 10 days of run time. There was no lag phase observed at the beginning of the test because the inoculum was collected from a digester that was already conditioned to this TWAS. This meant that the inoculum did not require a lag period to adapt to the feed. As depicted in this figure, the potential methane production from all pretreated samples were higher than that of the raw TWAS. The volume of methane that was produced increased with increasing the FNA dose from 0.35 to 1.4 mg N/L, after which, a reduction in methane production was observed.

The potential methane production from the raw TWAS was 117 mL. The methane production of 129, 137, 140, 152, and 147 mL were achieved for the pretreated samples with FNA doses of 0.0, 0.35, 0.7, 1.4, and 2.8, respectively. The methane yield normalized per gram of VS is shown in Figure 4-6. The methane yield produced from the raw TWAS was 158 mL/g VS. The second highest pretreatment level of 1.4 mg N/L FNA produced the highest methane yield of 203 mL/g VS. When the FNA dose increased to 2.8 mg N/L FNA, the methane yield dropped to 196 mL/g VS (which is still greater than the yield from the untreated TWAS). The highest cumulative methane yield that was achieved in the 1.4 mg N/L FNA sample was 28% higher than that of the methane produced from the untreated sample. Similarly, 31% improvement in methane yield per mass of TCOD added was observed when FNA dose of 1.4 mg N/L was used for pretreatment (Figure 4-6).

Wang et al. [8] conducted a BMP test for TWAS samples treated with FNA using doses ranging from 0 to 2.13 mg N/L FNA for 44 days. The sample that was treated with 1.78 mg N/L FNA produced the highest volume of methane for the first 15 days. However, they observed that after 15 days, the sample pretreated with 2.13 mg N/L FNA produced the highest methane yield. The sample that was treated with 2.13 mg N/L FNA showed 30% increase in methane production compared to the sample with only pH adjustment [8]. In the current study, the highest increase in methane yield was 17% which was observed in the 1.4 mg N/L sample (see Figure 4-6).

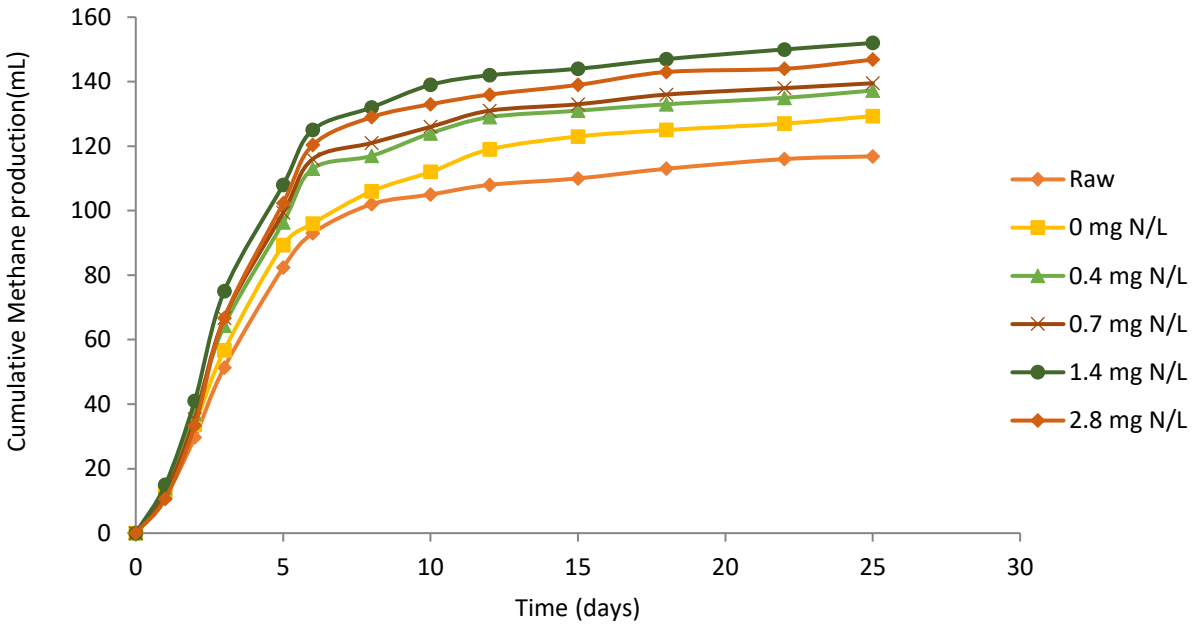

Figure 4-5. Cumulative methane production over BMP test period

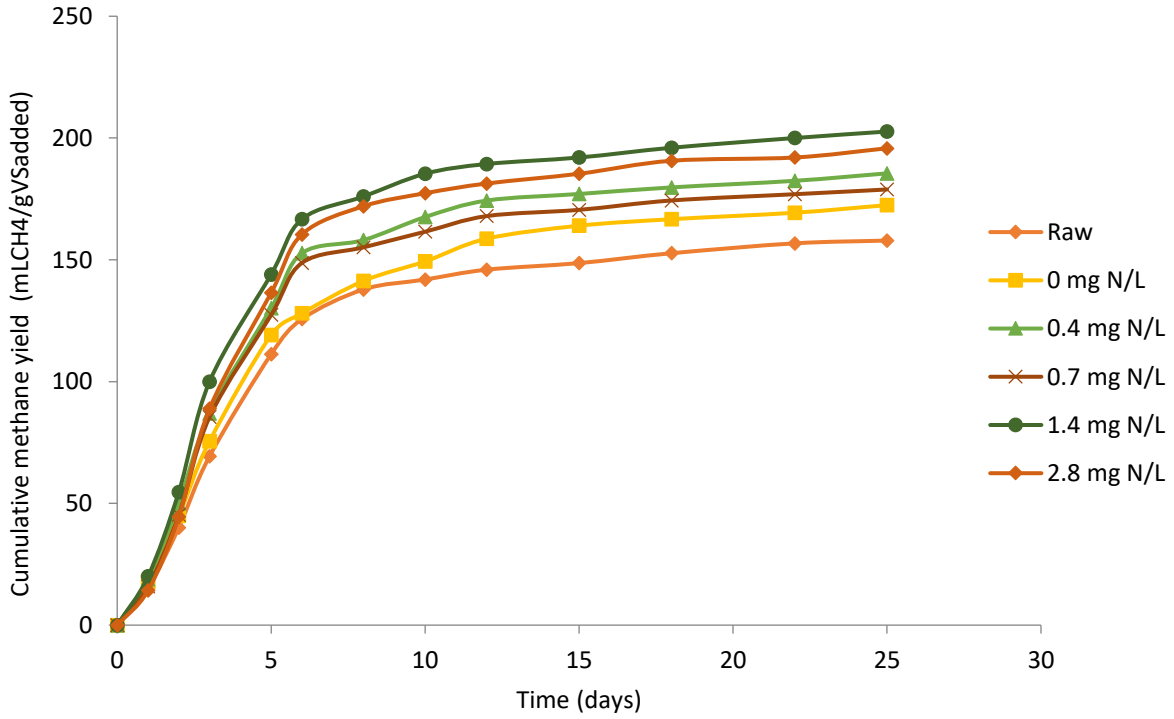

Figure 4-6. Normalized cumulative methane production trend per g VS

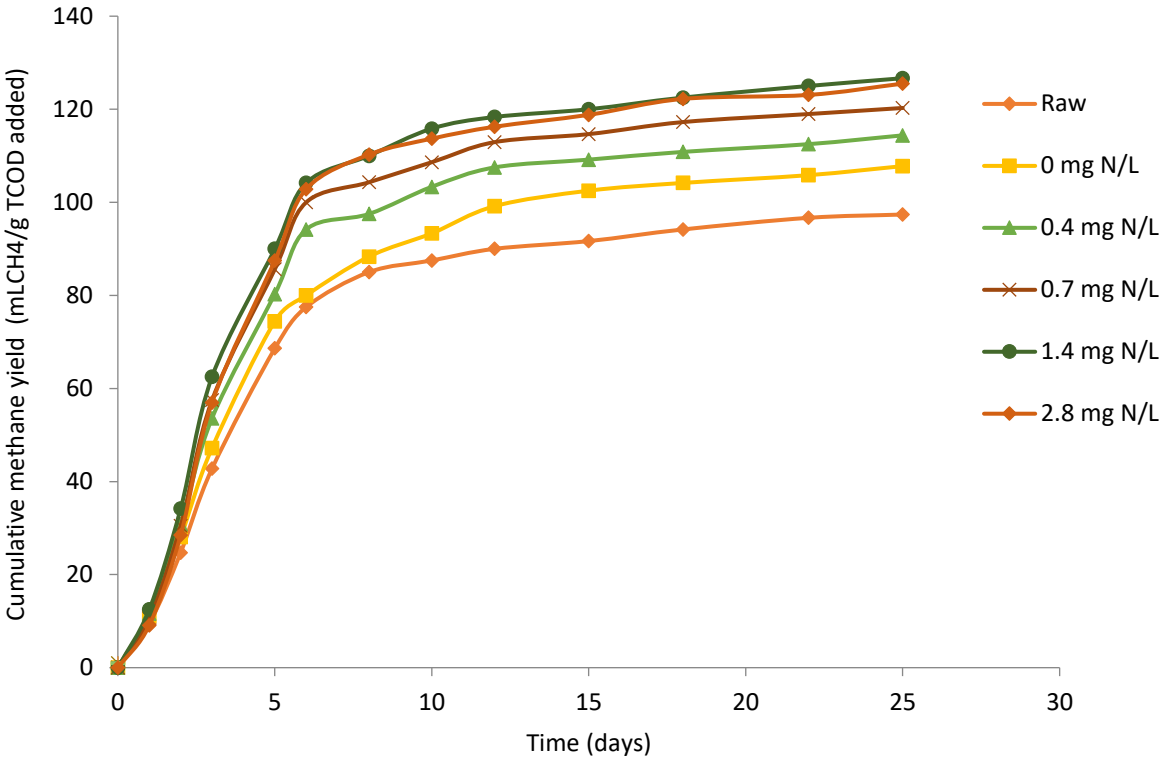

Figure 4-7. Normalized cumulative methane production per g TCOD added

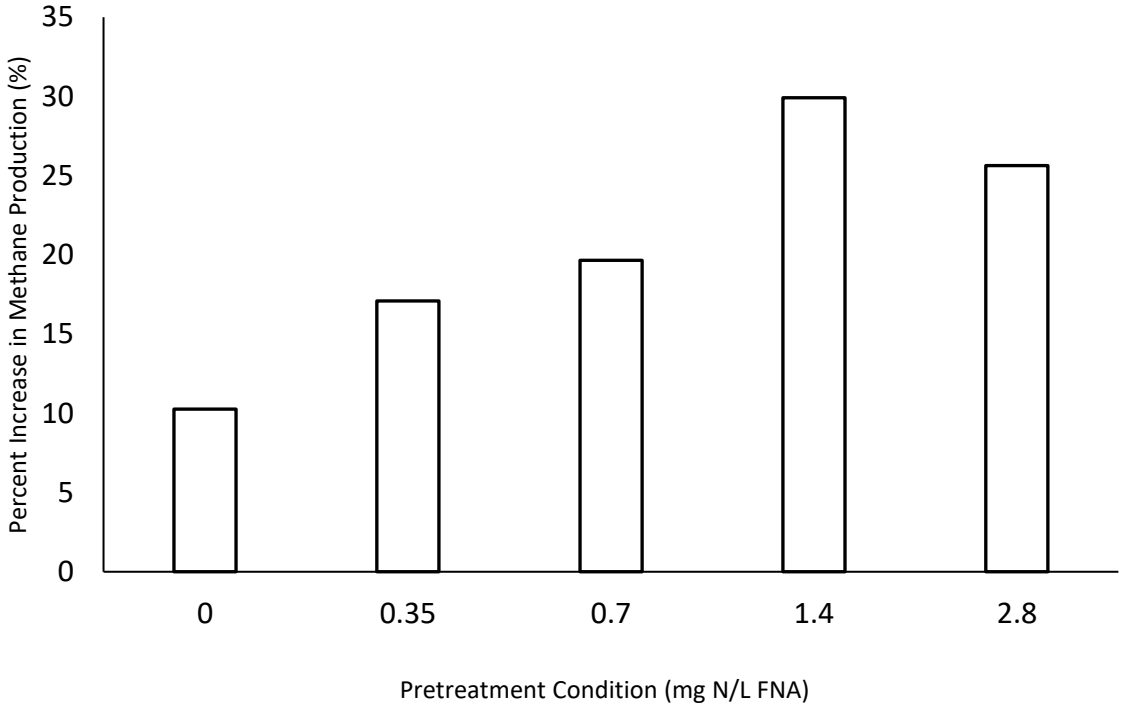

Figure 4-8. Percent increase in cumulative methane production corresponding to pretreatment levels

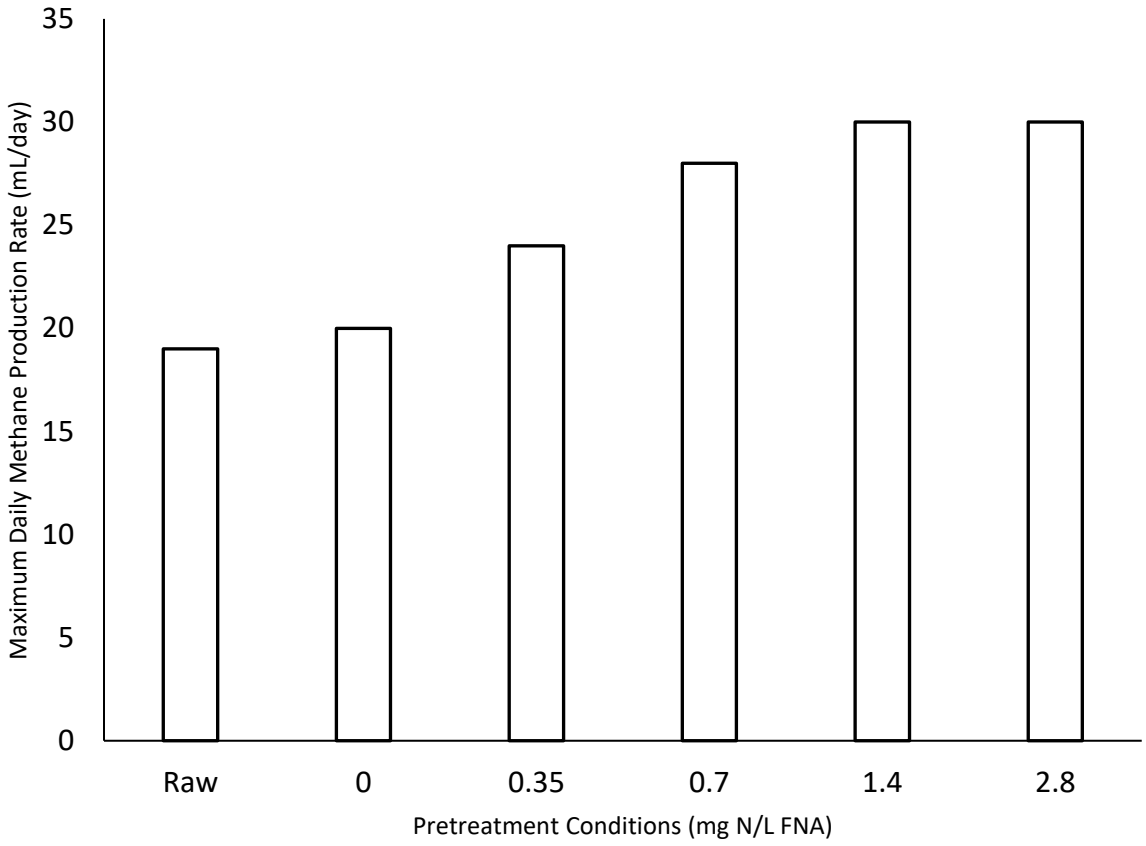

Figure 4-9. Maximum rate of daily methane production for samples with different pretreatment conditions

The maximum methane production rates during the exponential phase for the raw and the treated samples are shown in Figure 4-9. The maximum methane production rates for all the pretreated samples were higher than that of the untreated sample. The maximum methane production rate of 30 mL/d was achieved at pretreatment dose of 1.4 mg/L FNA-N compared to only 19 mL/d for the raw sample. The percentage increase in the maximum methane production rate compared to the raw sample is shown in Figure 4-10. The highest increase in the maximum methane production rate of 37% was achieved at FNA doses of 1.4 and 2.8 mg N/L compared to the raw sample.

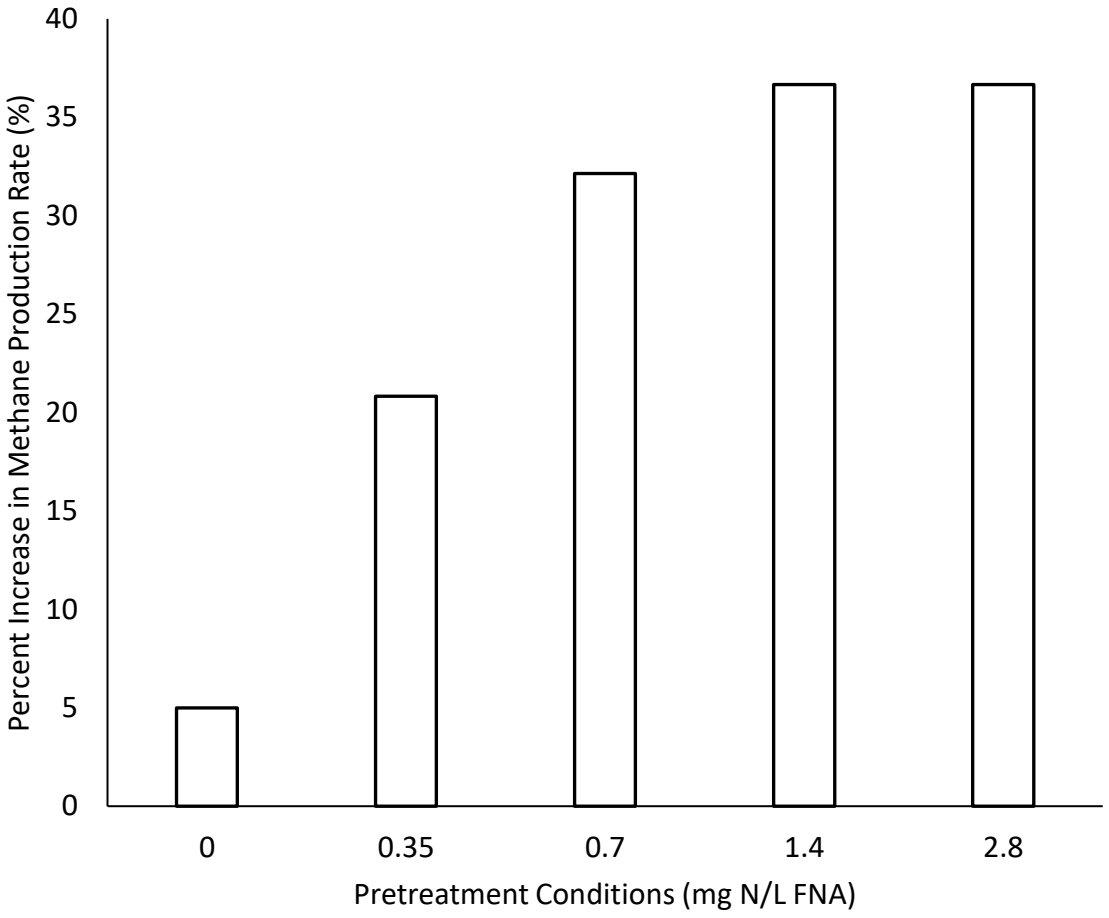

Figure 4-10. Percent increase of maximum daily methane production rate for pretreated samples compared with untreated sample

The methane yields normalized to per unit volume of TWAS and unit mass of TCOD added are shown in Figure 4-11 and Figure 4-12**,** respectively. The methane yields follow the same trend increasing from 4.7 L/L TWAS and 96 mL/g TCOD in the raw sample to 6.1 L/L TWAS and 128 mL/g TCOD for the pretreated sample with FNA dose of 1.4 mg N/L and then dropping slightly to 5.9 L/L TWAS and 125 mL/g TCOD when FNA increased further to 2.8 mg N/L.

The methane yield improvement of 28% that resulted from the application of 1.4 mg N/L FNA pretreatment to TWAS samples is comparable with values found in literature for other chemical pretreatment techniques. Indigenous iron activated peroxidation pretreatment of WAS using 50 mg H_2O_2/g TS and pH adjusted to 2 for 30 minutes led to 10% improvement in methane yield [54]. Alkali pretreatment of WAS to pH 10 at ambient temperature for 24 hours

using NaOH resulted in methane yield improvement of 15.4% [36]. A combined heat and alkali pretreatment in which 0.05g NaOH/g TS was added to WAS samples and maintained at 70°C for 9 hours yielded 83% more biogas than the untreated sludge [16]. Significantly higher biogas production, two times the amount of untreated sample, was obtained when 0.063 g O_3 /g TSS was applied as WAS pretreatment [55].

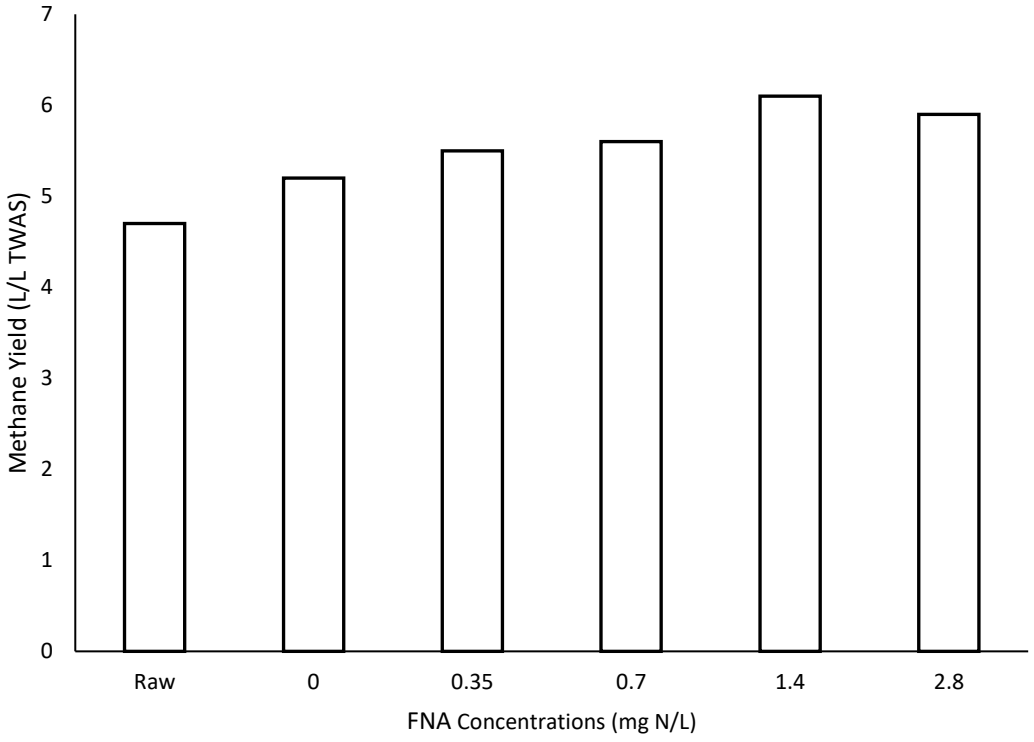

Figure 4-11. Total Methane yield normalized per volume of TWAS

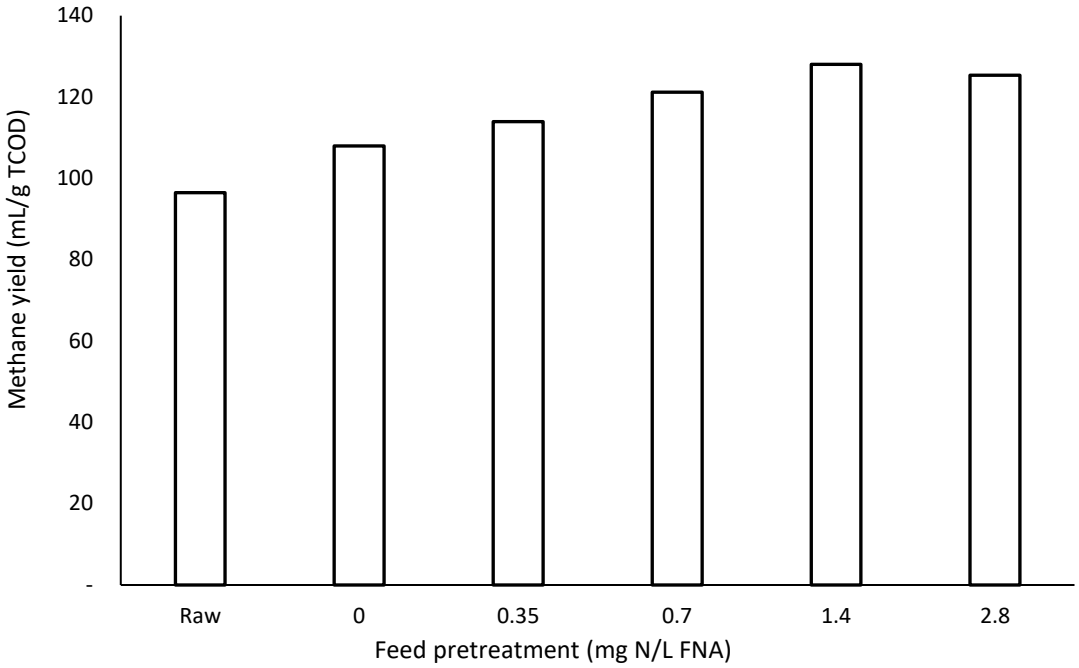

Figure 4-12. Total methane yield normalized per mass of total COD

4.3 Effect of FNA Pretreatment on Semi Continuous Anaerobic Digestion

4.3.1 Preliminary Semi-Continuous Study

At the initial stage of running the semi continuous AD systems, a 4 L system was used. It was heated by running a tubing around the bioreactor that conveyed hot water from a circulated heating bath. The tubing was covered with an aluminum heat shield wrap to prevent heat loss. The bioreactor was run using untreated TWAS at an SRT of 13 days. After 35 days had passed, the optimum FNA concentration for pretreatment that was selected from the batch tests, 1.4 mg N/L, was applied to the feed. The pH was not readjusted before feeding. A 55% reduction in the methane production was observed immediately following the first feed. The yield remained low over the next 20 days during which the feed was still pretreated. However, in order to recover the system, pretreatment was stopped and feed was reverted to untreated TWAS. Once the untreated TWAS was fed to the systems, the methane production improved immediately. The improvement continued for 27 days until the volume that was produced was similar to the levels prior to the FNA application.

When the system had recovered, the experiment was repeated to verify the observations. This time, the pH of the treated TWAS was adjusted to above 7 with NaOH before it was fed into the bioreactor. However, methane yield dropped immediately after the pretreatment started again and remained low while the feed was pretreated. Figure 4-13 shows the methane production throughout the experimental run as well as the pH of the effluent from the system. From the chart, it was evident that the effluent pH dropped as the methane production dropped albeit not as drastically. Recovery of pH was also observed once the feed pretreatment ceased. It was inferred that the methanogenesis stage was being inhibited which led to an increase in the VFA concentrations causing acidic conditions in the system that reduced the pH of the effluent. To confirm this hypothesis, all the FNA concentrations that were test in the batch test were retested under semi continuous conditions.

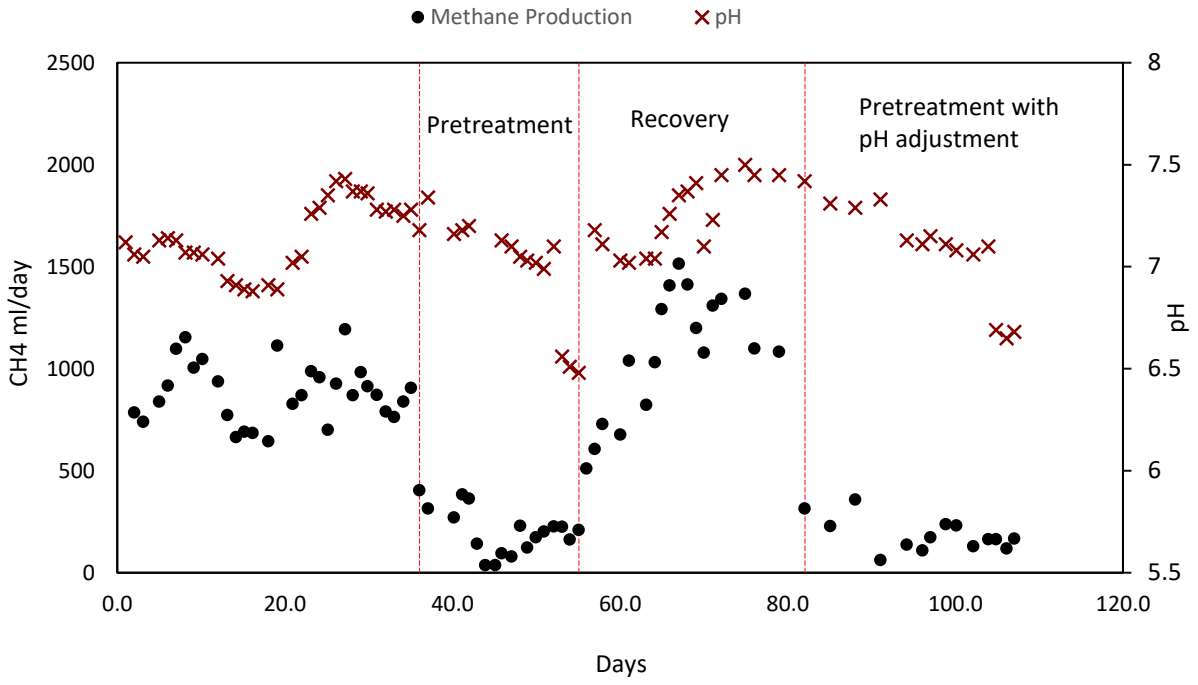

Figure 4-13. Methane production and pH trend for reactor fed with TWAS treated with 1.4 mg N/L

4.3.2 Sequential Semi-Continuous Study

Six bioreactors (2 L each) were then used to investigate the effect of FNA pretreatment on the anaerobic digestibility of TWAS in a semi-continuous (Fed-batch) process. The first reactor was fed with raw TWAS, the second reactor was fed with the control sample (in which the pH was adjusted to 5.5 without FNA addition), and the other four reactors were fed with TWAS that had been pretreated using FNA doses of 0.35, 0.7, 1.4, and 2.8 mg N/L. 100 mL of the untreated or treated TWAS was fed to each reactor every day in order to maintain the solid retention time at 20 days. The TWAS was pretreated every day prior to feeding it to the reactors.

The biogas content was analyzed and contained about 60% methane. This percentage was applied to the volume of biogas measured from the wet-tip gas meter. Over the experimental run of 72 days, the daily methane productions from the six reactors are shown in Figure 4-14. As shown in the figure, reactors 1, 2, 3, and 4 required about 20 days (one SRT) in order to stabilize and methane production to flatten out to a steady rate. Those reactors showed a steady increase in methane production until the 20^{th} day after which the daily methane production appeared to vary around mean values. This fluctuation was expected due the variation in the feed characteristics with time. On the other hand, the samples that were pretreated with 1.4 mg N/L FNA and 2.8 mg N/L started to fail about 10 to 12 days after the startup, yielding less methane production compared to bioreactor 1 which was fed with raw TWAS. Furthermore, the bioreactors that were fed with the control and the samples that were pretreated with FNA doses of 0.35 and 0.7 mg N/L produced higher methane compared to the reactor that was fed with raw sample.

Figure 4-15 shows the pH trend for each of the bioreactors over the course of the semi continuous experimental run. It is clear that while the four systems, bioreactors 1 to 4, that were successful maintained pH above 7. However, bioreactors 5 and 6 experienced a drop in pH below 7.

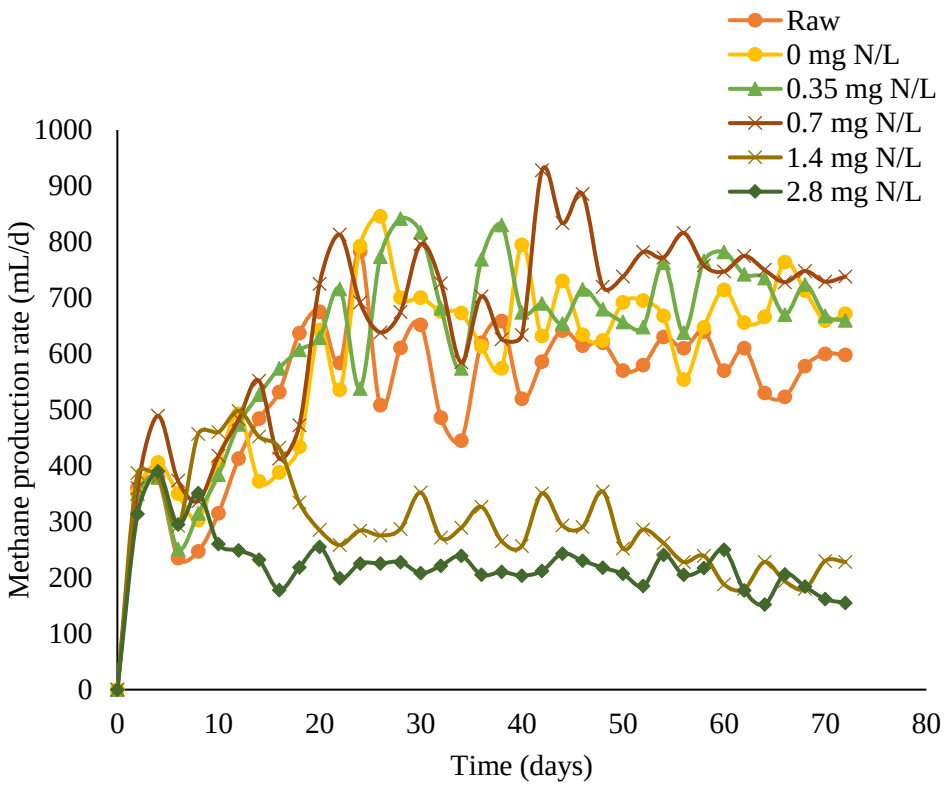

Figure 4-14. Methane production during semi-continuous process for TWAS samples pretreated with varying FNA concentrations

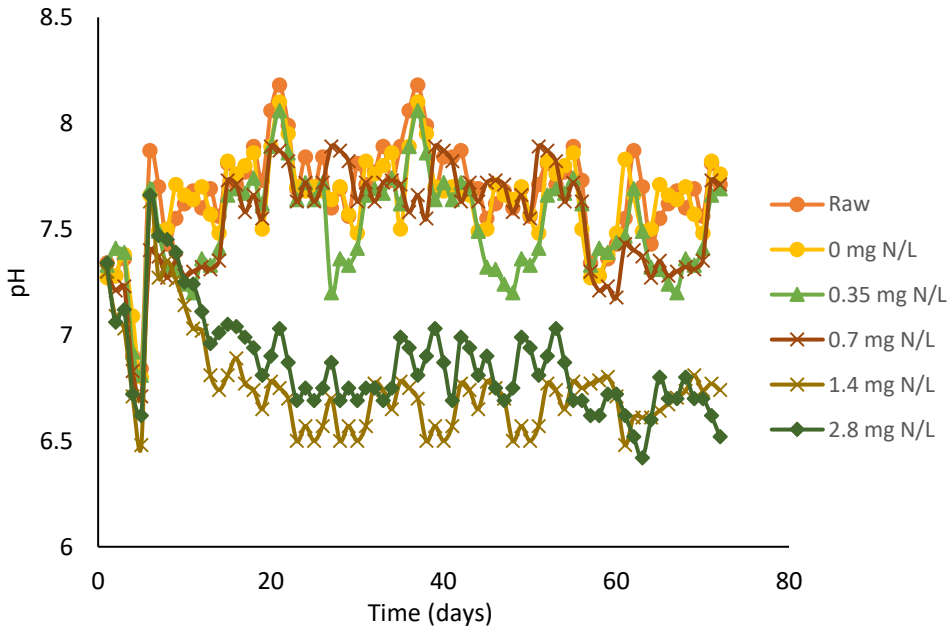

Figure 4-15. pH trend for bioreactors over experimental run

The average daily methane productions were considered for all systems after day 20 and are presented in Figure 4-16. The highest average methane production of 744 mL/day was observed in the system that was fed with pretreated TWAS with 0.7 mg N/L FNA. Surprisingly, the treatment level that yielded the highest methane production during the batch test, 1.4 mg N /L FNA, yielded 263 mL/day, which is almost a third of the maximum production observed during the semi-continuous process. Bioreactor 6, which was fed with 2.8 mg N/L FNA pretreated TWAS produced even less methane of 208 mL/day. Bioreactors 1 and 2 fed with raw and control sample produced 594 and 678 mL/day, respectively showing that the pH reduction does produce an effect on digestibility of TWAS. Methane production improved to 708 mL/day in bioreactor 3 which was fed with TWAS that was treated using 0.35 mg N/L FNA. The enhancement of the semi-continuous AD process was observed in this study when TWAS was pretreated using FNA up to a dose of maximum of 0.7 mg N/L.

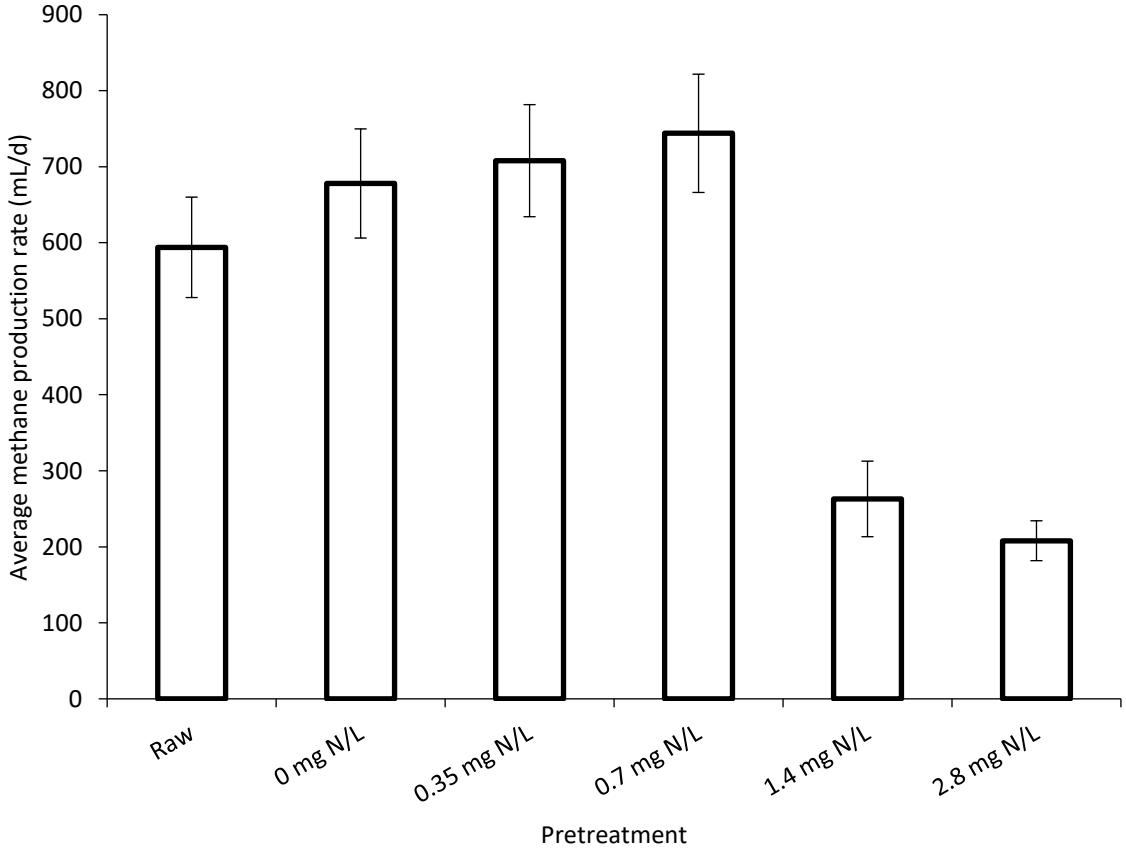

Figure 4-16. Average daily methane production during the steady state period

Methane yields (methane production per mass of TCOD fed in the influent) for each system were calculated and are shown in Figure 4-17. The raw system produced methane yield of 126 mL/g $TCOD_{added}$, this yield increased to 156 mL/g $TCOD_{added}$ when the TWAS was treated using 0.7 mg N/L FNA which was corresponding to 23% enhancement in yield. The methane yields for both the control bioreactor and bioreactor 3 (treated to 0.35 mg N/L FNA) was same (148 and 149 mL CH_4/g $TCOD_{added}$). However, when the FNA pretreatment was used in higher doses of 1.4 and 2.8 mL CH_4/g $TCOD_{added}$, the methane yield dropped significantly to 56 and 44 mL CH_4/ g $TCOD_{added}$, respectively.

This reduction in the methane yield in the two highest FNA concentrations may be attributed to the accumulation of FNA in the system. FNA was observed to reduce viability in microorganisms from anaerobic sewer biofilms at a concentration of 0.045 mg N/L. However, once FNA application ceased, recovery was observed within days [43]. This may be the reason that the pretreatment application of 1.4 mg N/L FNA resulted in enhanced digestion for the batch test (one dose) being that the destruction of microbial cell wall is one of the FNA mechanisms that is responsible for releasing organic matter. Subsequently, the same dosage led to the failure of the bioreactor in the semi-continuous mode, in which the feed was dosed every day.

The methane yields per volume of TWAS that was fed to the system and mass of VS added to the system are shown in Figure 4-18 and Figure 4-19, respectively. The methane yields show that the best performing system was that fed with pretreated TWAS with FNA dose of 0.7 mg N/L, produced 25% and 30% more methane per volume of TWAS and mass of VS, respectively, compared to bioreactor 1 (fed with raw TWAS).

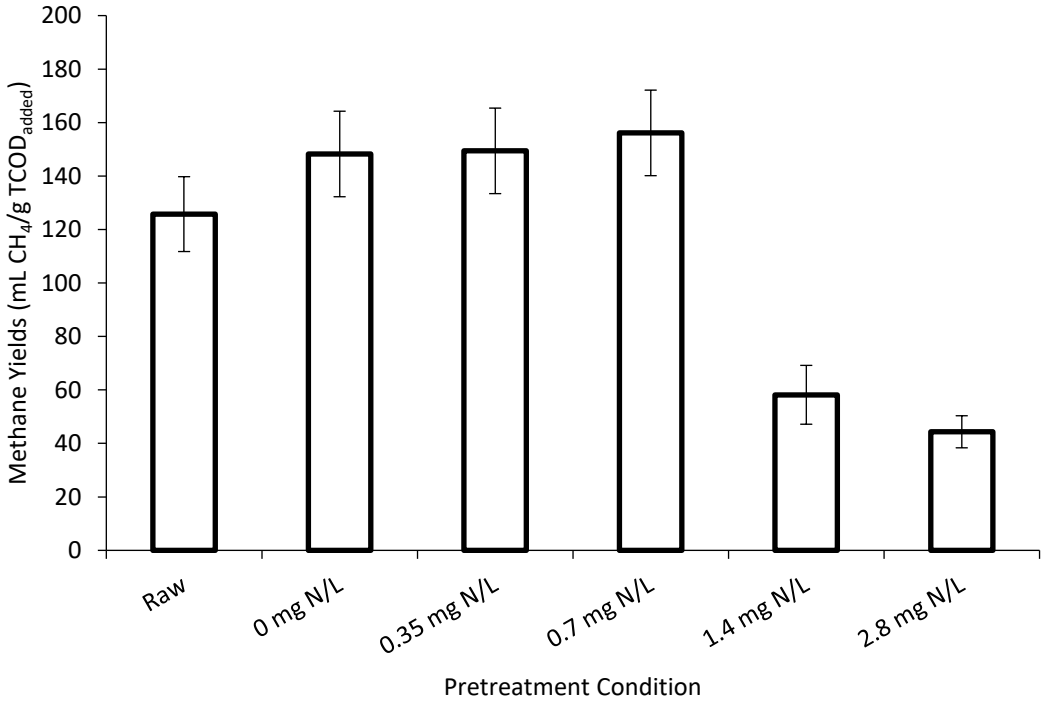

Figure 4-17. Methane yield per mass of TCOD added for semi-continuous system

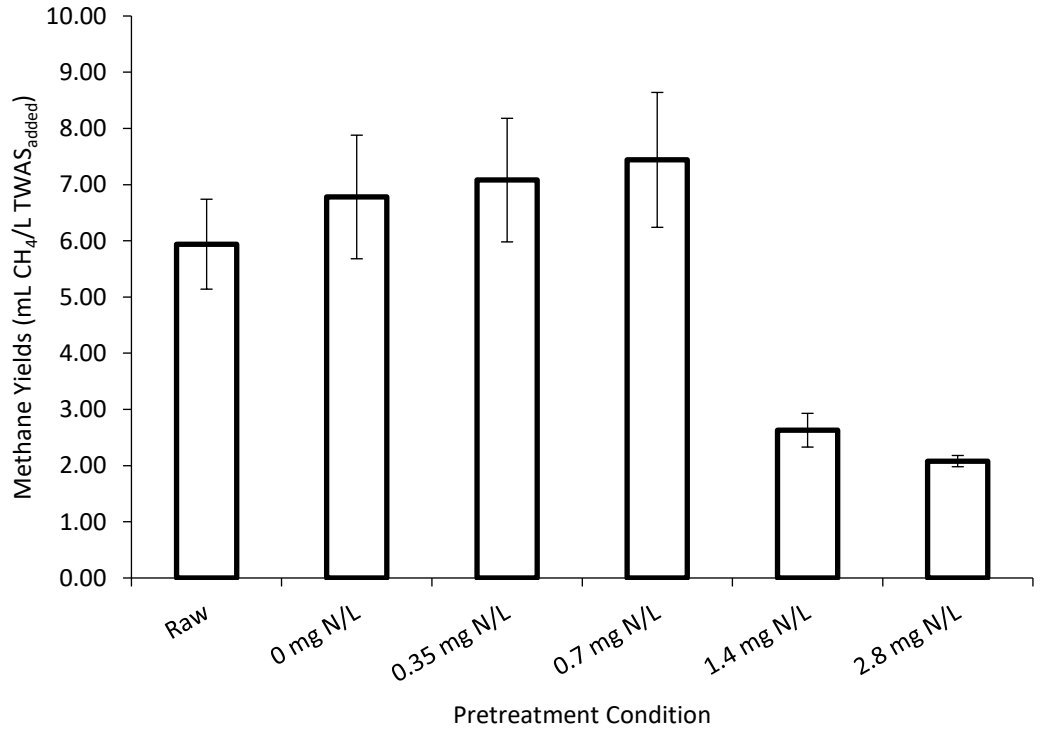

Figure 4-18. Methane yield per volume of TWAS fed into AD systems

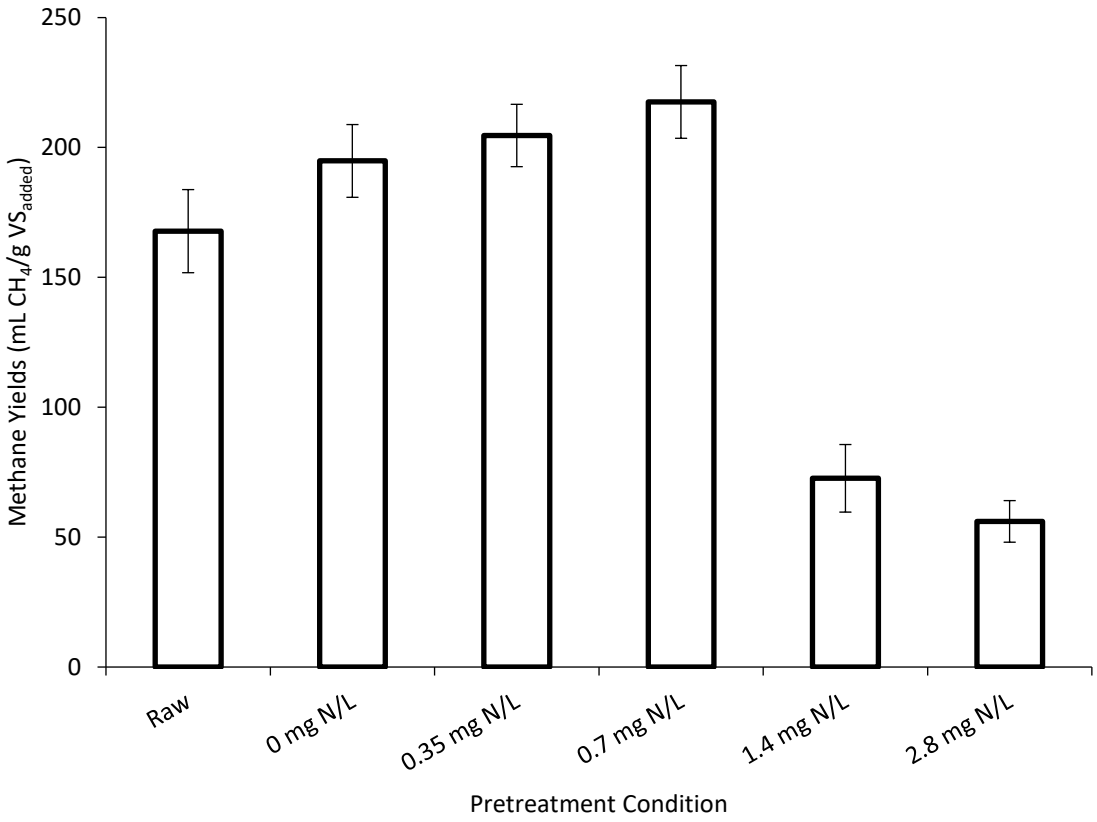

Figure 4-19. Methane yield per mass of VS added to AD systems

It should also be noted that the influence of FNA pretreatment appears to have a greater effect on methane production in the semi-continuous AD process than the batch process. As shown in Figure 4-6 and Figure 4-19, the highest methane yield per mass of VS in the batch after 25 days was 203 mL/g VS compared with the highest in the semi continuous reactors which was 218 mL/g VS using an SRT of 20 days.

The results from analysis on the samples of bioreactors were verified by conducting a mass balance on COD. Using the COD equivalent of methane and the TCOD concentrations of the influent and the effluent from the bioreactors, the percent mass balance was calculated using Equation (5).

$$Mass\ Balance\ \% = \frac{COD\ in\ Effluent + COD\ in\ Methane}{COD\ in\ Influent} * 100 \qquad \text{Equation (5)}$$

The mass balance for all reactors as shown in Table 4-2 were above 85%. This shows reliability of the data that was compiled.

Table 4-2. COD mass balance in bioreactors

Feedstock	TCOD			
	Influent (mg/d)	Effluent (mg/d)	Methane (mg/d)	COD mass Balance (%)
Raw	4723	2778	1492	90
0 mg N/L	4573	2449	1704	91
0.35 mg N/L	4738	2444	1779	89
0.7 mg N/L	4765	2324	1869	88
1.4 mg N/L	4520	3434	661	91
2.8 mg N/L	4690	3807	523	92

Following the data verification, the removal efficiency of TCOD during semi continuous anaerobic digestion was investigated.

$$Removal\ Efficiency\ (\%) = \frac{TCOD_{in} - TCOD_{out}}{TCOD_{in}} * 100 \qquad \text{Equation (6)}$$

Given by Equation (6), the effect of FNA addition on the TCOD removal efficiencies for all systems are illustrated in Figure 4-20. $TCOD_{in}$ is the concentration of total COD in the feed of the systems while $TCOD_{out}$ is the total COD concentration of the system effluent. Increasing the FNA concentration to 0.7 mg N/L during pretreatment appears to enhance the removal of TCOD during digestion from 41% (untreated) to 51%.

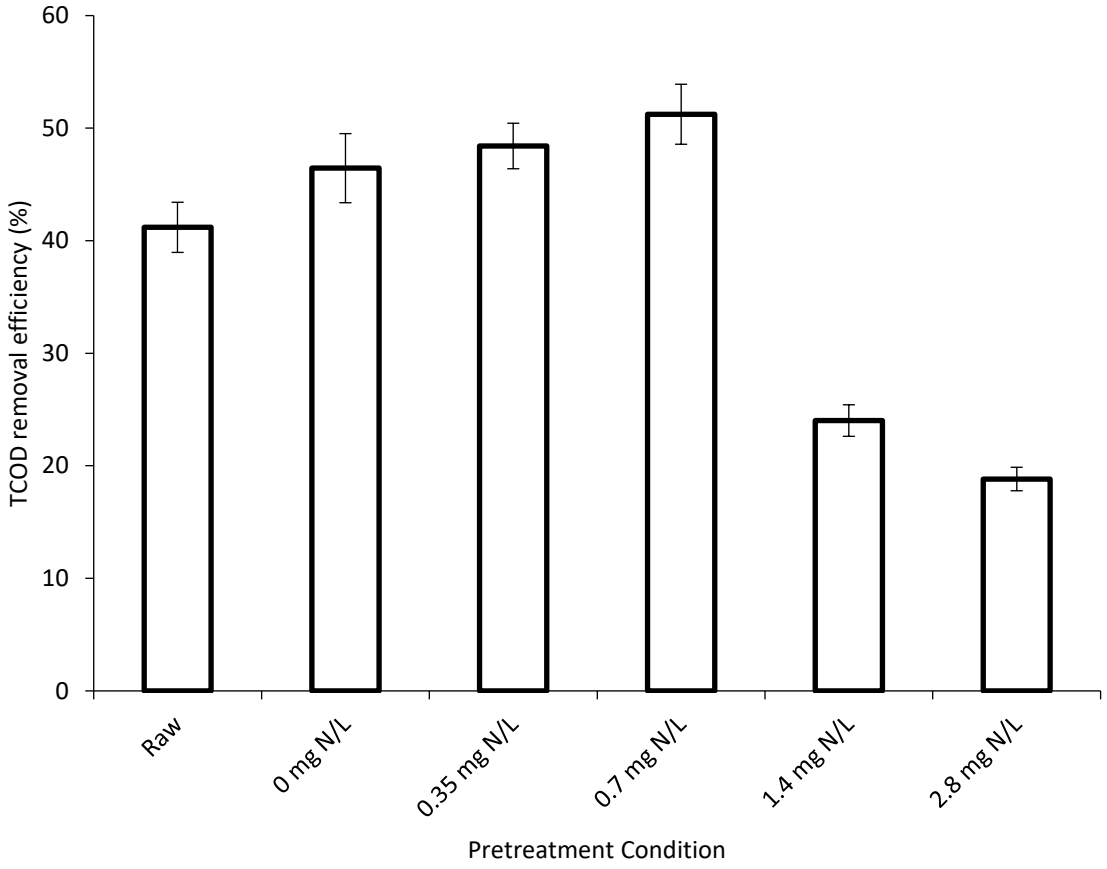

Figure 4-20. Removal efficiency of COD in semi-continuous systems

At concentrations that were higher than 0.7 mg N/L, the removal efficiency of the system drops below that of the untreated system to 19% at the highest FNA concentration of 2.8 mg N/L. Similarly, VS destruction of the AD systems were considered using a form of Equation (6) with TCOD replaced with VS. The highest solids destruction was observed in the 0.7 mg N/L system with 8% more VS destruction than the raw system. The negative effect of high FNA concentrations on VS destruction was less than that of COD removal but it was still significant with the 2.8 mg N/L system showing 18% less VS destruction than the raw system. This is displayed in Figure 4-21.

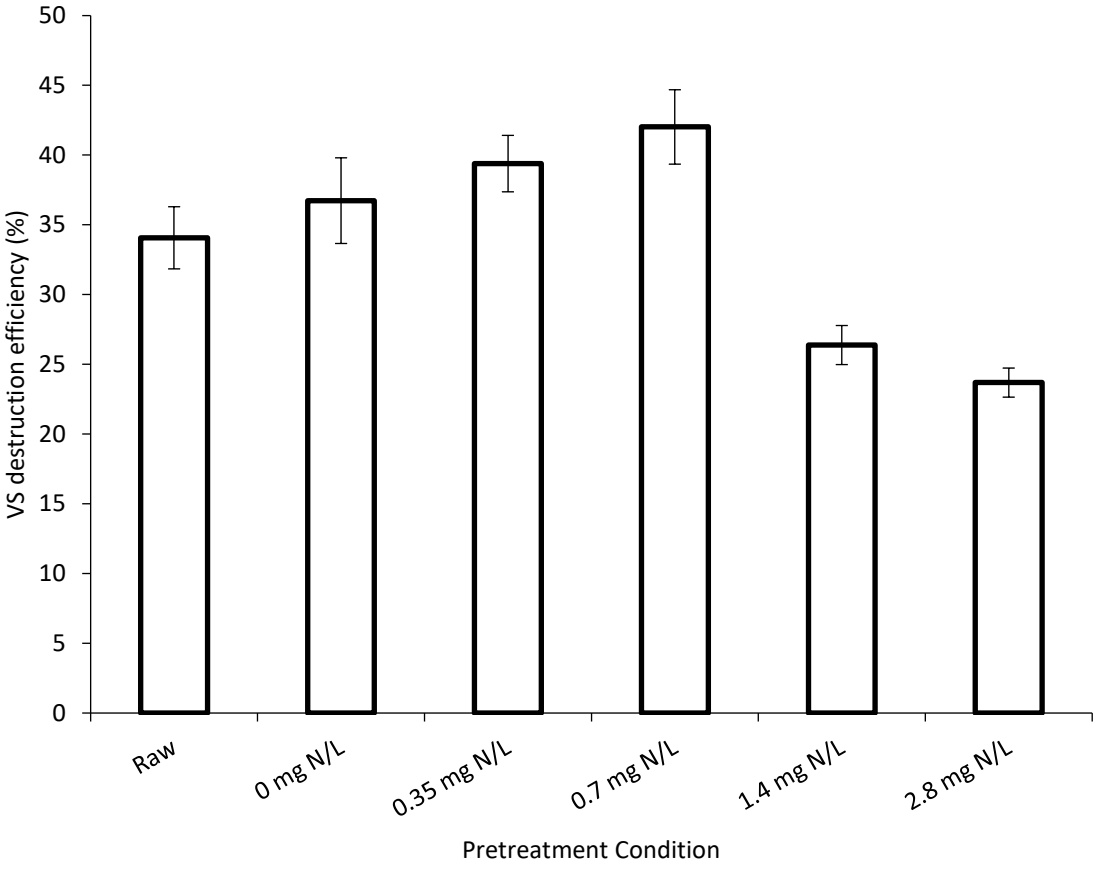

Figure 4-21. Effect of FNA pretreatment on VS destruction

5.0 Conclusions and Recommendations

Conclusions

Anaerobic digestion is one of the most common biological sludge handling methods. The potential for sludge stability, solids destruction, biogas production and low environmental impact make it attractive. WAS from the secondary aeration tank is held together by a complex structure of EPS that are resistant to degradation. One of the limitations of anaerobic digestion of sludge is the long retention time that is required to obtain a reasonably stable residue due to the complex structure that make up sludge. Techniques such as pretreatments are required in order to disrupt these structures and disintegrate the cells and thus shorten the retention time. Overall, these techniques can not only improve digestion efficiency but also improve biogas production. The pretreatment techniques are broadly classified into physical, biological, and chemical methods due to the mechanisms used for cell disintegration to improve the hydrolysis stage of anaerobic digestion.

Under the physical classification, thermal and mechanical methods such as conventional heating, microwave irradiation, high-pressure homogenization, and ultrasonication are typical technologies being used. The use of specific strains of enzymes to catalyze the digestion process lies under the biological method. Chemical pretreatment is the use of chemical reagents such as acids, bases, or oxidants to improve sludge disintegration before the anaerobic digestion process commences.

Many of the pretreatment techniques have negative environmental impacts, cause damage to facility equipment and can be energy intensive. Free nitrous acid (FNA) was seen to be a promising chemical technique for pretreatment since studies showed that it can work as a biocidal agent to disrupt cell walls making organic matter more accessible to microbes and will degrade in the wastewater treatment plant requiring no extra step for removal. Additionally, FNA can be produced in the wastewater treatment plant in a process that is beneficial to the biological nutrient removal stage.

Previous research into the use of FNA as a pretreatment for the anaerobic digestion of TWAS considered only the batch process. However, the semi continuous setup is more common

in full-scale applications. Therefore, the purpose of this study was to investigate the effect of FNA pretreatment on a lab scale, semi continuous anaerobic digestion process. The objective was to determine the optimum concentrations of FNA to improve biogas production and enhance digestion efficiency.

Four concentrations of FNA (0.35, 0.7, 1.4 and 2.8 mg N/L) were investigated in this study along with a blank and a control for pH. The pretreatment was conducted for 24 hours at pH 5.5 and temperature 25°C. The change in characteristics of thickened waste activated sludge due to this pretreatment were analyzed and discussed.

Based on the outcome of this study, the following conclusions can be made:

- No significant differences in TCOD concentrations were observed due to the pretreatment.
- SCOD increased with increasing FNA concentrations. At the second highest level of pretreatment, 1.4 mg N/L FNA, SCOD concentration increased by 140%.
- After the batch tests which were run for 25 days, the 1.4 mg N/L FNA pretreatment yielded the highest methane production of 203 mL/g VS.
- In the semi continuous process, the 1.4 mg N/L FNA pretreated system failed producing 56% less methane than the raw. However, the system that was pretreated to 0.7 mg N/L FNA yielded 218 mL/g VS of methane which is 30% more than the raw system. Digestibility of TWAS was enhanced by applying FNA pretreatment.
- Solids destruction improved by about 10% after FNA was used indicating the FNA may be able to lower transportation and disposal costs of TWAS.
- Methane production was more enhanced for the semi-continuous reactors with SRT of 20 days compared with the batch reactors which run for 25 days.
- It was suggested that the accumulation of FNA in the system due to continuous feeding might have led to the failure of the system since the chemical is a known biocidal agent.

Recommendations and Future Research

The TWAS that was used for this study contained some nitrite and so by adding extra nitrite, the true FNA concentration would have been higher than calculated. Therefore, it may

be useful to take consider the initial nitrite concentration of the substrate being studied to determine ideal FNA concentrations for pretreatment. Furthermore, while studies indicate that FNA is degraded in the system, it might be useful to track the nitrogen species cycle from pretreatment to AD effluent to better understand the implications of adding nitrogen to the system in form of FNA. In addition, dewatering is a primary post treatment of digester effluent before disposal of the solids portion. The application may or may not influence the dewaterability of digested sludge hence this effect should be investigated. Also, as one of the attractions of FNA is its ability to be produced in the wastewater treatment facility, the effect of using nitrite rich anaerobic digester liquor for WAS pretreatment should be studied. Finally, since this study has established that FNA can improve digestion efficiency under semi continuous flow, it would be useful to undertake a preliminary economic analysis of the cost of making changes to the plant to produce nitrite rich supernatant and the savings that are associated with using this liquid for WAS pretreatment.

Appendices

A. Pretreatment

Table A1. Condition of FNA pretreatment application

Nitrite and FNA doses								
Bottle	NO_2	pH	Temp	ka	FNA	Volume of pretreated sample	Nitrite added	Volume of Stock solution
	mg N/L		°C		mg N/L	mL	mg	mL
1	0		25		0.0	200	0	0
2	0	5.5	25		0.0	200	0	0
3	50	5.5	25	0.000445	0.35	200	10	2
4	100	5.5	25	0.000445	0.7	200	20	4
5	200	5.5	25	0.000445	1.4	200	40	8
6	400	5.5	25	0.000445	2.8	200	80	16

B. Batch Experiment

Table A2. Daily methane production during batch test

Time (day)	CH4 production mL					
	Raw	0 mg N/L	0.4 mg N/L	0.7 mg N/L	1.4 mg N/L	2.8 mg N/L
0	0	0	0	0	0	0
1	9	11	12	11	13	9
2	25	28	31	30	34	28
3	43	47	54	57	63	57
5	69	74	80	86	90	87
6	78	80	94	100	104	103
8	85	88	98	104	110	110
10	88	93	103	109	116	114
12	90	99	108	113	118	116
15	92	103	109	115	120	119
18	94	104	111	117	123	122
22	97	106	113	119	125	123
25	97	108	114	120	127	126

Table A3. Methane yield for each pretreatment level

System #	FNA dose (mg N/L)	Methane Production (mL)	Maximum daily production rate (mL/d)	Methane Yield (L/g $TCOD_{added}$)
1	Raw	117	19	96
2	0	129	20	108
3	0.35	137	24	114
4	0.7	140	28	121
5	1.4	152	30	128
6	2.8	147	30	125

C. Semi Continuous Experiment

Table A4. TCOD concentration of bioreactors influent with statistical analysis

	Influent TCOD (mg/L)						Av.	STD	CF
							mg/L	mg/L	%
Raw	48100	51700	45200	45200	47800	45400	47233	2556	5.4
FNA_0	49000	49000	42900	43100	47300	43100	45733	3023	6.6
FNA_0.35	50400	48600	46500	44800	46200	47800	47383	1980	4.2
FNA_0.7	49200	50400	47900	45200	49100	44100	47650	2479	5.2
FNA_1.4	47000	49400	44100	42200	45200	43300	45200	2634	5.8
FNA_2.8	47400	50900	44600	43900	48500	46100	46900	2598	5.5

Table A5. TCOD concentrations for effluent from bioreactors including statistical analysis

	Effluent TCOD (mg/L)							Av.	STD	CF
								mg/L	mg/L	%
Raw	25600	27750	27750	29200	28000	28550	27600	27779	1114	4.0
FNA_0	20900	24000	24000	25100	24350	26350	26750	24493	1929	7.9
FNA_0.35	21350	26000	26000	24200	25400	24900	23250	24443	1684	6.9
FNA_0.7	24150	22650	22650	24100	24100	22550	22450	23236	827	3.6
FNA_1.4	32350	35150	35150	35350	33950	35150	33300	34343	1168	3.4
FNA_2.8	36000	40600	40600	37350	37900	37350	36700	38071	1827	4.8

Table A6. Effluent SCOD concentrations from the semi-continuous bioreactors

Pretreatment FNA dose (mg N/L)	Effluent SCOD (mg/L)						Av.	STD	CF
							mg/L	mg/L	%
Raw	3,980	3,880	2,100	2,220	2,290	2,310	2,797	882	31.5
0	2,330	2,400	2,320	2,310	2,560	2,590	2,418	126	5.2
0.35	4,110	4,060	2,360	2,390	2,900	2,860	3,113	786	25.3
0.7	5,410	5,460	4,340	4,220	4,160	4,190	4,630	627	13.5
1.4	9,590	9,550	13,290	13,160	12,440	12,320	11,725	1712	14.6
2.8	11,610	11,350	14,680	14,860	14,040	14,020	13,427	1547	11.5

Table A7. Effluent VS concentrations from the semi-continuous bioreactors

Pretreatment	Effluent VS (g/L)					Av.	STD	CF
						g/L	g/L	%
Raw	22.12	23.75	23.22	23.37	24.24	23.34	0.79	3.4
FNA_0	21.41	23.34	20.45	21.97	22.92	22.02	1.16	5.3
FNA_0.35	18.73	21.86	21.90	21.59	20.78	20.97	1.33	6.4
FNA_0.7	16.77	20.64	20.52	19.65	21.57	19.83	1.84	9.3
FNA_1.4	24.24	24.52	27.65	26.09	30.74	26.65	2.66	10.0
FNA_2.8	26.86	27.32	25.38	28.51	33.48	28.31	3.10	11.0

Table A8. Effluent TS concentrations from the semi-continuous bioreactors

Pretreatment FNA dose (mg N/L)	Effluent TS (mg/L)					Av.	STD	CF
						mg/L	mg/L	%
Raw	20.16	21.26	21	21.04	21.62	21.0	0.5	2.6
0	21.36	23.22	22.52	22.54	23.38	22.6	0.8	3.5
0.35	19.68	22.56	22.86	22.34	22.48	22.0	1.3	5.9
0.7	21.48	23.88	23.56	22.86	23.2	23.0	0.9	4.0
1.4	20.66	23.68	24.52	23.52	24.92	23.5	1.7	7.1
2.8	25.1	26.12	25.2	25.24	27.98	25.9	1.2	4.7

Table A9. VS content of effluent from bioreactors including statistical analysis

Pretreatment	Effluent VS (g/L)					Av.	STD	CF
						g/L	g/L	%
Raw	22.12	23.75	23.22	23.37	24.24	23.34	0.79	3.4
FNA_0	21.41	23.34	20.45	21.97	22.92	22.02	1.16	5.3
FNA_0.35	18.73	21.86	21.90	21.59	20.78	20.97	1.33	6.4
FNA_0.7	16.77	20.64	20.52	19.65	21.57	19.83	1.84	9.3
FNA_1.4	24.24	24.52	27.65	26.09	30.74	26.65	2.66	10.0
FNA_2.8	26.86	27.32	25.38	28.51	33.48	28.31	3.10	11.0

Table A10. Daily methane production from the semi continuous bioreactors

	CH_4 (mL/day)					
Time (d)	Raw	0 mg N/L	0.35 mg N/L	0.7 mg N/L	1.4 mg N/L	2.8 mg N/L
0	0	0	0	0	0	0
2	360	351	350	360	387	314
4	379	406	379	489	380	390
6	235	350	251	373	293	295
8	247	303	315	337	457	351
10	315	404	384	418	460	260
12	413	493	474	483	498	249
14	484	372	527	552	452	232
16	532	388	574	413	432	178
18	637	434	607	472	334	218
20	675	642	629	725	285	255
22	584	536	716	813	258	199
24	784	792	538	692	284	226
26	508	846	774	638	276	226
28	611	700	842	675	287	228
30	652	700	817	796	352	208
32	486	676	682	726	271	221
34	445	673	574	585	289	239
36	620	613	769	703	327	205
38	658	574	830	626	265	210
40	520	795	674	633	257	204
42	586	632	690	928	351	212
44	641	730	654	834	293	243
46	614	634	715	886	290	230
48	620	624	680	720	354	218
50	570	692	657	738	252	207
52	580	695	648	782	286	185
54	630	668	763	772	262	241
56	610	554	638	816	228	205
58	640	647	766	758	239	217
60	570	714	782	747	188	250
62	610	656	742	775	180	177
64	530	666	735	750	228	152
66	523	764	670	728	194	206
68	578	713	724	748	180	184
70	600	659	668	729	230	162
72	598	672	660	738	228	155

Table A11. Removal efficiency of TCOD in bioreactors

Pretreatment FNA dose (mg N/L)	TCOD		Removal efficiency	
	Influent	Effluent	Average	STD
	mg/L	mg/L	%	%
Raw	47233	27779	41	2.2
0	45733	24493	46	3.1
0.35	47383	24443	48	2.0
0.7	47650	23236	51	2.7
1.4	45200	34343	24	1.4
2.8	46900	38071	19	1.0

Table A12. Maximum daily methane production rate

Pretreatment FNA dose (mg N/L)	Average methane production rate	
	Av.	STD
	mL/d	mL/d
Raw	594	66
0	678	72
0.35	708	74
0.7	744	78
1.4	263	50
2.8	208	26

Table A13. Methane yield per mass of TCOD

Pretreatment FNA dose (mg N/L)	$TCOD_{in}$		CH_4	Methane yields	
				Average	STD
	mg/L	mg/d	mL/d	mL CH_4/g $TCOD_{added}$	mL CH_4/g $TCOD_{added}$
Raw	47233	4723	594	126	14
0	45733	4573	678	148	16
0.35	47383	4738	708	149	16
0.7	47650	4765	744	156	16
1.4	45200	4520	263	58	11
2.8	46900	4690	208	44	6

Table A14. Methane yield per volume of TWAS added

Pretreatment FNA dose (mg N/L)	CH_4 mL/d	Methane yields	
		Average	STD
		L CH_4/L $TWAS_{added}$	mL CH_4/L $TWAS_{added}$
Raw	594	5.94	0.8
0	678	6.78	1.1
0.35	708	7.08	1.1
0.7	744	7.44	1.2
1.4	263	2.63	0.3
2.8	208	2.08	0.1

Table A15. Methane yield per mass of VS added

Pretreatment FNA dose (mg N/L)	VS_{in}		CH_4	Methane yields	
				Average	STD
	mg/L	mg/d	mL/d	mL CH_4/g VS_{added}	mL CH_4/g VS_{added}
Raw	35400	3540	594	168	16
0	34800	3480	678	195	14
0.35	34600	3460	708	205	12
0.7	34200	3420	744	218	14
1.4	36200	3620	263	73	13
2.8	37100	3710	208	56	8

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