# FERMENTATION OF THICKENED WASTE ACTIVATED SLUDGE FOR VOLATILE FATTY ACIDS

by

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Fermentation of Thickened Waste Activated Sludge for the Production of Volatile Fatty Acids, Tolulope Oludemi, M.Eng, Civil Engineering, Ryerson University, 2018

# ABSTRACT

The pre-treatment of sludge has gained a lot of attention for treating and handling waste, especially in wastewater treatment plants. Different technologies are being investigated to improve how waste can be reduced, a certain type of pre-treatment used is Free Nitrous Acid (FNA). This technology has been used as a chemical pre-treatment in the anaerobic digestion process to improve the fermentation/hydrolysis stage. The effect of utilizing different doses of the FNA pre-treatment was investigated for this study, and the effect of the pre-treatment on the characteristics of thickened waste activated sludge (TWAS) were identified. It was observed that as the doses of FNA increased, the concentration of soluble chemical oxygen demand (SCOD) increased, with the highest values noticed at an FNA concentration of 0.7 mg N/L for both experiments. The volatile suspended solids (VSS) decreased as the doses of FNA increased, the highest decrease in VSS was observed at FNA dose of 2.8 mg N/L.

# ACKNOWLEDGMENTS

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# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# INTRODUCTION

The populations in urban areas are increasing which makes wastewater treatment vital, but through treating wastewater, an extensive quantity of sewage sludge is produced. Treating and disposing sludge generated from sewage has been proven to be costly to the societies that acquire treatment plants. Most of the waste management approach used, only caters to the regulations concerning the environment, without considering utilizing waste by recovering valuable resources in the process. By paying attention to strategies involving resource recovery from waste, it minimizes the amount of end products from the treatment of waste and it also increases the production of useful resources (Lee, Chua, Yeoh & Ngoh, 2014, p. 84). The typical method used in the management of sewage sludge is anaerobic digestion, which aids in restoring energy from the sludge. Anaerobic digestion involves an array of biochemical mechanisms that transforms organic matter into biogas through intricate microbial systems. In biogas, there is methane which is vital in renewable energy (Peces et al, 2016, p. 631), more information on the anaerobic digestion process will be discussed in this paper, and a major factor used in the anaerobic process is sludge. During the process, volatile fatty acids are produced during the first two stages, sludge is utilized due to its high volume of organic carbon (Morgan, 2011, p.3089).

There is a critical need for wastewater and water treatment processes that results in the decrease of sludge production, and research has shown that the fermentation of primary sludge produces a large amount of volatile fatty acids (VFA) in comparison to other sludge types like activated sludge (Ucisik & Henze, 2008). The conventional method for producing VFAs is using natural gas and petroleum, but the fermentation of primary sludge proves to be a more economical and beneficial means of producing VFAs. It transforms the organic wastes into essential substances used in bioprocesses like the production of biogas, biopolymer and biological nutrient removal from wastewater treatment (Morgan, 2011, p.3089).

The production of volatile fatty acid is important because it is critical to the elimination of specific nutrients in wastewater, especially biological nutrients. The excess volume of nutrients in water bodies can be prevented due to the fatty acids that restrict the discharge of phosphorus and nitrogen (Maharaj, 1999, p. 24). This paper will discuss the production of volatile fatty acids through the fermentation of primary sludge, focusing on the factors affecting fermentation and different types of procedures used in the fermentation process. Primary sludge will also be characterized, and the production and applications of VFAs will also be discussed.

# WASTEWATER TREATMENT AND SOLIDS PRODUCTION

Wastewater can be derived from many components in the society, but it comes mostly from the waste produced by humans. Other components could be from industries, institutions, storm water, etc. The amount of waste produced from all these components vary in types and quantities, it usually depends on the way the society is living as well as behavioral situations. Another factor that influences the production of waste is the structure of the juridical system in the society and technical aspects as well (Henze, 2011, p. 33). Wastewater is composed of physical, biological and chemical components. The physical components comprise of the color, smell and the opacity of the wastewater. Chemical components are the different solids (total and suspended) found in the wastewater, in addition to the chemical and oxygen demand (COD and BOD); soluble and total organic carbon (SOC and TOC) and all the various compounds discovered like phosphorus, nitrogen, etc. (Banerjee, 1997, p. 5). Treated wastewater obtained from societies and municipalities should be discharged back to the rivers, streams or the land. There is a certain degree of treatment that ensures the treated wastewater is clean enough to return to receiving waters, to assure a preservation of the environment and the health of communities. This specified degree of treatment is obtained through vigorous analysis, applying substantial knowledge from scientific aspects, critical reasoning in terms of engineering applications, which can be situated on previous experiences, and finally, the review from the juridical system (Metcalf & Eddy Inc. et al, 2003, p. 10-11).

There will be issues concerning the health of the environment and public if the wastewater is not treated properly and is sent back to receiving water bodies, which is used as water supply for majority of municipalities (Metcalf & Eddy Inc. et al, 2003, p. 7). The treatment of wastewater is important because after the wastewater is treated, the clean water can then be discharged back into water bodies. The common way that wastewater is treated can be classified into three different processes: chemical, physical and biological. It can also be an integration of the three processes to eliminate solids, organic substances and nutrients found in the wastewater. There different categories of treatment are: preliminary, primary, secondary and advanced or tertiary treatment (Pescod, 1992).

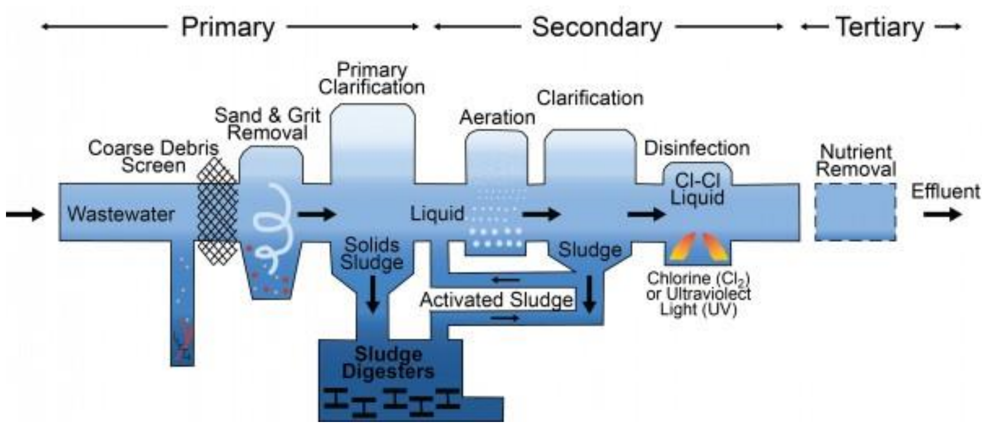


**Figure 1: Wastewater Treatment Process (Center for Sustainable Systems, 2016)**

## PRELIMINARY TREATMENT

The preliminary treatment is used to eliminate rough and large solids that are present in the raw form of the wastewater. This step is important because it aids in ensuring the performance and preservation of the other treatment systems. During this process, the screening of coarse solids and grit removal is done, with the addition of disintegrating larger solids if necessary (Pescod, 1992).

## PRIMARY TREATMENT

For primary treatment, organic solids that settle are removed, as well as the inorganic solids. Sedimentation, floatation, screening, and filtration are some methods used to complete primary treatment. Sedimentation involves the use of different settling tanks to guarantee that the significantly high-density particles settle at the bottom of the tank for their removal (Stephenson & Stuetz, 2009, p. 48). With the use of these tanks, primary sludge (could also be referred to as solids that are settled) is obtained from the settled solids located at the bottom of the tank, where it is pumped into a processing system for further treatment (Pescod, 1992).

## SECONDARY TREATMENT

Secondary treatment deals with the treatment of effluents obtained from primary treatment, during this level of treatment, suspended solids and organic matter leftover is removed, in some cases, disinfection is also performed during the treatment. Disinfection can include the act of introducing chlorine into the basin, depending on the concentration of the wastewater. Biological treatment is used during this level of treatment to eliminate all the soluble organic substance that was not treated during the primary treatment phase (Pescod, 1992, Banerjee, 1997, p. 4).

## ADVANCED/TERTIARY TREATMENT

The tertiary or advanced treatment deals with the effluent from the secondary treatment, it eliminates the leftover suspended solids with the use of micro screens. For situations where the treated wastewater is needed for reuse, the dissolved materials remained after the biological treatment is removed. There are some processes at this level that are integrated with both primary and secondary treatment, where chemicals are added to the clarifiers or the basins to eliminate phosphorus (Pescod, 1992, Metcalf & Eddy Inc. et al, 2003, p. 11).

The importance of treating wastewater is eminent, it aids in preserving the water bodies, protecting the environment and the health of the community. For societies where the availability of fresh water supply is diminishing, it would be resourceful to reuse treated wastewater (Metcalf & Eddy Inc. et al, 2003, p. 20).

# SLUDGE PRODUCTION AND TREATMENT

Sludge management is very vital to the health of the environment and if done improperly, it can lead to severe consequences, it is however significantly complicated and expensive to effectively manage sludge generated from wastewater treatment plants (WWTP). Due to the rapid growth and development of urban areas, many WWTPs are needed, which then increases the amount of produced sludge, resulting in the need to enforce effective management of waste and sludge (Andreoli, Sperling & Fernandes, 2007, p. 1). The product from the wastewater treatment plants in solid form can be referred to as sludge, there are different types of solids produced during the different levels of treatment, and some can be used as a valuable resource in other applications, while the non-valuable solids are transferred to the landfill or taken for incineration. To have an effective sanitation system, the applied use of bio solids is required, but unfortunately, sludge is usually neglected in many countries (Andreoli, Sperling & Fernandes, 2007, p. 2).

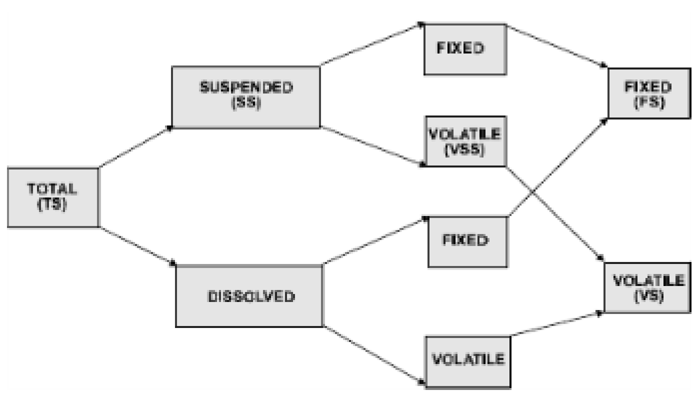

**Figure 2: Solids distribution in Sludge (Andreoli et al, 2007, p. 13)**

Sludge is basically a mix of both water and solids and it comprises of suspended solids (SS) and dissolved solids (DS) resulting in total solids (TS), these are then divided into fixed solids (FS) or volatile solids (VS) (Andreoli et al, 2007, p. 13). In wastewater treatment plants, various methods are used to treat wastewater, which results in the production of primary sludge, chemical sludge and secondary sludge (Foladori et al, 2010, p.7). Primary sludge is derived from the remnant solids from the primary clarifiers, after the primary treatment is completed, and it contains about 2 to 7 percent of total solids, however, more details on the characteristics of primary sludge will be discussed further in this paper (Andreoli et al, 2007, p. 16) (Foladori et al, 2010, p. 8).

Secondary sludge can also be referred to as biological sludge, it comprises of bio solids which are produced as the organic matter is eliminated during the treatment, and through the acquired inert solids obtained from sewage in raw form (Andreoli et al, 2007, p. 17), and the total solids in secondary sludge usually ranges from 0.5 to 1.5 percent. Chemical sludge is generated from the precipitation of distinctive substances and suspended solids; an example of such substances is phosphorus. The three different types of sludge introduced above can be classified as sludge handling units. Before sludge can be identified as primary, secondary or chemical sludge, it is defined as raw sludge which is basically untreated sludge, after it has passed through any, or the combination of the different handling units, it can then be identified as a specific type of sludge (Foladori et al, 2010, p. 8).

As stated above, sludge can be comprised of total suspended solids (TSS), TS, VS, volatile suspended solids (VSS) and total or suspended chemical oxygen demand (COD). Total solids consist of: total suspended and soluble solids; inorganic and organic solids - also known as volatile solids. The total COD considers only the soluble and suspended part of the solids, and the suspended COD assesses the amount of organic substance that the suspended solids acquire (Foladori et al, 2010, p. 9).

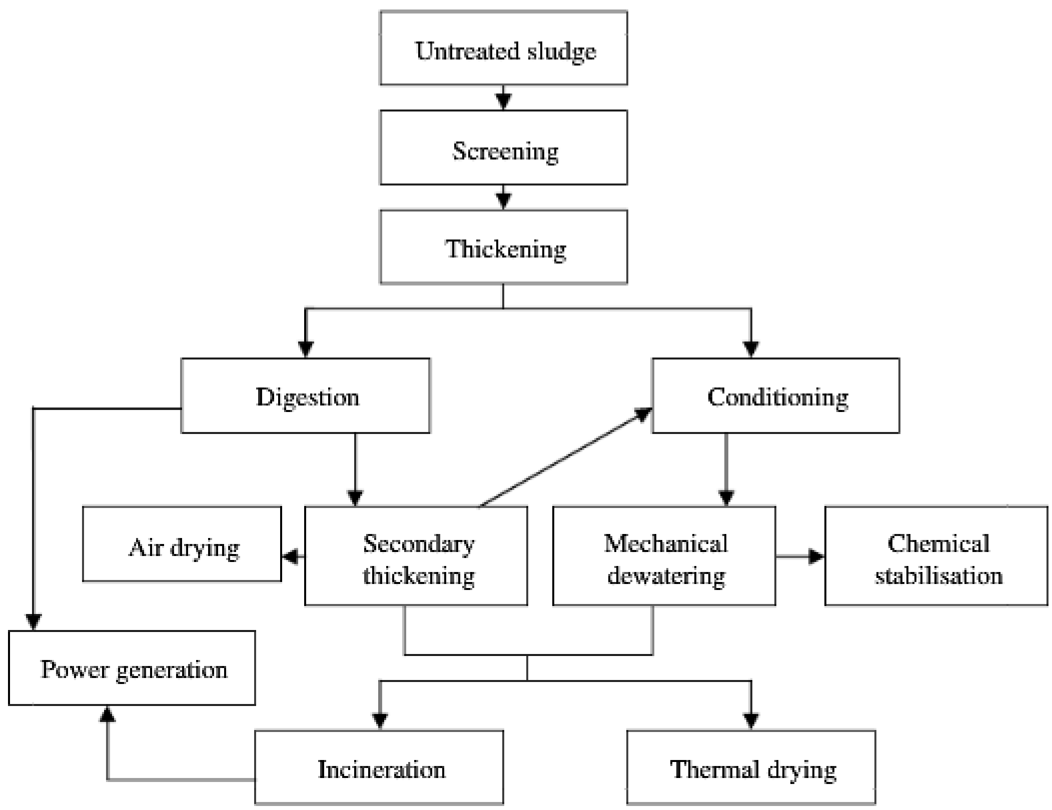


**Figure 3: Sludge Treatment Processes (Stephenson & Stuetz, 2009, p. 170)**

There are various ways that sludge can be treated, it can be achieved by thickening, dewatering, thermal drying and stabilization. Thickening is attained by eliminating a percentage of water from the sludge to increase the mass of the suspended solids, but ensuring that the sludge can still be in liquid form, by applying gravity, air floatation, etc. Dewatering essentially has similar goals as the thickener, it deals with reducing the moisture in the sludge, to reduce the volume sludge with the addition of chemicals (Stephenson & Stuetz, 2009, p. 170).

The stabilization of sludge improves the amount of organic matter readily available in sludge, resulting in the decline of risking decomposition and reducing the accumulation of pathogens. The three processes of stabilizing sludge are: biological, chemical and thermal. Chemical stabilization involves the use of adding chemicals to achieve oxidation, while thermal is the act of adding heat to sustain the volatile portion of sludge, they are both used in agriculture. The method used more frequently is biological stabilization, which involves the use of bacteria to enhance the biodegradable portion of the organic substance; this method is divided into two processes: anaerobic digestion and aerobic digestion, with the former as the most popular method (Andreoli et al, 2007, p. 48).

# ANAEROBIC DIGESTION

Anaerobic digestion is the process by which organic matter (that can be derived from both plants and animals) is decomposed, to produce methane, carbon dioxide and water. This method is achieved with the use of two microorganisms called bacteria and archaea, which can only strive with the absence of oxygen, allowing the procedure to be called anaerobic ("What is AD? |ADBA|Anaerobic Digestion & Bioresources Association", n.d.). This system is used to produce biogas, classified as renewable energy, which aids in the reduction of the cost regarding the treatment of wastewater, and it greatly reduces the amount of generated sludge from wastewater plants as well (Madsen et al, 2011, p. 3142).

An easy way of understanding the process of anaerobic digestion is that organic matter or substance, which is usually food waste, manure and many more, are used as a feedstock, also referred to as substrate. The organic matter is secured in a tank (also known as digester), to prevent oxygen from penetrating, and it is stirred in the digester. In the digester, a bacterium is used to decompose the organic matter, which results in the production and release of biogas. The generated gas can then be utilized to produce electricity and heat, biogas as similar compositions to that of natural gas. The left-over matter in the digester is now referred to as digestate and it can be used in agriculture as a fertilizer or conditioner due to its high nutrients content, like potassium and nitrogen (Pullen, 2015, p. 2).

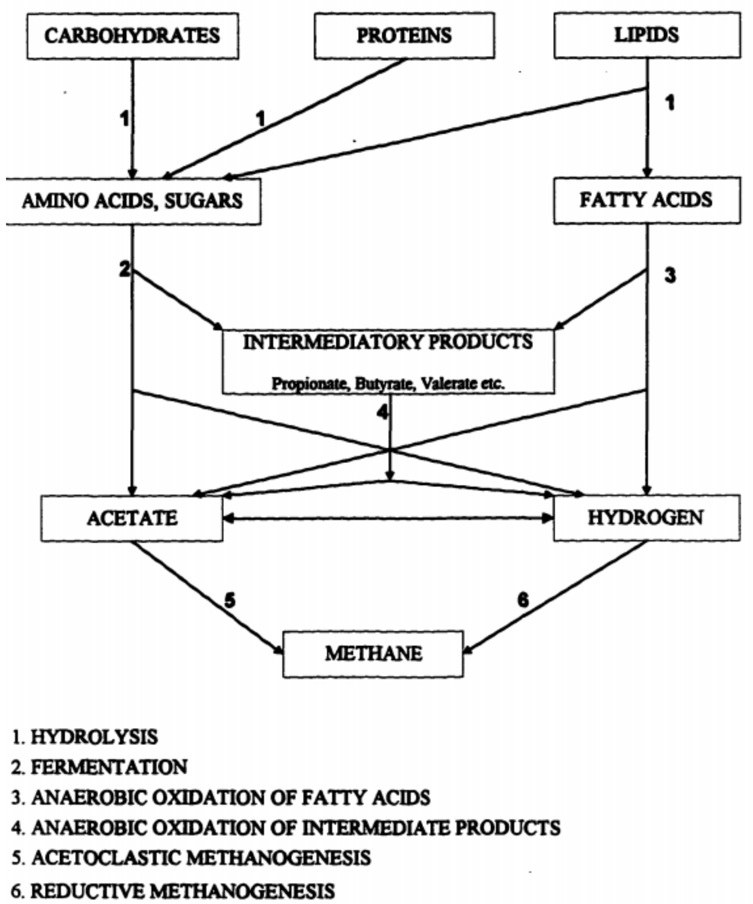


**Figure 4: Anaerobic Process (Banerjee, 1997, p. 11)**

This system works in four stages to disintegrate bio-waste and produce biogas, they are: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The image above showcases the anaerobic digestion process in six different ways. The first being the hydrolysis of the organic substrates: carbohydrates, proteins and lipids (also known as fats). The second step is the fermentation of the products from the hydrolysis of the biopolymers, which are amino acids and sugars. The first two steps (hydrolysis and fermentative process) deals with the disintegration of complex polymers to short and long fatty acids (Stephenson & Stuetz, 2009, p. 136), while the third step is the anaerobic oxidation of the fatty acids into intermediates. The fourth is then the oxidation of the intermediary products which produces volatile fatty acids. The fifth and sixth step involves the transformation of acetate and hydrogen to methane (which can be referred to as methanogenesis), which ends the anaerobic digestion process (Banerjee, 1997, p. 9-10).

Therefore, the first two steps are deemed to be acidogenesis, which ends in the production of volatile fatty acids, while the others are known as methanogenesis. Acidogenesis was both hydrolysis and fermentation, but it was realized that the end products were more efficient in production when it was considered as a two-phase system in comparison to having it as a two-step system (Banerjee, 1997, p. 10). The first two processes, hydrolysis and acidogenesis, of anaerobic digestion is focused on in this paper, because that is where volatile fatty acids are produced.

## ANAEROBIC SYSTEMS AND REACTORS

The systems utilized for anaerobic digestion varies at different temperatures, the most common temperature used is normally around 35 degrees Celsius, which is known as mesophilic. Nonetheless, temperature around 55 degrees Celsius is also used, which can be referred to as thermophilic. It has been noted that using thermophilic temperatures are more efficient in comparison to mesophilic conditions, especially regarding utilizing distillery, pharmaceutical, ice cream wastes and many more in anaerobic treatment. The application of thermophilic temperatures in anaerobic systems enables substantially greater growth rate and reaction, aiding in a quick decomposition of wastes while also reducing the growth of microorganisms that can cause any diseases due to high temperatures. The use of thermophilic temperatures demands more energy than mesophilic, employing produced biogas can be adopted (Stephenson & Stuetz, 2009, p. 138). There are some disadvantages of utilizing thermophilic systems include the inability to effectively removal chemical and biochemical oxygen demands, and reducing the amount of methane and gas production, making the system less dependable. The strength of the sludge is also affected in thermophilic systems; research has also been conducted to conclude that more harmful toxins from the wastewater can result in problems in thermophilic systems in comparison to mesophilic temperature (Stephenson & Stuetz, 2009, p. 139).

Some reactors used in anaerobic treatments are: suspended growth and fixed film reactors. The conventional reactors however, are anaerobic digesters, where a continuously stirred tank reactor (CSTR) is utilized to mainly treat sewage sludge, while the other reactors are usually used to treat wastewater. Some anaerobic reactors used for wastewater treatment include: anaerobic filters, contact reactors, fluidized bed and up flow sludge blanket reactors. These reactors usually remove about 70 to 80 percent of chemical oxygen demand (Stephenson & Stuetz, 2009, p. 139).

## ANAEROBIC CONTACT REACTOR

This reactor is identical to activated sludge, the influent goes into the reactor, where the gas is withdrawn and is then taken to a sedimentation tank (or a sort of separation system) where the sludge is settled and is recycled back into the reactor to preserve a larger amount of biomass.

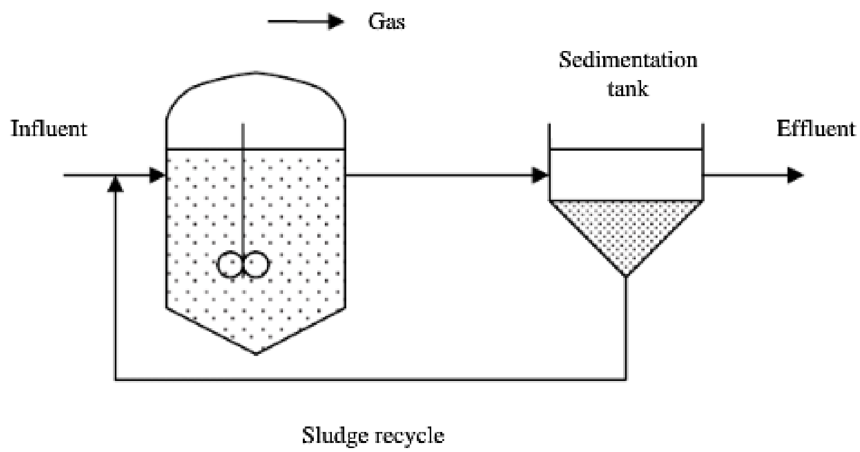


**Figure 5: Anaerobic Contact Reactor (Stephenson & Stuetz, 2009, p. 139)**

## ANAEROBIC FILTER

A fixed film is used to keep the biomass produced, where the filter used in the system is designed to deny the access of oxygen from penetrating into the reactor, to keep the reactor anaerobic, anaerobic filters are widely used during the denitrification phase (Stephenson & Stuetz, 2009, p. 139).

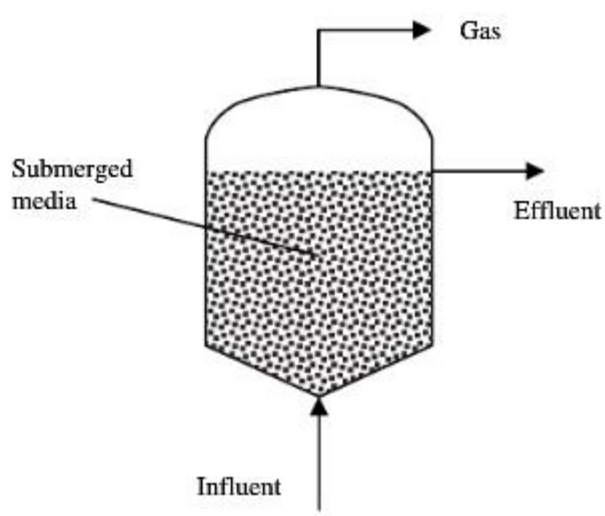


**Figure 6: Anaerobic Filter (Stephenson & Stuetz, 2009, p. 140)**

# PRIMARY SLUDGE CHARACTERISTICS

As discussed previously, wastewater compromises of fundamental aspects which include physical, biological and chemical. The physical aspect consists of the smell and color, while the chemical aspect deals with the different solids like total solids and total suspended solids (TS and TSS). It also consists of the biochemical and chemical oxygen demand (BOD & COD); soluble and total organic carbon (TOC & SOC) and other components of phosphorus and nitrogen. Volatile solids (VS) are composed of the three-main organic matter found in primary sludge, which are carbohydrate, lipids and proteins.

Carbohydrates found in most municipal wastewaters include cellulose, starch, sugar and many more. The components of carbohydrates cannot dissolve in water, with sugar being an exception. Proteins can be referred to as polymers, it comprises of nitrogen, hydrogen and carbon. There are two types of proteins: globular, which can dissolve in water and fibrous, which cannot dissolve in water and is strong. Lipids is a kind of fat, which varies from simple to complex particles, it can also be known to be an organic composite which is only soluble in specific solvents and does not dissolve in water. Lipids in wastewater are often obtained from margarine, butters, oils and the like (Banerjee, 1997, p. 5 - 6).

Wastewater in treatment plants is treated with different methods which produce primary, secondary and chemical sludge (Foladori, Andreottola & Ziglio, 2010, p. 7). Sludge can be defined as the debris recovered from wastewater treatment processes, and primary sludge is produced from the first phase of processing, in the primary clarifier. The primary clarifier is conducted by sedimentation, where the wastewater is treated through pumping into settling tanks (Scott, & Smith, 1995). When wastewater comes into the treatment plant, it undergoes screening to get rid of debris and rough solids to enable the preservation of the pumps. When the debris and solids are removed, the next phase is to discard grits and sand. It is then transported out for disposition, the organic solids that have settled like total suspended solids and biological oxygen demand are disposed of as the wastewater moves through the primary clarifier, and volatile fatty acids are then produced after the primary sludge is transferred to fermenters (ACRWC, "Process Description").

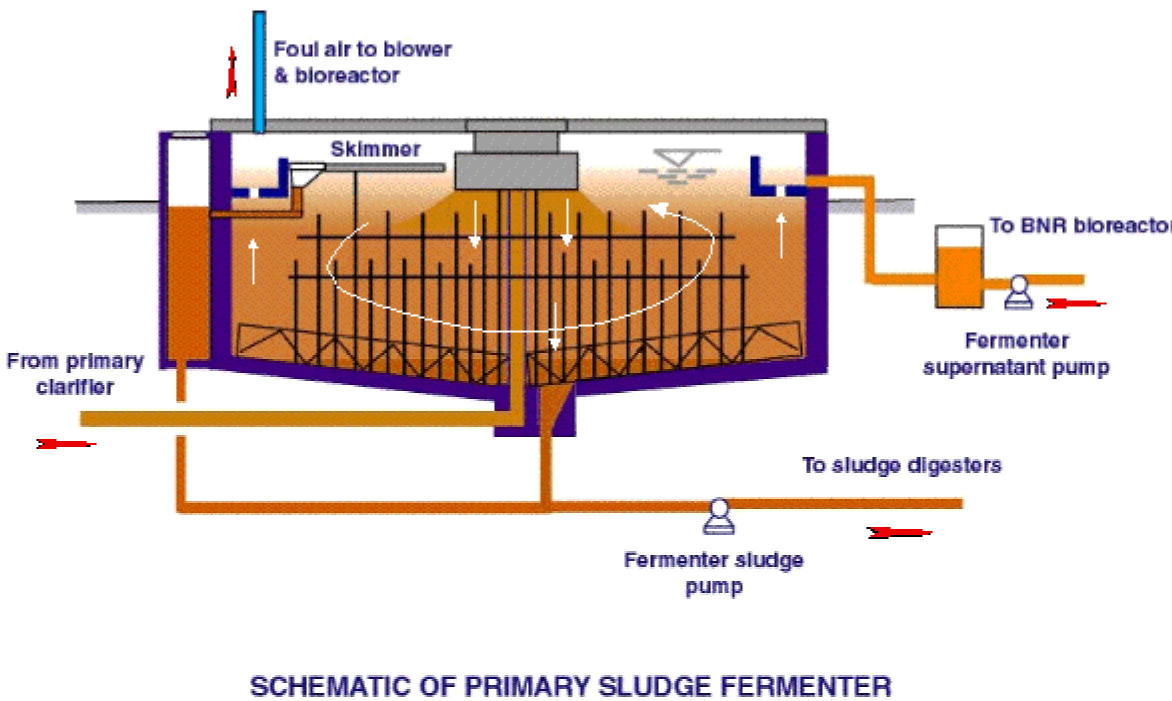


**Figure 7: Primary Sludge Fermenter (ACRWC, "Primary Sludge Fermenters")**

Primary sludge is usually gray or a light brown color varying in sizes and compositions; it decomposes quickly and becomes toxic, which is noticeable by its change in color into a dark gray or black with an unpleasant odor (Turovskiy & Mathai, 2006, p. 32). The amount of sludge produced during primary treatment (where primary sludge is generated), is dependent on the competence of suspended solids removed while in the primary clarifier (Andreoli, 2007, p. 16). After the sludge is pumped into the fermenters, big confined tanks are utilized to rotate and stir the sludge where the VFA are produced (ACRWC, “Primary Sludge Fermenters”). Primary sludge is critical to the process of biological nutrients removal (BNR), it is used as a carbon source for the removal process (Min et al, 2005, p. 910). The fermentation of primary sludge also aids in decreasing the quantity of sludge (Ucisik, & Henze, 2008, p. 3730).

Waste activated sludge can also be fermented to produce volatile fatty acids, but primary sludge is more favorable in comparison to waste activated sludge, because it contains a larger amount of readily biodegradable organic matter, which is utilized in the fermentation to produce VFA in a reasonable amount of time. Whereas, the activated sludge consists of mostly bacterial weight which requires more complex processes with a lengthy fermentation time to produce VFA, so therefore, under the same solids and hydraulic retention time, primary sludge still produces more VFA than waste activated sludge with less time (Yuan, 2012, p. 40).

## FERMENTATION PROCESS

Fermentation is the process by which a product is created with the use of microorganisms (Pumphrey, & Julien, 1996). It does not require oxygen when decomposing glucose, which makes it an anaerobic method ("Fermentation and anaerobic respiration") and it is a part of the anaerobic digestion process. Fermentation is crucial to the generation of short chain fatty acids (also known as VFAs) which enables the production of an outside carbon source that can be utilized in the removal of biological nutrients in the wastewater treatment plant. During the process of fermentation, pH plays a vital role in the chemical reaction leading to the separation of varying compounds of sludge, resulting in the production of volatile fatty acids (Katsou, 2014).

Primary sludge fermenters are utilized to impair the intricate organic matter in the sludge to generate VFAs. The fermentation of primary sludge can be done by activated primary tanks, separate complete thickeners, static fermenter and complete mix fermenter. The chosen configuration for fermenting primary sludge depends on the intensity of the wastewater and fermentation in the sewage system, as well as a feasible design used for conversion in the current plant (Atherton, 1995, p. 1).

## ACTIVATED PRIMARY TANKS

Activated primary tanks can be used to generate the outcome of fermentation which aids in the elimination of biological phosphorus. After the influent flows into the primary clarifier, the undercurrent is then taken for recycling back to the basin of the clarifier, to preserve the thickness of the sludge located in the primary clarifier. The underflow is recycled for the conservation of the sludge cover as well as the mixing or purification of the acids, therefore, the volume of the clarifier is feasible for the fermentation process. However, research shows that using activated primary tanks is not a very effective fermentation system in comparison to other systems, in a way that using the activated tanks did not affect the increase in concentrations of volatile fatty acids when compared to conventional primary tank which is usually designed as a rectangular shaped tank with a flat bottom (Atherton, 1995, p. 14).

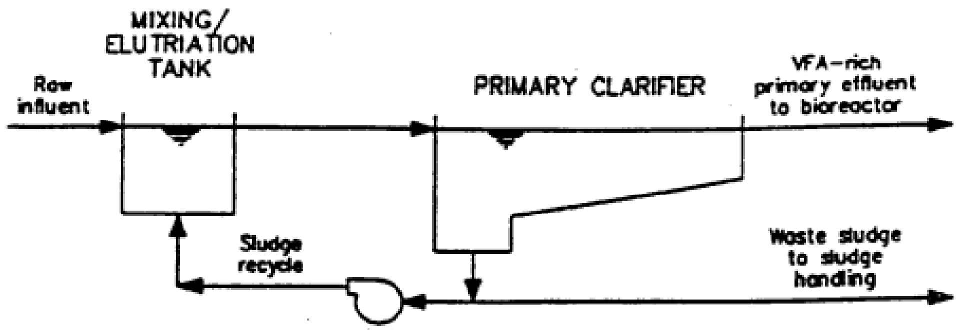


**Figure 8: Activated Primary Tank (Atherton, 1995, p. 14)**

Although the system might not be might not be as effective as others, it is a cheaper alternative when the large amounts of the thickness of sludge need to be modified, and the recycling of the solids needed in the fermentation of the sludge. The disadvantages of using this system includes the difficulty of maintaining the thickness of the sludge which results in the decline of the quality of the sludge, another is the loss of VFAs due to the failure in transportation through the outflow pathway in the primary clarifiers (Atherton, 1995, p. 15).

## SEPARATE COMPLETE THICKENERS

This system is also called a thickener fermenter, which is known to be the most effective fermentation system used. It includes the combination of both the complete mix and static fermenter. The movement of the primary sludge is achieved by a pump to the completely mixed tank, where the excess discharges to the gravity thickener by gravity. The complete mix tank is then used to take in the thickener sludge derived from the thickener resulting in a large portion of sludge being stored in the mixed tank, unlike the thickener (Atherton, 1995, p. 20).

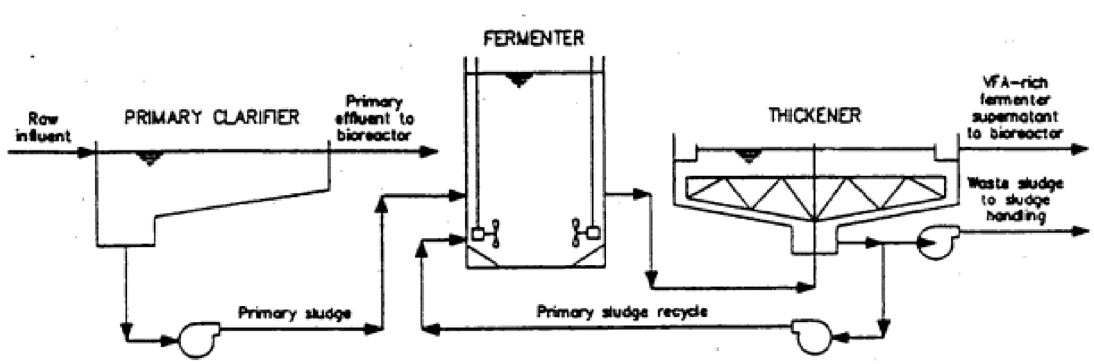


**Figure 9: Separate Complete/Mix Thickener (Atherton, 1995, p. 21)**

It has been observed that this system has produced higher and optimal VFA than static fermenter and even complete mixed fermenters. It provides optimal supervision of both HRT and SRT; it also reduces the decline of VFAs when it is being transported to the biological nutrient removal reactor. Although this system has great advantages, it is highly expensive in comparison to the other fermenters (Atherton, 1995, p. 21).

## STATIC FERMENTER

This system has an identical idea to that of the activated primary tank; it has a gravity thickener which aids in the fitting of the sludge volume. The primary sludge is stirred into the system to thicken the sludge, during this process, specific liquids that have essential products from fermentation is released to the anaerobic area where the biological nutrient removal reactor resides (Atherton, 1995, p. 16).

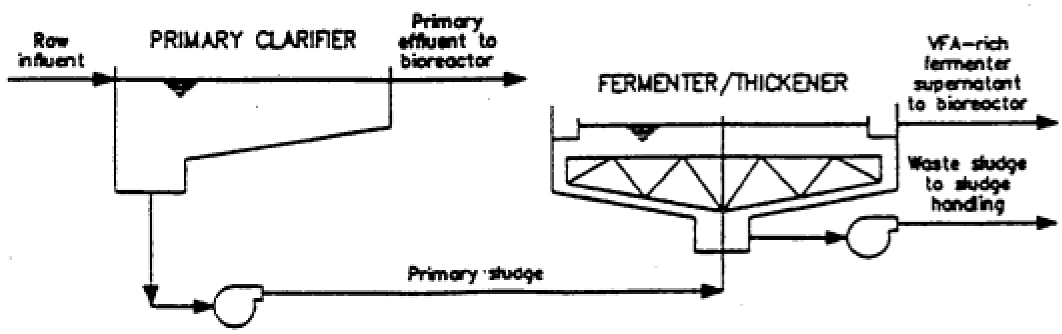


**Figure 10: Static Fermenter (Atherton, 1995, p. 16)**

The use of this system maintains the volume of generated VFA because it is being released directly to the reactor, which enables the essential use of sources for an efficient removal of biological phosphorus. However, the disadvantage of utilizing this system is the absence of the control over solids retention time (Atherton, 1995, p. 17).

## COMPLETE MIX FERMENTER

After the primary sludge is taken from the primary clarifier, it is taken to the mixed tank resulting in the acid fermentation of the sludge; the overflow from the fermenter is pumped back by gravity into the primary clarifier to separate the supernatant and the sludge. Thereafter, the surplus primary sludge is then transferred to the sludge handling unit (Atherton, 1995, p. 18).

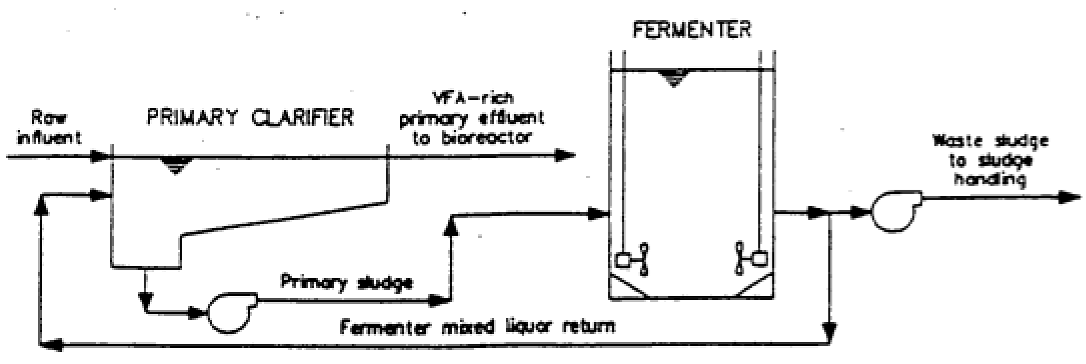


**Figure 11: Complete Mix Fermenter (Atherton, 1995, p. 18)**

This system aids in the accuracy over the control of hydraulic retention and solids retention time, thus resulting in the reduction of the production of sulphide and methane. The disadvantages of using this system is like the activated primary tank because significant volumes of generated volatile fatty acids are lost because of the movement through the clarifier, and the aerobic action in the system (Atherton, 1995, p. 19).

## FACTORS AFFECTING FERMENTATION

There are various factors that affect fermentation: the availability of nutrients; temperature; sulfite; ethanol; acidity and pH ("Factors Impacting the Fermentation | Viticulture & Enology", 2014). The strength of fermentation is dependent on the pace of flow in the sewers, as well as, the temperature and the infiltration of groundwater (Atherton, 1995, p. 1).

## SOLIDS AND HYDRAULIC RETENTION TIME

The solids retention time (SRT) is a parameter used when wastewater treatment plants are designed. It is utilized for the real time that microorganisms remain in the reactor; it also outputs the rate at which the microorganisms grow (Clara et al, 2005, p. 98). Hydraulic retention time (HRT) can be defined as the time at which the waste is kept in the reactor; it helps to find out the volume of the reactor (Lee, Chua, Yeoh & Ngoh, 2014, p. 90-91). The products originated from the acid-phase digestion are affected by both SRT and HRT (Elefsiniotis & Oldham, 1993, p. 7).

During the hydrolysis process, it was discovered that the hydrolysis of carbohydrates and lipids intensifies with SRT. There are different ways that SRT can be increased; it is proportional to the increase in sludge concentration located in the fermentation system and the size of the fermenter (Sanchez Rubal et al, 2012, p. 2). Experiments have shown that the reduction of SRT improves the production of VFA, but there should be a good amount of SRT to fully hydrolyze the sludge. Like the other parameters, there should be a specific range of values that produce a good amount of VFA, because going above or below the specified range can result in a negative effect of the generation of VFAs.

The application of a higher HRT proves to generate optimal amounts of VFA since there is sufficient time for the waste and microorganisms to react, but it is highly dependent on the type of waste used, which aids in the specification of an effective range for the HRT to produce VFA efficiently. However, it is important to know that to guarantee a higher value of HRT, a larger reactor will be needed, which is costly (Lee, Chua, Yeoh & Ngoh, 2014, p. 91).

## TEMPERATURE

The increase of temperature is proportional to the enhancement of the hydrolysis of lipids, carbohydrates and proteins. As the temperature increases, the higher the hydrolysis for primary sludge fermentation (Sanchez Rubal et al, 2012, p. 2). The fermentation of primary sludge is conducted at atmospheric temperatures, usually between the ranges of 10 to 30 degrees Celsius.  Through the experiment, it was noticed that there was a vast improvement as the temperature increased between the ranges noted (Atherton, 1995, p. 11).

Emine et al also conducted an experiment to observe the effect that temperature has on the competence of primary sludge fermentation. Variety of reactors used in the fermentation process ranging from 10 to 24 degrees Celsius in 4-5 increments. Bench scale reactors and reactor temperature were utilized to ensure the accuracy of the temperature calibrated (Emine et al, 2009, p. 381). The results of the experiment showed that the VFA generation rate increased as the temperature increased, it was observed that at a temperature of 14 degrees Celsius, the generation rate was about 42 percent lesser than the temperature at 21 degrees Celsius. The composition of VFA was also affected by the increase in temperature, where the acetic acid, which is a component of VFA composition was proportional to temperature, therefore the acetic acid content increased when the temperature increased (Emine et al, 2009, p. 384).

## pH

During batch test experiments of primary sludge, it was discovered that the higher the pH, the higher the result for the acetogenesis fermentation. pH also impacted the category and concentration of the generated VFA (Atherton, 1995, p. 11). An experiment conducted by Emine et al, showed that specific ranges of pH values affect the performance of primary sludge fermentation. The primary sludge was obtained from a wastewater treatment plant located in Turkey with an initial pH value ranging from 6.72 to 6.84, the pH values used for the experiments ranged from 5.5 to 7.5 with 0.5 increments. The results from the experiments were assessed based on data gotten from five different sets of specimens over the period of a year, the first two runs were conducted with pH values, 6.5 and 7.0, the other two runs were 6.0 and 5.5 while the last was 7.0, which was the highest pH value for this experiment, it was also conducted by running lateral experiments with controlled and uncontrolled conditions. The uncontrolled experiment entailed that no pH adjustments will be done, unlike the controlled condition where the anaerobic reactor was constantly altered and managed at the range of the pH values mentioned above (Emine et al, 2009, p. 381).

The results of the experiment showed that in the uncontrolled pH values, the volatile fatty acids produced were the highest with the range of 6.72-5.84. It was also noticed that the amount of volatile suspended solids (VSS) also affected the production of VFA, where the higher the initial VSS, the greater the amount of VFA production (Emine et al, 2009, p. 382). In conclusion to the experiment, there was an effective amount of VFA production at the range like the initial pH values before testing and the composition of VFA was affected by VSS and pH, where the higher the VSS level and pH value, the lower the acetic acid formed in the VFA (Emine et al, 2009, p. 385).

## PRE-TREATMENT METHODS

According to European Union Regulation EC1772/2002, substrates such as municipal solid waste (MSW), food waste (FW), and slaughterhouse wastes need to be pasteurized or sterilized before and/or after AD. Taking this regulation into account, pre-treatment methods could be applied, thus obtaining a higher energy recovery and eliminating the extra cost for pasteurization and/or sterilization (Hendriks, Zeeman, 2009; Eggeman, Elanderb, 2005). Pre-treatment methods could nevertheless be unsustainable in terms of environmental footprints, even if they enhance the AD process performance (Carballa, Duran & Hospido, 2011). The effects of various pre-treatment methods are highly different depending on the characteristics of the substrates and the pre-treatment type. Hence, it is difficult to compare and systematically assess the applicability and sustainability of such methods at a full scale.

## MECHANICAL PRE-TREATMENT

Mechanical pretreatment disintegrates and/or grinds solid particles of the substrates, thus releasing cell compounds and increasing the specific surface area. An increased surface area provides better contact between substrate and anaerobic bacteria, thus enhancing the AD process (Carrere et al, 2010; Skiadas et al, 2005; Elliot et al, 2012). Esposito et al. suggested that a larger particle radius results in lower chemical oxygen demand (COD) degradation and a lower methane production rate (Esposito et al, 2011). Likewise, Kim et al. showed that particle size is inversely proportional to the maximum substrate utilization rate of the anaerobic microbes (Kim et al, 2000). Therefore, mechanical pretreatments such as sonication, lysis-centrifuge, liquid shear, collision, high-pressure homogenizer, maceration, and liquefaction are conducted to reduce the substrate particle size. In addition to size reduction, some methods result in other effects depending on the pretreatment. Hartmann et al. reported that the effect of maceration is more due to shearing than cutting of the fibers (Hartmann et al, 2000). Sonication pretreatment generated by a vibrating probe mechanically disrupts the cell structure and floc matrix (Elliott et al, 2007). The main effect of ultrasonic pretreatment is particle size reduction at low frequency (20–40 kHz) sound waves (Chua et al, 2002). High-frequency sound waves also cause the formation of radicals such as OH , HO 2, H , which results in oxidation of solid substances (Bougrier et al, 2006).

A high-pressure homogenizer (HPH) increases the pressure up to several hundred bars, then homogenizes substrates under strong depressurization (Mata-Alvarez et al, 2000). The formed cavitation induces internal energy, which disrupts the cell membranes (Barjenbruch et al, 2003). These pretreatment methods are not common for OFMSW, but they are more popular with other substrates such as lignocellulosic materials, manure and WWTP sludge. Size reduction through beads mill, electroporation and liquefaction pretreatments of OFMSW has been studied at lab scale, whereas rotary drum, screw press, disc screen shredder, FW disposer and piston press treatment are successfully applied at full scale. Both electroporation and liquefaction pretreatments cause cellular structure damage, thus the effect on the AD process is like maceration (Shepherd, 2006; Carlsson, 2008). The advantages of mechanical pretreatment include no odour generation, an easy implementation, better dewaterability of the final anaerobic residue and a moderate energy consumption. Disadvantages include no significant effect on pathogen removal and the possibility of equipment clogging or scaling (Toreci et al, 2009; Perez-Elvira et al, 2006).

## THERMAL PRE-TREATMENT

Thermal treatment is one of the most studied pretreatment methods, and has been successfully applied at industrial scale (Carrere et al, 2010; Carlsson et al, 2012; Cesaro et al, 2014). Thermal pretreatment also leads to pathogen removal, improves dewatering performance and reduces viscosity of the digestate, with subsequent enhancement of digestate handling (Ariunbaatar et al, 2014, p. 146). Various temperatures (50–250 C) to enhance the AD of different OSW (mainly WWTP sludge and lignocellulosic substrates) have been studied. However, to the best of our knowledge, no systematic research on various temperature and treatment times to enhance AD of OFMSW has been conducted.

The main effect of thermal pre-treatment is the disintegration of cell membranes, thus resulting in solubilization of organic compounds (Ariunbaatar et al, 2014, p. 146). COD solubilization and temperature have a direct correlation. Higher solubilization can also be achieved with lower temperatures, but longer treatment times. Mottet et al. compared different thermal pretreatment methods and found no significant difference between steam and electric heating, whereas microwave heating solubilized more biopolymers (Mottet et al, 2009). The higher rate of solubilization with microwave pretreatment can be caused by the polarization of macromolecules (Toreci et al, 2009; Marin et al, 2010). Concerning the lignocellulosic substrates, temperatures exceeding 160 C cause not only the solubilization of hemicellulose but also solubilization of lignin.

The released compounds are mostly phenolic compounds that are usually inhibitory to anaerobic microbial populations. Bougrier et al. suggested that thermal pretreatment at high temperatures (>170 C) might lead to the creation of chemical bonds and result in the agglomeration of the particles. One of the most known phenomena is the Mallaird reaction, which occurs between carbohydrates and amino acids, resulting in the formation of complex substrates that are difficult to be biodegraded. This reaction can occur at extreme thermal treatment at temperatures exceeding 150 C, or longer treatment time at lower temperatures (<100 $^{o}$C) (Ariunbaatar et al, 2014, p. 146).

In addition to these chemical reactions, thermal pretreatment can also result in loss of volatile organics and/or potential biomethane production from easily biodegradable substrates. Therefore, the effects of thermal pretreatment depend on the substrate type and temperature range (Ariunbaatar et al, 2014, p. 146).

## THERMAL PRE-TREATMENT AT LOWER TEMPERATURES (<110 $^{O}$C)

Protot et al. suggested that thermal pretreatment at temperatures below 100 C did not result in degradation of complex molecules, but it simply induces the deflocculation of macromolecules (Protot et al, 2011). Barjenbruch and Kopplow obtained a similar conclusion with pretreatment at 90 C. Their results showed that the filaments are not disintegrated, but they were only attacked with thermal pretreatment (Barjenbruch et al, 2003). Neyens and Bayens reported that thermal pretreatment resulted in the solubilization of proteins and increased the removal of particulate carbohydrates (Neyens et al, 2003). Thermal pretreatment of sludge even at lower temperature (70 C) has a decisive effect on pathogen removal (Skiadas et al, 2005).

Probably based on such results, the EU Regulation EC1772/2002 requires OSW to be pretreated at least an hour at 70 C. In this regard, numerous studies on thermal pretreatment at 70 C were conducted. For instance, pre-treating household waste and algal biomass at 70 C for 60 min and 8 h, respectively, did not result in enhancement of the biogas production (Gonzalez-Fernandez et al, 2012; Chamchoi et al, 2011). Appels et al. obtained a negligible increase of biogas production from sludge pretreated at 70 C for 60 min, whereas the biogas production was improved 20 times when applying a 60-min pretreatment at 90 $^{o}$C (Appels et al, 2010).

Rafique et al. achieved a maximal enhancement of 78% higher biogas production with a 60% methane content by pretreatment at 70 C (Rafique et al, 2010). Ferrer et al. obtained a 30% higher biogas production with a 69% methane content (Ferrer et al, 2008), whereas Climent et al. obtained a 50% biogas volume increase with pretreatment at 70 C prior to thermophilic AD (Climent et al, 2007). Gavala et al. reported that pretreatment of primary and secondary WWTP sludge at 70 C has a different effect on the thermophilic and mesophilic methane potential. Thermal pre-treatment at 70 C was shown to have a positive effect on mesophilic AD of primary sludge, but not on its thermophilic AD; whereas it enhanced both the thermophilic and mesophilic methane production of secondary sludge. This can be explained by the chemical composition of the OSW substrates: primary sludge contains higher amounts of carbohydrates, whereas secondary sludge contains higher amounts of proteins and lipids (Raposo et al, 2011).

## THERMAL PRE-TREATMENT AT HIGHER TEMPERATURE (>110 $^{O}$C)

Liu et al. studied the thermal pre-treatment of FW and fruit and vegetable waste at 175 C; they obtained a 7.9% and 11.7% decrease of the biomethane production, respectively, due to the formation of melanoidins. Ma et al. obtained a 24% increase of the biomethane production with FW pretreated at 120 C. Rafique et al. studied pretreatment of pig manure at temperatures higher than 110 C. They observed hardening and darkening of manure, which resulted in a low biogas yield. Hardening and the dark brownish color development of the substrate indicated the occurrence of Mallaird reactions (Ariunbaatar et al, 2014, p. 146).

## BIOLOGICAL PRE-TREATMENT

Biological pretreatment includes both anaerobic and aerobic methods, as well as the addition of specific enzymes such as peptidase, carbohydrolase and lipase to the AD system. Such conventional pretreatment methods are not very popular with OFMSW, but have been applied widely on other types of OSW such as WWTP sludge and pulp and paper industries. The hydrolytic-acidogenic step (first step) of a two-phase AD process is considered as a biological pretreatment method by some researchers (Ariunbaatar et al, 2014, p. 147), while others consider it as a process configuration of AD, but not a pretreatment method (Carlsson et al, (Carlsson et al, 2012). Physically separating the acidogens from the methanogens can result in a higher methane production and COD removal efficiency at a shorter hydraulic retention time (HRT) as to conventional single-stage digesters (Hartmann, Ahring, 2006). Parawira et al. reported that optimizing the first hydrolysis stage could stimulate the acidogenic microbes to produce more specific enzymes, thus resulting in more extended degradation of substrates (Parawira et al, 2005). Therefore, in this review paper the first step of the two-phase AD systems is considered as a pretreatment method.

## CONVENTIONAL BIOLOGICAL TREATMENT

Aerobic pretreatment such as composting or micro-aeration prior to AD can be an effective method to obtain a higher hydrolysis of complex substrates due to the higher production of hydrolytic enzymes, which is induced by the increased specific microbial growth (Lim et al, 2013). Fdez-Guelfo et al. reported that pretreatment by composting resulted in a higher specific microbial growth rate (160–205% as compared to untreated OFMSW) than by thermochemical pretreatment (Fdez-Guelfo et al, 2011). Lim and Wang also affirmed that the aerobic pretreatment yielded a greater VFA formation due to the enhanced activities of the hydrolytic and acidogenic bacteria (Lim et al, 2013). However, according to the results obtained by Brummeler and Koster, a pre-composting treatment of OFMSW resulted in a 19.5% VS loss (Brummeler et al, 1990). Mshandate et al. also observed a loss of potential methane production with a longer aerobic pretreatment of sisal pulp waste (Mshandete et al, 2005). Miah et al. investigated the biogas production of SS pretreated with aerobic thermophilic bacteria closely related to Geobacillus thermodenitrificans (Miah et al, 2005).

According to their results, the highest J. Ariunbaatar et al. / Applied Energy 123 (2014) 143–156 147 amount of biogas (70 ml/gVS) with an 80–90% methane content was achieved at 65 C. Melamane et al. studied the AD of wine distillery wastewater pretreated with the fungus Trametes pubescens. This fungal pretreatment obtained a 53.3% COD removal efficiency, which increased the total COD removal efficiency of the AD system up to 99.5% (Melamane et al, 2007). Muthangya et al. used pure cultures of the fungus Trichoderma reseei to aerobically pre-treat sisal leaf decortication residues. Their results showed that aerobic incubation for 4 days resulted in a 30–40% cumulative biogas increase with a higher (50–66%) methane content (Muthangya et al, 2009). Romano et al. studied two types of enzymes capable to hydrolyze plant cell walls to enhance the bio methanation of Jose Tall wheat grass (Romano et al, 2009). They did not obtain a significant biogas enhancement or VS reduction, though the hydrolysis step was accelerated (Miah et al, 2005).

## CHEMICAL PRE-TREATMENT

Chemical pre-treatment is used to achieve the destruction of the organic compounds by means of strong acids, alkalis or oxidants. AD generally requires an adjustment of the pH by increasing alkalinity, thus alkali pretreatment is the preferred chemical method (Li, Chenchen et al, 2012). Acidic pretreatments and oxidative methods such as ozonation are also used to enhance the biogas production and improve the hydrolysis rate. The effect of chemical pretreatment depends on the type of method applied and the characteristics of the substrates. Chemical pretreatment is not suitable for easily biodegradable substrates containing high amounts of carbohydrates, due to their accelerated degradation and subsequent accumulation of VFA, which leads to failure of the methanogenesis step (Wang et al, 2011). In contrast, it can have a clear positive effect on substrates rich in lignin (Fernandes et al, 2009).

## ALKALI PRE-TREATMENT

During alkali pretreatment, the first reactions that occur are solvation and saphonication, which induce the swelling of solids (Carlsson et al, 2012). As a result, the specific surface area is increased, and the substrates are easily accessible to anaerobic microbes (Ariunbaatar et al, 2014, p. 146). Then, COD solubilization is increased through various simultaneous reactions such as saponification of uronic acids and acetyl esters, as well as neutralization of various acids formed by the degradation of the particulates (Kim, Park et al, 2003). When substrates are pretreated with alkali methods, an important aspect is that the biomass itself consumes some of the alkali (Hendriks et al, 2009), thus higher alkali reagents might be required for obtaining the desired AD enhancement.

## ACID PRE-TREATMENT

Acid pretreatment is more desirable for lignocellulosic substrates, not only because it breaks down the lignin, but also because the hydrolytic microbes are capable of acclimating to acidic conditions (Mussoline et al, 2012). The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose into perspective monosaccharides, while the lignin condensates and precipitates (Hendriks et al, 2009; Mata-Alvarez, 2003). Strong acidic pretreatment may result in the production of inhibitory by-products, such as furfural and hydroxymethylfurfural (HMF) (Modenbach et al, 2012; Mussoline et al, 2012). Hence, strong acidic pretreatment is avoided and pretreatment with dilute acids is coupled with thermal methods. Other disadvantages associated with the acid pretreatment include the loss of fermentable sugar due to the increased degradation of complex substrates, a high cost of acids and the additional cost for neutralizing the acidic conditions prior to the AD process (Modenbach et al, 2012; Taherzadeh et al, 2008; Kumar & Murthy, 2011).

# FREE NITROUS ACID (FNA) PRE-TREATMENT

A significant number of organics found in wastewater are usually converted to waste activated sludge, also known as WAS during biological wastewater treatment. Some of the methods used in the disposal of WAS includes the utilization of the sludge in agriculture and incineration. These disposal methods require or benefit from biological stabilization, which destroys organics and controls pathogens. Anaerobic digestion is attracting extensive attention since it converts organics in WAS to a renewable bioenergy resource in the form of methane. However, methane production through anaerobic digestion is often limited by the slow hydrolysis rates and poor biochemical methane potential of the WAS.

Thus, effective improvement of methane production in anaerobic digestion, particularly through thermal, chemical, and mechanical pretreatments has become an important research topic and an industrial application area. These technologies destroy cells and/or extracellular polymeric substances (EPS) with the release of intracellular and/or extracellular constituents to the aqueous phase. The released constituents are more easily biodegraded during anaerobic digestion, thereby enhancing methane production. For example, it was observed that methane production in anaerobic digestion increased by 42% after pretreating WAS at 175 °C for 60 min (Wang et al, 2013, p. 11897).

However, most of the above-mentioned approaches are cost intensive due to high energy and/or chemical requirements and have negative environmental consequences (e.g., higher net CO2 emissions compared with the case without pretreatment). Thus, alternative methods to enhance methane production in anaerobic digestion are needed. Free nitrous acid (FNA or

HNO2), at parts per million (ppm) levels, had a strong biocidal effect on anaerobic sewer biofilms13 and microorganisms in WAS.14 It was reported that WAS treatment using FNA at 1−2 mg N/L for 24−48 h reduced WAS metabolic activity to zero and killed 50−80% of the cells (i.e., damaged cell membrane) in WAS.14 Aerobic digestion tests showed that the degradation of WAS with FNA pretreatment was more than two times higher in comparison to the WAS without FNA pretreatment. More recently, WAS production in a reactor operated under alternating anoxic-aerobic conditions was reduced by 28%, by treating part of the returned activated sludge with FNA for 24 h at an FNA level of 2.0 mg N/L.15 However, the FNA induced improvement in WAS degradation, as revealed by the above research, all occurred under aerobic/anoxic conditions. No information has been reported so far on the effect of WAS pretreatment using FNA on methane production in anaerobic digestion of WAS (Wang et al, 2013, p. 11897).

FNA is a renewable and low-cost chemical that can be produced on site by nitritation of the anaerobic digestion liquor, thus achieving sustainable use of the anaerobic digestion liquor otherwise requiring extra treatment (Wang et al, 2013, p. 11898).

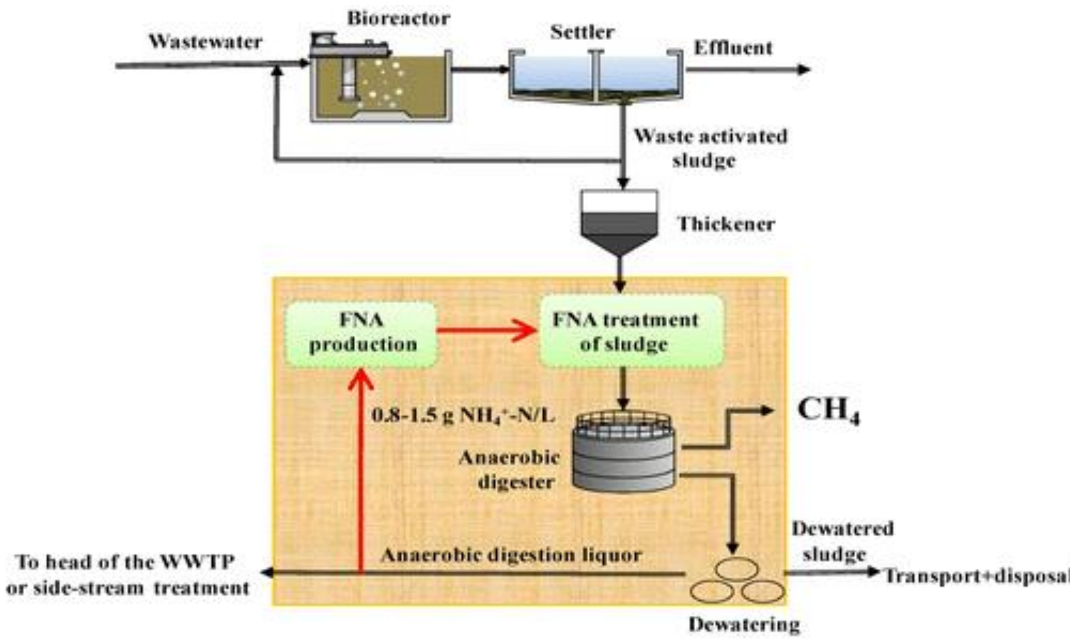


**Figure 12: "Closed-loop" FNA pre-treatment technology for enhancing methane production in a wastewater treatment plant (Wang et al, 2013, p. 11902)**

# VOLATILE FATTY ACIDS PRODUCTION

There are two types of volatile fatty acids: short-chain and long-chain. Short chain VFAs include: propionic, iso-butyric, butyric and acetic acids. Long chain VFAs are mostly valeric acids. Short chain VFAs are derived from the fermentation of the major compounds (carbohydrates, proteins and lipids), while long chain is generated mainly from the fermentation of just proteins (Cokgor, Oktay, Tas, Zengin & Orhon, 2009, p. 380). Volatile fatty acids consist of propionic and acetic acid, which are produced from primary sludge generated in treatment plants (Min et al, 2005, p. 910). VFA can be produced by varieties of microorganisms, the difference though is the shape and degree of the acid which is usually dependent on the microbial breed and the substrates affected in the process (Atherton, 1995, p. 4). VFA production involves anaerobic procedures which include hydrolysis and acidogenesis (which is also called acidogenic or dark fermentation) (Lee, Chua, Yeoh & Ngoh, 2014, p. 92). The process to produce VFA is accomplished simultaneously during anaerobic digestion, there are various ways involving the collection of VFA. They include the time of digestion and even the reactions of biochemicals in the fermentation phase (Peces, Astals, Clarke & Jensen, 2016, p. 631).

VFAs can be produced with the varieties of wastes, solid, liquid and even the combination of both. Solid wastes that can be used include: sludge, organic portions of solid wastes derived from municipalities and food waste. Liquid wastes can be classified as wastewater generated from pulp and paper, agricultural and dairy industries, as mentioned before, primary sludge and waste activated sludge are the most common types of wastes used to produce VFAs. These two types of wastes are utilized due to their large amount of produced fatty acids and extensive use during biological treatment for wastewater (Lee, Chua, Yeoh & Ngoh, 2014, p. 86).

There are two steps involved with sludge fermentation to produce volatile fatty acids; they are **hydrolysis and acidogenesis** (Sanchez Rubal et al, 2012, p. 1). The application of anaerobic treatment of primary sludge through hydrolysis and fermentation generates volatile fatty acids, resulting in the production of valuable resources like the removal of biological nutrients and nitrification. To fully restrict anaerobic digestion to just the hydrolysis and fermentation phase, it is necessary to control both the hydraulic retention time and temperature, while the primary sludge is being treated (Hatziconstantinou et al, 1996, p. 418).

## HYDROLYSIS

Hydrolysis is the first stage in anaerobic digestion, during this process, substantial organic polymers, like carbohydrates, proteins and lipids are broken down and dispersed into little molecules (like amino acids, fatty acids and sugars), and then into a solution through microorganisms. This process is a vital first step, because it breaks down compound organic molecules into more uncomplicated amino acids, fatty acids and sugars (Sanchez Rubal et al, 2012, p. 2). Through this process, proteins are converted to amino acids; carbohydrates into sugars or glucose and lipids are converted to long chain fatty acids (LCFA), these products are now made ready to be used in the acidogenesis process (van Lier, Mahmoud & Zeeman, 2008, p. 405).

### CARBOHYDRATES

Various complex types of carbohydrates like starch, cellulose and pectin undergo hydrolysis for the conversion into more uncomplicated simple sugars, which consists primarily of glucose, together with mannose, galactose and arabinose. The various types of microorganisms used for the hydrolysis of carbohydrates include: fungi, clostridia and *acetivibrio celluliticus*, starch can be used as a decomposing material, hence why four different types of enzymes are utilized to allow the conversion of starch to glucose (Banerjee, 1997, p. 13).

$$\textbf{Carbohydrates} \xrightarrow[\text{Hydrolysis}]{} \textbf{Sugars (primarily glucose)}$$

### PROTEINS

Proteolytic enzymes are derived from bacteria; it is used in the hydrolysis of proteins to produce amino acids. Amino acids can also be used in cells as substrates for fermentation, the microorganisms used for the hydrolysis process is called proteus vulgaris, in addition to specific types of *clostridia* (Banerjee, 1997, p. 15).

$$\textbf{Proteins} \xrightarrow[\text{Proteolytic Enzymes}]{\text{Hydrolysis}} \textbf{Amino Acids}$$

## LIPIDS

Bacillus, serratia and clostridium are microorganisms utilized for the hydrolysis of lipids, which results in the production of long chain fatty acids. The enzymes used are: lipases and phospholipases; the former allows the fatty acids to be hydrolyzed into more uncomplicated lipids, while the latter is used for the hydrolysis of phospholipids (Banerjee, 1997, p. 19).

$$\textbf{Lipids} \xrightarrow{\text{Hydrolysis}} \textbf{Glycerol and Long Chain Fatty Acids}$$

Hydrolysis is considered as the rate-limiting step during the process of anaerobic digestion (Skalsky & Daigger, 1995, p.230), meaning that the hydrolysis process needs to be amplified to generate optimal amounts of volatile fatty acids (Zhang et al, 2016, p. 100). This process can be applied using primary sedimentation tanks, gravity thickeners or completely mixed (closed) reactors. The volatile fatty acids can then be purified from the sludge with different methods, whether mechanically or physically. Physically, the elutriation is accomplished by utilizing primary sedimentation tanks or thickeners, where gravity is used to circulate the sludge constantly to enhance elutriation (Hatziconstantinou et al, 1996, p. 418). During this process, it is observed that solids retention time (SRT) acts as a factor. It was realized that the hydrolysis of carbohydrates and lipids was heightened due to SRT (Sanchez Rubal et al, 2012, p. 2).

## ACIDOGENESIS

After the hydrolysis process, acidogenesis decomposes the other components left over (during hydrolysis) to intermediary products. This process is where VFAs are formed, with the inclusion of other side products like ammonia, hydrogen sulfide, etc. (Sanchez Rubal et al, 2012, p. 2). In this process, the major compounds utilized include carbohydrates, proteins and lipids. Acidogenic fermentation deals with an array of microorganisms called acidogenic bacteria, which aids in the decomposition of the production of hydrolysis to simpler organic acids like **butyric**, **acetic** and **propionic** acids. A massive number of microorganisms are needed to decompose the three complex organic substrates, to produce VFAs. The major compounds are used as substrates to produce VFA using anaerobic metabolic direction (Banerjee, 1997, p. 12-13). This process is where sludge is fermented, and is usually distinguished by the decline in pH during the process (Sanchez Rubal et al, 2012, p. 2). To have optimal production of VFAs with the use of sludge collected from wastewater, factors like hydraulic retention time (HRT); characteristics of sludge; pH; operational criterion; temperature; composition of the reactor; knowledge of metal content and the possibility of reducing oxidation are considered (Min et al, 2005, p. 910).

### GLUCOSE

The product from the hydrolysis of carbohydrates is primarily glucose; it is fermented with the use of microorganisms. These microorganisms use glucose to provide carbon and energy to combine all the components of the cells. Pyruvate is then formed through the conversion of glucose, with the Embden-Meyerhof-Parnas (EMP) pathway - which is a type of glycolysis, making pyruvate one of the intermediate products of the fermentation of glucose, VFAs and other alcohols are then generated from pyruvate with dependency on the type of microorganisms used (Banerjee, 1997, p. 14).

**Glucose** $\longrightarrow$ **Pyruvate** $\longrightarrow$ **Volatile Fatty Acids**

As mentioned previously, acidogens are the non-complex organic acids, formed from the decomposition of the end-products of hydrolysis. They are acetic, butyric and propionic acids. Acetic acid is generated from the fermentation of glucose, propionic acid is formed due to the fermentation of pyruvate, along with various other end products, while butyric acid is formed from the conversion of acetoacetate (formed by converting acetyl) (Banerjee, 1997, p. 14-17).

## AMINO ACIDS

The first phase in the fermentation of amino acids, is to remove the amino group from the amino acids for it to be converted into ammonia, this is also referred to as deamination. This is achieved using an acceptor, which results in the production of an organic acid. This organic acid is then converted into pyruvate or acetyl; the product can be utilized as a source of energy and carbon. The formation of some VFAs can also be achieved without having to convert the organic acid into pyruvic acid (Banerjee, 1997, p. 18).

**Amino Acid** $\xrightarrow{\text{Deamination}}$ **Organic Acid** $\longrightarrow$ **Pyruvate** $\longrightarrow$ **VFA**

## Fatty Acids

After the hydrolysis of lipids to form glycerol, pyruvic acid is retrieved via glycolysis (EMP pathway), which goes through the fermentation process to form VFAs (Banerjee, 1997, p. 19).

# FACTORS AFFECTING VFAs PRODUCTION

As mentioned previously, the hydrolysis process is the rate-limiting step during the acid production stage, but it was also observed that the conversion from the intermediary products to volatile fatty acids was a vital criterion to deciding the rate-limiting step. Through Perot et al research conducted in 1988, it was determined that a pH of 5 produced the lowest amount of VFA, while the most optimal amount was at a pH of 6 and higher, with a relatively high temperature in the high 50's and an agitation speed of 500 rpm and higher. Another experiment conducted by Gupta in 1985 with the use of batch systems, also suggested that a pH at 7 and higher did not prove to produce optimal volumes of VFAs, while there was a great development of VFAs at a temperature range of 10 to 30 degrees Celsius (Banerjee, 1997, p. 20).

pH is an important factor in the production of VFA because the acidogens used in the process cannot sustain high acidity and alkalinity levels which usually surround a pH value of 3 and 12. Therefore, the optimal range for an efficient production of VFA usually varies from 5 to 11, but the specific range is determined by the type of waste used for the generation of the fatty acids (W.S. Lee et al, 2014, p. 89). For temperature, it was observed at four different ranges: thermophilic, which ranges from 50 to 60 degrees Celsius; mesophilic, which is from 20 to 50 degrees Celsius; psychrophilic, from 4 to 20 degrees Celsius and hyper-thermophilic, from 60 to 80 degrees Celsius. It was realized that higher concentrations of VFAs were produced when there was a temperature increase in the mesophilic and psychrophilic range, an increase in both the momentum at which the VFA were produced and the VFA yield were observed as well (W.S. Lee et al, 2014, p. 90).

# APPLICATIONS OF VFAs

Volatile fatty acids can be applied in different ways, applications utilized in the production of VFAs consists of the creation of bioenergy, bioplastics and the elimination of biological nutrients found in wastewater, which helps in the reduction of utilizing pricey carbon sources like methanol (Wee, Adeline, Hak & Gek, 2014, p. 84). Some advanced applications of volatile fatty acids include polyhydroxyalkanoates, which are biodegradable polymers that can be incorporated by microorganisms with the use of renewable resources like VFA. This polymer is used extensively in industries, it is better for the environment in comparison to the usual plastics generated from petroleum (W.S. Lee et al, 2014, p. 92). The production of VFAs is critical to the avoidance of eutrophication of water bodies, which is where excessive nutrients in the receiving waters are present. It aids in the process of eliminating biological nutrients by reducing the amount of nitrogen and phosphorus into lakes, streams or rivers (Maharaj, 1999, p. 24).

In this current century, one of the challenges societies face is the increased price for oil and the decline of storage spaces for oil produced, hence why the application of VFAs for the creation of bioenergy is vital, to extend supplies of energy sources, with the advantage that VFAs derived from waste is an inexpensive approach to generating different sources of energy (W.S. Lee et al, 2014, p.93). Biogas is used to generate both power and heat due to its large amount of methane (W.S. Lee et al, 2014, p.94).

# OBJECTIVES OF THE EXPERIMENT

A semi continuous (fed-batch) experiment was conducted, to understand the fermentation process of TWAS and observe the effect on the TWAS characteristics. In order to fully understand the effect, two different HRT's were analyzed, 2 HRT and 3 HRT. For both HRT's, the reactor was fed with the pretreated sludge every 24 hours, for 2 HRT, the liquid samples were collected on the $6^{th}$ day and every day after that until the $12^{th}$ day, where the experiment was concluded. For 3 HRT, the liquid samples were collected on the $9^{th}$ day and everyday after that until the $18^{th}$ day, when the experiment was conducted. The dosage of FNA was similar for both HRTs (0.35, 0.7, 1.4, and 2.8 mg N/L), the difference was the volume of sample being fed into the reactor, for 2 HRT, the volume was 600 mL was used for each of the 6 reactors (making a total of 3600 mL of sludge) and for 3 HRT, the volume used was 400 mL for each of the 6 reactors (total of 2400 mL of the sample). The liquid samples collected for analysis were gotten from day 3 and 6 of the experiment.

# MATERIALS AND METHODS

## MATERIALS

TWAS samples were retrieved from the Ashbridges Bay Wastewater Treatment Plant located in Toronto, Ontario, Canada. These samples were used to conduct the semi continuous experiments. Ashbridges Bay is the biggest WWTP out of the 4 others located in Toronto, the waste activated sludge is collected from the final clarifier, where it is sent to the air flotation tanks to be thickened with a thickening polymer. This process results in the TWAS having approximately 3.4% of Total Solids, and 71% of the Volatile Solids of the Total Solids. After the thickening process, the samples were collected from the air flotation tanks. The Ashbridges Bay WWTP receives almost 7,000 $m^3$ of sludge every day from 20 digesters, two thirds of the sludge are primary sludge, while one third is TWAS. The digester tanks that hold the sludge varies from 30 to 33 diameters, with a temperature varying from 34 to 38 degrees Celsius. The plant produces about 64,560 $m^3$ of biogas everyday with an average SRT of 23 days, this biogas is then used as fuel in the plant, while the remnant of the biogas is burned (City of Toronto, 2016).

## TWAS PRE-TREATMENT WITH FNA

When the samples are collected from the plant, it is placed in the refrigerator until it is time to be used. The samples with FNA pre-treatment were set at a pH of 5.5 +/- 0.2 for bottle 2 through 6, while the temperature was set to 25 degrees Celsius for all the samples. To ensure a pH of 5.5, the TWAS samples were attuned with the use of 3M HCl to reduce the pH and 1M NaOH to increase the pH.

Table 1: Pre-treatment conditions for semi-continuous systems

| Bottle (#) | Nitrite conc. (2 HRT) | Nitrite conc. (3 HRT) | FNA (mg N/L) |
|---|---|---|---|
| **1 (raw)** | 0 | 0 | 0 |
| **2 (control)** | 0 | 0 | 0 |
| **3** | 8 mL | 4 mL | 0.35 |
| **4** | 16 mL | 8 mL | 0.7 |
| **5** | 32 mL | 16 mL | 1.4 |
| **6** | 48 mL | 32 mL | 2.8 |

Once the FNA pre-treatment was prepared and added to the TWAS bottles, the bottles were then placed in a shaker, which mixed the contents of the bottle at 100 to 150 rpm for 24 hours every day for 12 days for 2 HRT and 18 days for 3 HRT, the pH was supervised, and it was ensured that 5.5 was maintained with the use of HCl when needed. After 24 hours in the shaker, samples were collected for wet chemistry analysis and the rest were fed to the semi continuous system for 12 days (2 HRT) and 18 days (3 HRT).

## SEMI CONTINUOUS EXPERIMENT SETUP AND PROCEDURE

Six glass reactors, with a volume of 2 L each were used for the semi continuous experiment. These reactors came with fitted airtight caps, stirrers and two spouts, as shown in the figure below. A PolyScience General Purpose water bath, Model WB28 was used to hold the sex glass reactors. The water bath was filled with water and the temperature was set to 40 degrees Celsius, ensuring that the temperature inside the reactors were in the high 30's, the temperature for the sludge in the reactors were measured with the use of a thermometer everyday. Due to evaporation occurring in the water bath, the water level had to be sustained everyday during the experiment. The top of the reactors had caps with tubes connected to gas meters from Tyogn-S3$^{TM}$ B-44-3 tubing, this was used to measure the amount of gas being produced by the bioreactors.

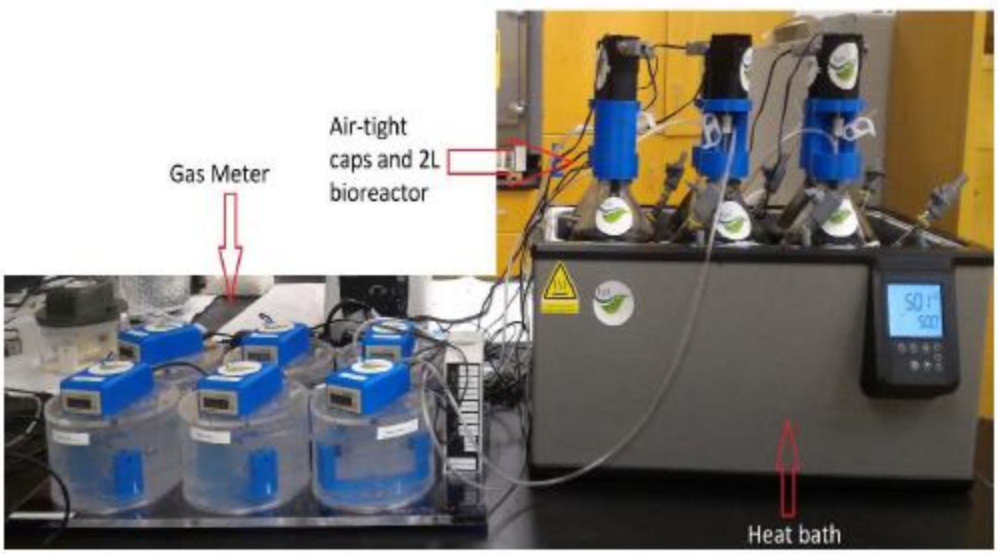


**Figure 13: Semi Continuous Experiment Set Up (Okoye, 2017, p. 27)**

The six reactors contained 1.2 L of digested sludge collected from Ashbridges Bay WWTP. Every 24 hours, for 2 HRT, 600 mL of digested sludge was collected from the lower part of the reactors, while the same amount of pretreated TWAS was fed into the reactor with the use of the top sprout. For 3 HRT, 400 mL of digested sludge was collected from the lower sprout of the reactors, while the same amount of pretreated TWAS was fed into the reactor with the use of a digital pump system and tubing. During the process of extracting and feeding the sludge, the stirrer was kept on. The first reactor was fed with untreated raw sludge, the second reactor was fed with pH adjusted sludge, the sludge was adjusted with the use of HCl to adjust the pH to 5.5 +/- 0.2. Reactor 3 through to 6 were fed with FNA pretreated sludge with the different doses discussed previously. The sludge was pretreated every day before feeding it into the reactors for the span of the experiment. The semi-continuous process was run for 12 days for 2 HRT, with liquid samples being collected everyday after the $6^{th}$ day, and 18 days for 3 HRT, with liquid samples being collected everyday after the $9^{th}$ day. When the liquid samples were collected, the pH and temperature were measure immediately, then stored in a plastic sample bottle before placing it in the refrigerator. The amount of gas produced were also measured via the gas meters each day.

## LIQUID SAMPLING ANALYSIS

The analyses that were conducted for the collected liquid samples are as follows: pH, temperature, TS (Total Solids), VS (Volatile Solids), alkalinity, ammonia, total phosphorus (TP), carbohydrate, protein, nitrite, nitrate, total soluble nitrogen (SN), total nitrogen (TN), total and soluble chemical oxygen demand (TCOD and SCOD). The raw, pretreated and effluent samples were filtered with the use of a 0.45 µm VWR Vacuum Filtration Systems, Model 10040-462 to conduct the soluble experiments, except for the TS, VS tests. The samples were filtered by diluting it in a ratio of 1 to 10 with Deionized Distilled Water (DDW) to aid in reducing the filtration time. The following describes the procedure of some of the experiments conducted.

- **pH**: Immediately after the liquid samples were retrieved, the pH was measured with a VWR Benchtop pH Meter and a glass probe, model B10P.

- **Temperature**: Using a Durac Bi-metallic thermometer thermal pin, the temperature was measured immediately after it was retrieved from the bioreactors, to ensure that the temperatures were under the right conditions for the semi continuous experiment
- **TS and VS**: The solids content for the liquid samples were measured by placing 5 mL of each sample in aluminium dishes and using standards given in Methods 2540B and 2540E for TS and VS
- **Nitrogen**: For Nitrite-nitrogen, Method 8153 from the HACH manual was used, the ferrous sulfate procedure. This method measured the nitrite content in the samples, NitriVer Nitrite Reagent Powder pillows were used in the sample and the HACH 3900 was used to measure the nitrogen content.
- **Ammonia**: Method 10031 from the HACH manual was used, AmVer$^{TM}$ Diluent Reagent Test for High-Range Ammonia was utilized and ammonia salicylate and ammonia cyanurate reagent powder pillow was used in the HACH sample vials and HACH 3900 was used to measure the amount of ammonia.
- **Total Phosphorus**: Method 8190 from the HACH manual was used, PhosVer$^{R}$ 3 with acid persulfate digestion method was utilized. Potassium persulfate and PhosVer 3 powder pillow reagents were added to vials and the HACH 3900 was used to measure the total phosphorus content.
- **TCOD and SCOD**: Reagent vials with High Range from 20 to 1500 mg/L from HACH were used to conduct the TCOD and SCOD experiments. Steps outlined in Method 8000 were utilized, it was created by Carter and Jirka, it is a reaction digestion based method. The

amount of COD was then measured with the use of the HACH DR3900 spectrophotometer.

# RESULTS

## EFFECT OF FNA ON TWAS CHARACTERISTICS

Different doses of FNA were added to the pre-treatment in a semi-continuous (fed-batch process) to understand how it would affect the TWAS characteristics. The six bottles contained, one bottle with raw sludge (no FNA additions and pH was left as is), the second bottle contained a "controlled" sample, where the pH was adjusted to be 5.5 +/- 0.2 but FNA was not used as a pre-treatment. The third bottle through to the sixth bottle contained the pH adjusted sample, the only difference was the variation of the FNA doses. The third bottle had a dose of 0.35 mg N/L, the fourth had 0.7 mg N/L, the fifth had 1.4 mg N/L and the sixth had 2.8 mg N/L. It was ensured that all the samples were at a constant temperature of 25$^oC$ while in the stirrer, except for the raw sample, which was stored in a refrigerator with a temperature of about 4$^oC$. the second bottle with no FNA was used to analyze how the pH adjustments affected were different from the effect of the variations of FNA doses. Two HRTs were used in this analysis: 2 HRT, where the experiment was conducted in 12 days, and the liquid samples were retrieved after the 6$^{th}$ day. 3 HRT was conducted in 18 days, and the liquid samples of the treated effluent were collected after the 9$^{th}$ day.

## EFFECT OF FNA PRE-TREATMENT ON VFAs PRODUCTION IN FERMENTATION PROCESS AT HRT OF 2 DAYS

Table 2 below shows the different characteristics for the raw and digester effluent samples for the different FNA doses. These results represent the analysis of liquid sample of the digester effluent for 2 HRT, the liquid samples were collected every day after the 6$^{th}$ day until the 12$^{th}$ day, the values are the average for day 3 and day 6. As shown in the table, there is not much of a difference with the raw sample and the treated samples up until the 6$^{th}$ batch where there is approximately a 23% drop for TCOD. It is also observed that the TCOD increases as the dosage of FNA increases until the dosage is 2.8 mg N/L, where it drops to 33,500 mg/L. The SCOD decreased as the FNA doses increased from 0.35 to 2.8 mg N/L. the highest SCOD was 5,600 mg/L, which was for the raw sample, but with the FNA dose, the highest was 5,470 mg/L for a dose of 0.35 mg N/L.

Table 2: Characteristics of the TWAS for 2 HRT

| Batch # | FNA (mg N/L) | TCOD (mg/L) | SCOD (mg/L) | TS (%) | VS (%) | Ammonia (mg N/L) | Alkalinity (mg/L $CaCO_3$) | TN (mg/L) | SN (mg/L) | TP (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Raw | 45,675 | 5,600 | 2.32 | 65.97 | 940 | 1,710 | 2,300 | 625 | 3,460 |
| 2 | 0 | 45,925 | 5,525 | 2.47 | 66.92 | 880 | 2,050 | 2,575 | 710 | 3,510 |
| 3 | 0.35 | 46,450 | 5,470 | 2.22 | 63.57 | 875 | 2,345 | 2,400 | 775 | 3,670 |
| 4 | 0.7 | 49,700 | 5,130 | 2.33 | 65.10 | 948 | 2,265 | 2,325 | 655 | 3,510 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1.4 | 40,775 | 4,945 | 2.12 | 60.63 | 830 | 2,525 | 2,000 | 565 | 3,430 |
| 6 | 2.8 | 33,500 | 4,160 | 1.75 | 50.24 | 848 | 1,970 | 1,925 | 635 | 3,145 |

The total solids (TS) from the table above shows that as the doses of FNA increases, the total solids decrease. The highest value for TS is when the dose of 0.7 mg N/L FNA was added to the sample. For the volatile solid (VS), it is also clear from Table 2 that it decreases as the dosage of FNA increases, with the highest value being 65.10% for 0.75 mg N/L of FNA and the lowest, 50.24% for 2.8 mg N/L dosage. The same trend is noticed for the ammonia as well, where the highest value is 948 mg/L for 0.75 mg N/L dosage of FNA. For the rest of the experiments, the trend varies, but the constant trend is that the values decreases when the FNA dosages are added.

The transformation from the raw sample to the FNA pretreated samples with a dosage of 2.8 mg N/L, are as follows: the TCOD decreased by 27% from 45,675 mg/L to 33,500 mg/L; the SCOD reduced by 26% from 5,600 mg/L to 4,160 mg/L. There was a reduction in the total solids by 57% from 2.32 to 1.75, and a reduction in the volatile solids by 24% from 65.97 to 50.24. For ammonia, there was a decrease by 10% from 940 mg N/L to 848 mg N/L. Total Nitrogen decreased from 2,300 mg/L to 1,925 mg/L making it a 16% increase, while Soluble Nitrogen increased only by 2% from 625 mg/L to 635 mg/L and Total Phosphorus decreased by 9% from 3,460 mg/L to 3,145 mg/L. The only major increase was for alkalinity, where the concentration increased by 15% from 1,710 mg/L $CaCO_3$ to 1,970 mg/L $CaCO_3$.

The table below shows the percent solubility for carbohydrate and protein for 2 HRT, it is observed that the percent solubility for carbohydrate increases when the pH is adjusted, and as the variety of FNA dosage is added to the pre-treatment, it reduces, but spikes up after a dosage of 0.7 mg N/L of FNA, with the highest value being 53.21% of carbohydrate for the highest dosage of 2.8 mg N/L.

Table 3: Carbohydrate and Protein Percent Solubility for 2 HRT

| Batch # | FNA (mg N/L) | Carbohydrate (%) | Protein (%) |
|---|---|---|---|
| 1 | Raw | 16.87 | 14.11 |
| 2 | 0 | 31.21 | 12.24 |
| 3 | 0.35 | 21.56 | 15.24 |
| 4 | 0.7 | 32.69 | 18.05 |
| 5 | 1.4 | 37.67 | 7.77 |
| 6 | 2.8 | 53.21 | 7.24 |

For protein, the percent solubility decreased when the pH was adjusted from the raw sample and like that of carbohydrate, the percent solubility increased after a dosage of 0.35 mg N/L of FNA was added and it significantly decreased after a dosage of 1.4 mg N/L was added, where the highest value is 18.05% (0.7 mg N/L) and the lowest value is 7.24% (2.8 mg N/L). Therefore, to conclude with 2 HRT, from the results of the experiment, there is a significant decrease from the “controlled” sample (having no dosage of FNA) to the digested effluent of the highest dosage (2.8 mg N/L).

## EFFECT OF FNA PRE-TREATMENT ON VFAs PRODUCTION IN FERMENTATION PROCESS AT HRT OF 3 DAYS

The liquid samples analyzed for 3 HRT were collected every day after the 9th day of the experiment, till the 18th day, the values shown in Table 4, are the average values for day 3 and day 6. The trend for this HRT is a little different to that of 2 HRT, for TCOD starts to increase as the FNA dosage increases until 1.5 mg N/L FNA is added, where TCOD then reduces to 37,475 mg/L (2.8 mg N/L) and the highest value is 42,325 mg/L (0.7 mg N/L). This is also observed for SCOD, where it increases until it gets to a dosage of 0.7 mg N/L then it decreases to 5,335 mg/L (2.8 mg N/L) and the highest value occurred at 0.7 mg N/L FNA, with a value of 6,850 mg/L. For TS and VS experiments, the values increase when the pH is adjusted and later starts to decrease as it gets to a dosage of 1.4 mg N/L of FNA, then at 2.8 mg N/L, it decreases significantly to 2.26 mg/L and 61.42 mg/L. However, the highest values for TS and VS are 2.35 mg/L and 66.78 mg/L (for controlled sample with no FNA) respectively, and the lowest values are 1.71 mg/L and 51.02 mg/L (for 1.4 mg N/L) for TS and VS respectively.

Table 4: Characteristics of the TWAS for 3 HRT

| Batch # | FNA (mg N/L) | TCOD (mg/L) | SCOD (mg/L) | TS (mg/L) | VS (mg/L) | Ammonia (mg N/L) | Alkalinity (mg/L $CaCO_3$) | TN (mg/L) | SN (mg/L) | TP (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Raw | 28,875 | 2,510 | 2.05 | 65.68 | 1,415 | 2,760 | 2,150 | 180 | 3,510 |
| 2 | 0 | 37,525 | 2,655 | 2.35 | 66.78 | 1,760 | 2,705 | 2,625 | 735 | 3,720 |
| 3 | 0.35 | 39,300 | 5,105 | 2.05 | 61.03 | 1,588 | 2,600 | 2,675 | 760 | 3,765 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 0.7 | 42,325 | 6,850 | 2.05 | 61.27 | 2,030 | 2,890 | 2,325 | 880 | 4,275 |
| 5 | 1.4 | 40,675 | 6,370 | 1.71 | 51.02 | 1,740 | 3,735 | 2,400 | 845 | 3,430 |
| 6 | 2.8 | 37,475 | 5,335 | 2.26 | 61.42 | 1,925 | 2,780 | 2,225 | 842 | 3,480 |

For TN and SN, the values increase after the Ph is adjusted from the raw sample to the controlled sample. Afterwards, the values start to increase and for TN, the lowest value was 2,225 mg/L (2.8 mg N/L) and the lowest value for SN was 735 (controlled sample – with no FNA). With ammonia, the liquid sample for the raw sample to the highest dosage (2.8 mg N/L), there was an increase from 1,415 mg N/L to 1,925 mg N/L. For alkalinity, when the pH was adjusted, the alkalinity reduced until a dosage of 0.7 mg N/L was added, then it increased to 3,735 mg/L for a dosage of 1.4 mg N/L which is the highest value, and then it reduces to 2,780 mg/L for the highest dosage.

Table 5: Carbohydrate and Protein Percent Solubility for 3 HRT

| Batch # | FNA (mg N/L) | Carbohydrate (%) | Protein (%) |
|---|---|---|---|
| 1 | Raw | 15.67 | 12.56 |
| 2 | 0 | 24.00 | 10.51 |
| 3 | 0.35 | 37.14 | 18.17 |
| 4 | 0.7 | 35.29 | 18.95 |
| 5 | 1.4 | 27.20 | 18.68 |
| 6 | 2.8 | 40.88 | 12.99 |

For the percent solubility of carbohydrate, there is a significant increase from the raw sample and the controlled sample to the highest dosage, from 15.67% to 40.88%. While, for protein, there is a slight decrease from the raw sample to the controlled sample, before it increases to 18.17% (0.35 mg N/L) and then decreases at the highest dosage to 12.99%. Therefore, for most of the experiments, it was observed that the values significantly increase when a dosage of 0.7 mg N/L FNA was used for the pre-treatment, and then reduces at the highest dosage of 2.8 mg N/L.

## COMPARISON BETWEEN THE TWO HRTs (2 DAYS VS 3 DAYS)

Now that the effect of the FNA pre-treatment and different HRTs have been discussed, the following figures analyze and compare the experimental results for 2 and 3 HRT.

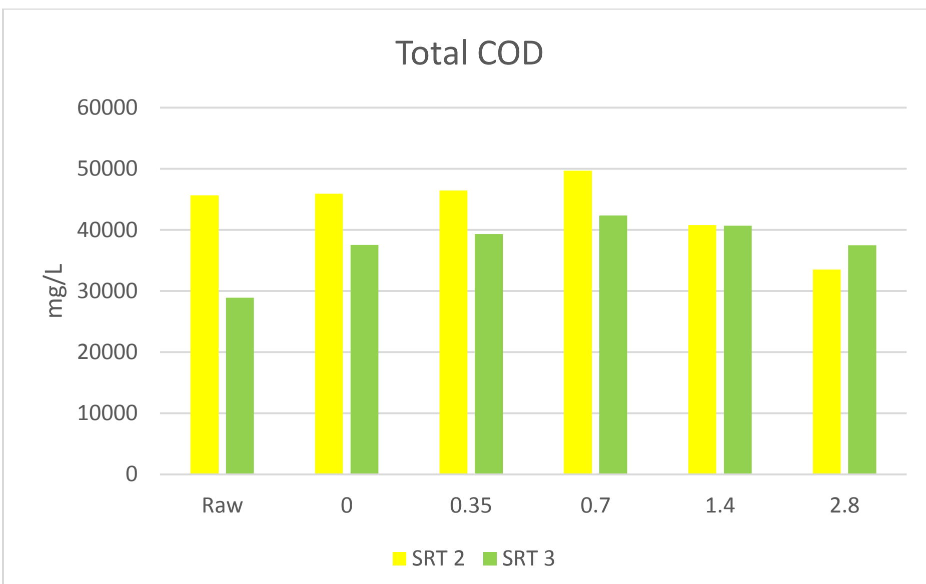


**Figure 14: Total COD for 2 and 3 HRT**

For total COD, it is shown that the values for 2 HRT is higher than that of 3 HRT, expect when the FNA dosage reaches 1.4 to 2.8 mg N/L where 3 HRT is higher than that of 2 HRT, and it is observed that as the FNA dosage increases, the concentrations for TCOD increases as well.

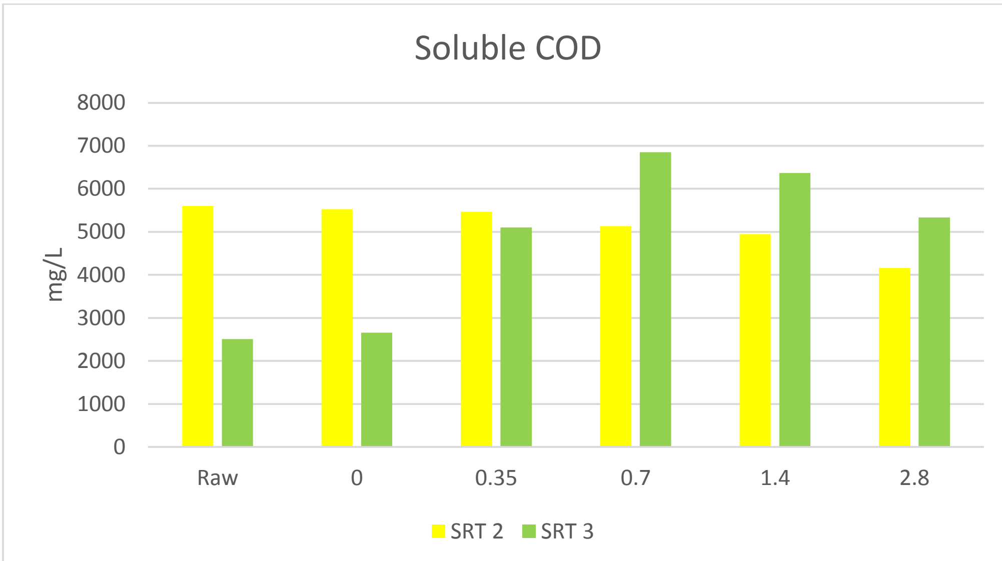


**Figure 15: Soluble COD for 2 and 3 HRT**

For soluble COD, it has a similar trend like total COD, where the values for 2 HRT is higher until a dosage of 0.7 mg N/L is reached where the values for 3 HRT surpasses that of 2 HRT. Therefore, it is observed that as the dosage of FNA increases, the SCOD concentrations increases as well.

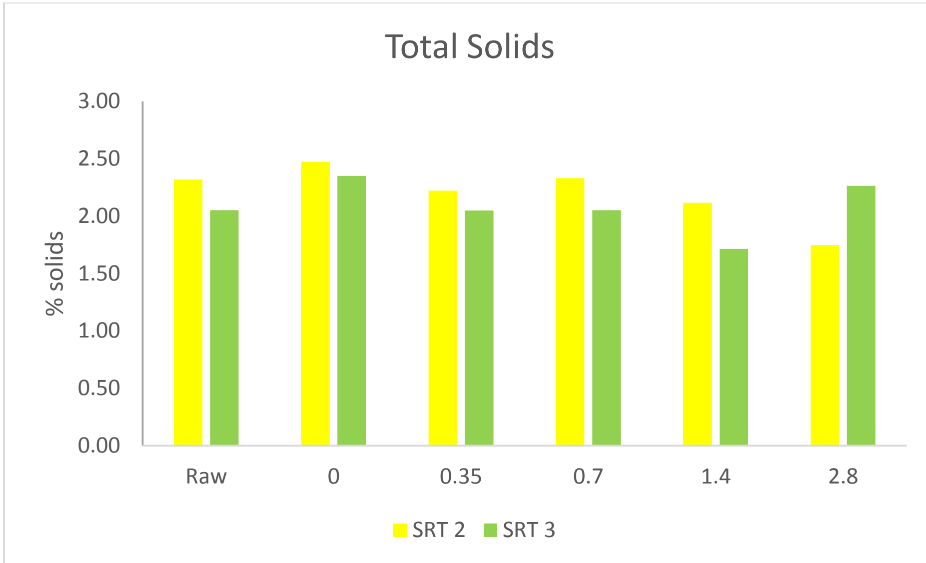


**Figure 16: Total Solids for 2 and 3 HRT**

For total solids (TS), the values for 2 HRT is higher than that of 3 HRT, until a dosage of 2.8 mg N/L, which is the highest dosage of FNA for this experiment, there, the value for 3 HRT is higher at 2.26%. Therefore, based on the observations made from the analysis, higher concentrations of FNA yield to lower percentage of the total solids.

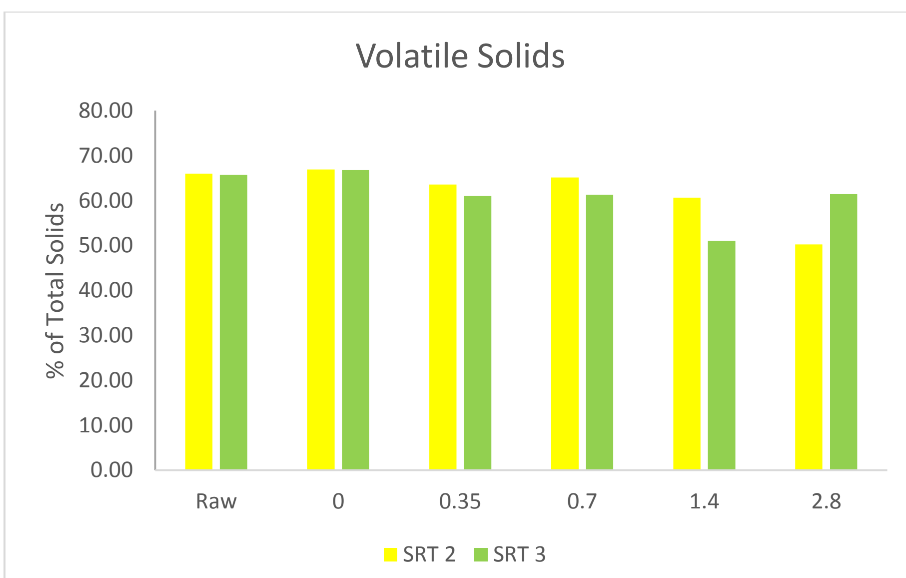

**Figure 17: Volatile Solids for 2 and 3 HRT**

Similar to that of TS, the values for 2 HRT is higher than that of 3 HRT, until the highest dosage of FNA is reached at 2.8 mg N/L, where 3 HRT is higher than 2 HRT. Otherwise, like the percentage of total solids, the values for volatile solids reduce as the concentration of FNA doses increases.

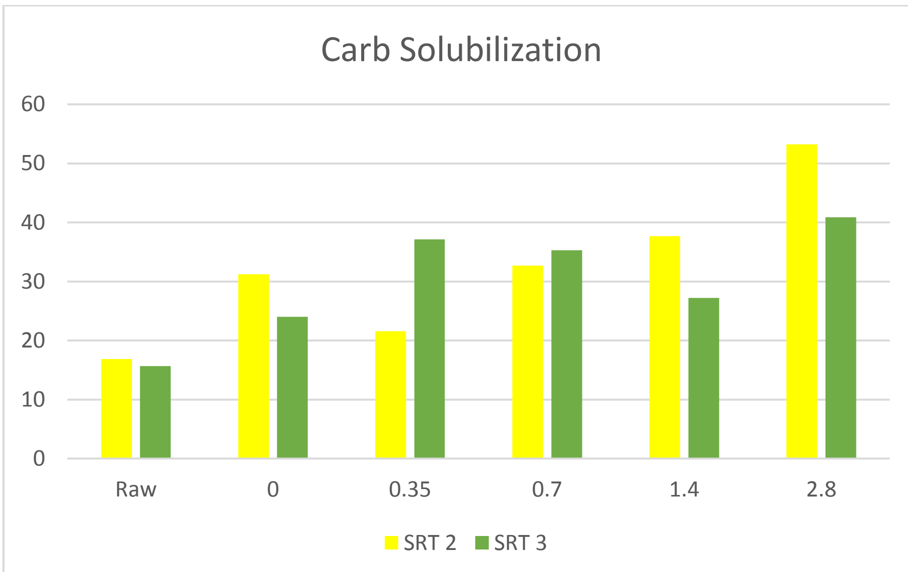


**Figure 18: Carbohydrate Solubilization for 2 and 3 HRT**

The percent solubilization of carbohydrate varies from the other observed experiments, here, the values for 2 HRT increases until it reaches a dosage of 0.35 mg N/L where 3 HRT is higher, before it reduces again at a dosage of 1.4 mg N/L and 2.8 mg N/L. The percent solubilization for protein, shown in Figure 19, also has a similar trend to that of carbohydrate, except that after a dosage of 1.4 mg N/L was reached, the percent solubilization for 3 HRT increased and was higher than that of 2 HRT.

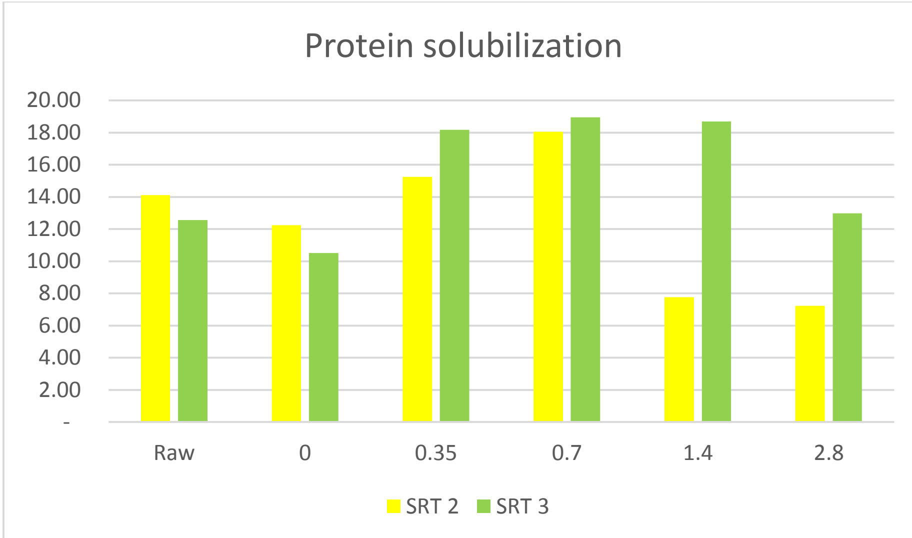


**Figure 19: Protein Solubilization for 2 and 3 HRT**

The experiments conducted for the Nitrogen species can be observed in Figure 20, 21 and 22, it shows the concentrations for total nitrogen, soluble nitrogen and nitrogen solubilization. For this experiment, there was a significant change in concentrations from samples collected in Day 3 and Day 6.

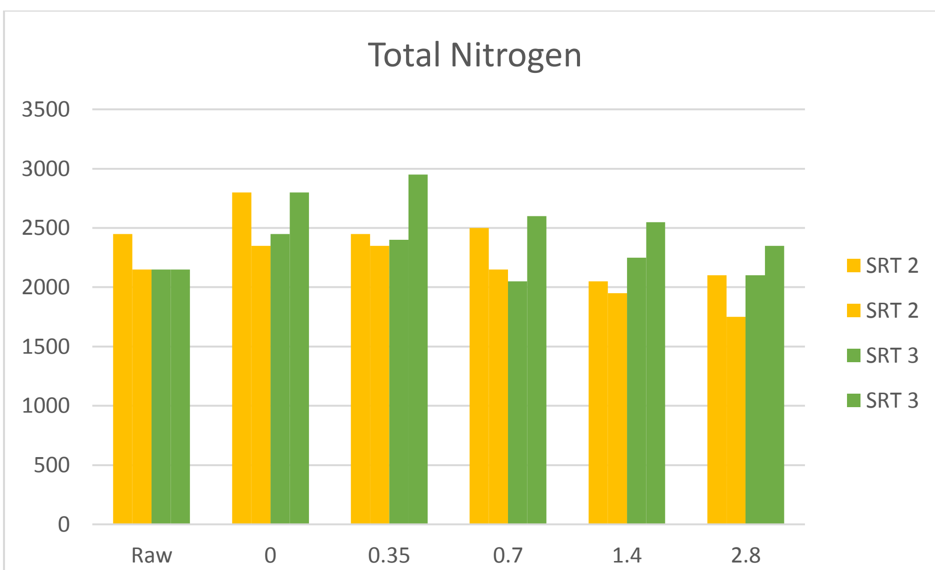


**Figure 20: Total Nitrogen for 2 and 3 HRT**

For total nitrogen, shown in Figure 20, the liquid samples collected for 2 HRT on day 3 was significantly higher than collected samples on day 6. In contrary to that observation, it was observed that for 3 HRT, the collected samples for day 3 was significantly lower to the samples retrieved on day 6 of the experiment. With that in mind, for both HRTs, the concentration for total nitrogen started to decrease after an FNA dosage of 0.35 mg N/L was added.

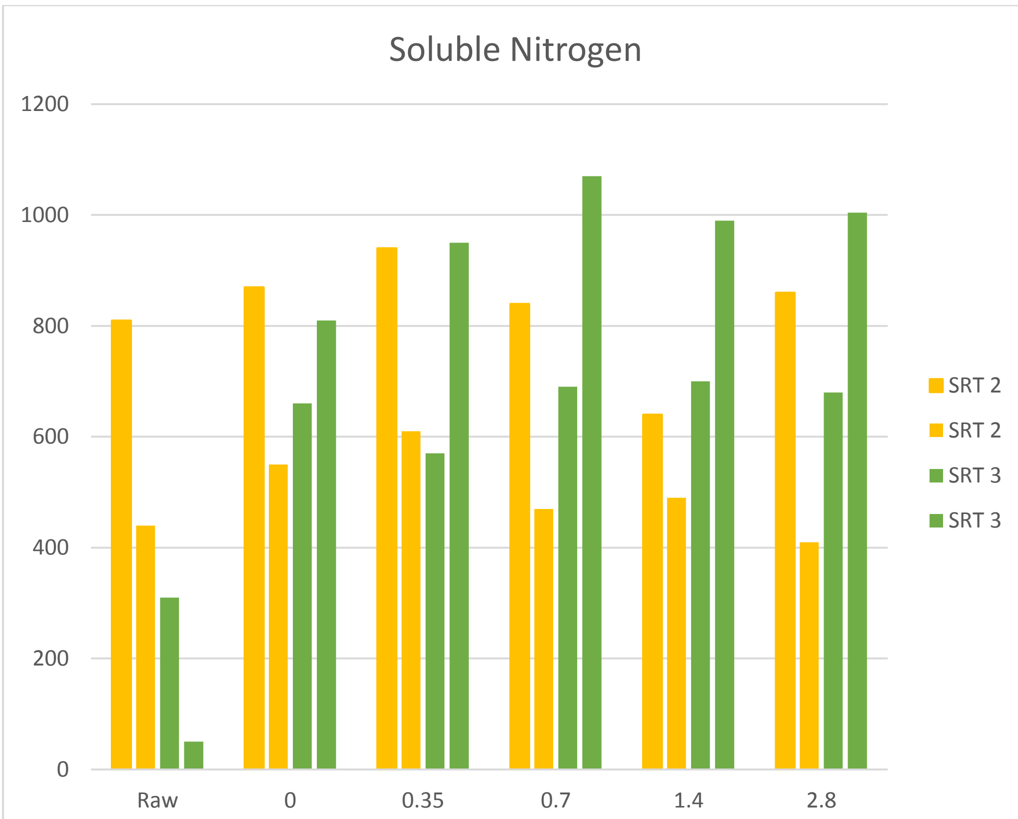


**Figure 21: Soluble Nitrogen for 2 and 3 HRT**

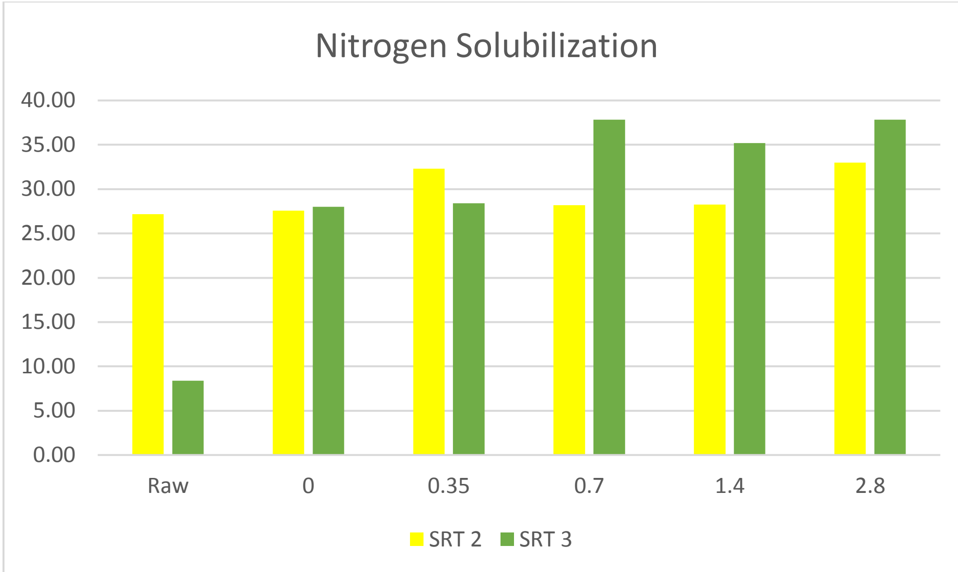


**Figure 22: Nitrogen Solubilization for 2 and 3 HRT**

For soluble nitrogen, the same trend applies to that of total nitrogen, for 3 HRT, the concentrations for soluble nitrogen for day 3 is observed to be significantly lower than that of the concentrations for day 6. While for 2 HRT, the concentrations for day 3 is substantially higher than the concentrations for day 6. The highest concentration for 3 HRT is when a dosage of 0.7 mg N/L was applied, and the lowest (apart from the raw sample) concentration is the controlled sample (where no FNA dosage was applied). For 2 HRT, the highest concentration is observed at a dosage of 0.35 mg N/L and the lowest concentration is observed at a dosage of 0.7 mg N/L (apart from the raw sample).

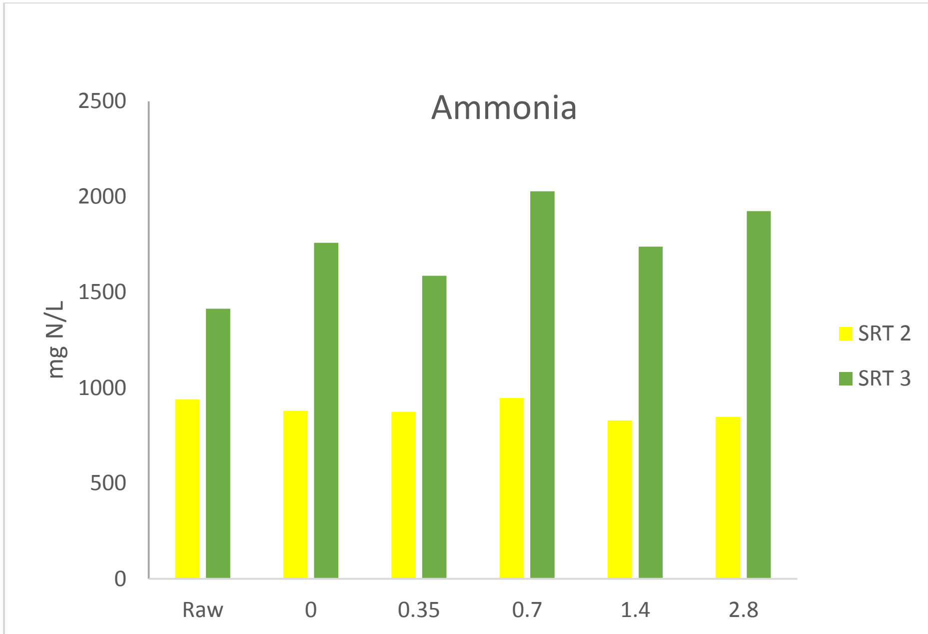


**Figure 23: Ammonia for 2 and 3 HRT**

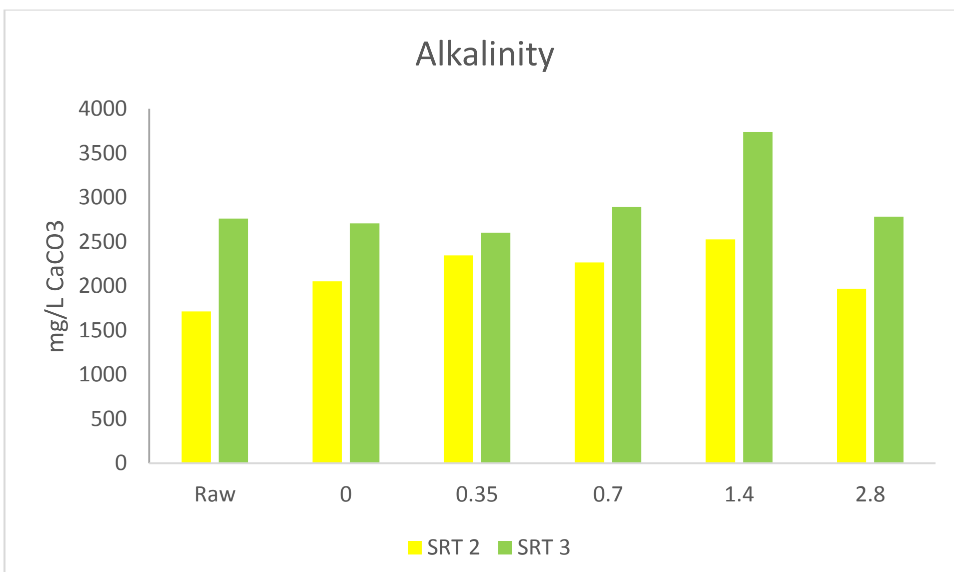


**Figure 24: Alkalinity for 2 and 3 HRT**

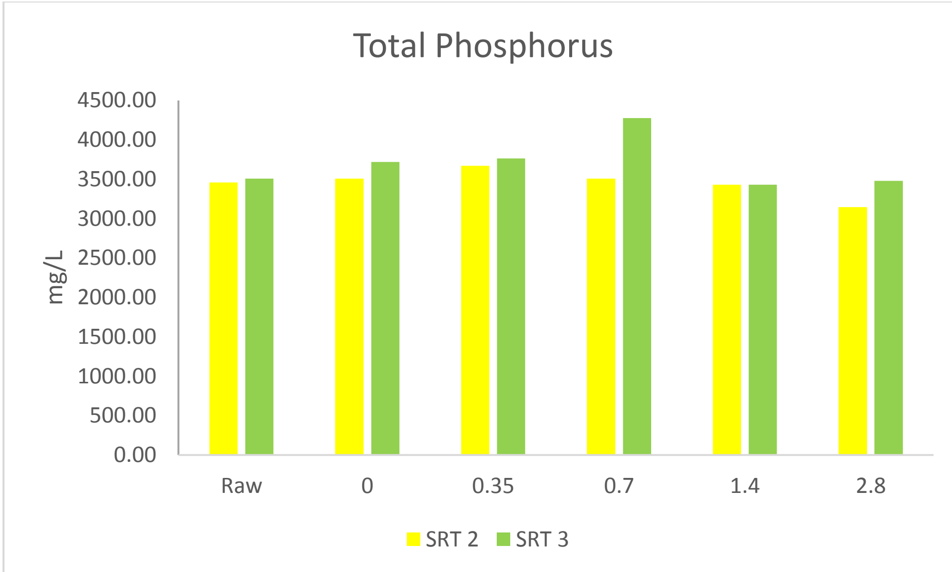


**Figure 25: Total Phosphorus for 2 and 3 HRT**

Experiments conducted for ammonia, alkalinity and phosphorus, shown in Figure 23, 24 and 25, have similar trends, meaning that for all three experiments, the concentration from 3 HRT is higher than the concentrations for 2 HRT. The only difference is that for total phosphorus, when a dosage of 1.4 mg N/L of FNA was reached, the concentration for 2 HRT and 3 HRT were almost equal. By adjusting the pH, the concentration of ammonia increases for both HRTs, then there is a spike when a dosage of 0.35 mg N/L of FNA is added, but by applying a dosage of 0.7 mg N/L, the concentration increases, before it declines when the next dose of FNA is applied. The concentration for alkalinity was more constant until a dosage of 1.4 mg N/L of FNA was reached, which reduced later for 2.8 mg N/L.

# CONCLUSION

The objective of this paper was to analyze the fermentation of FNA pretreated thickened waste activated sludge (TWAS) in order to produce volatile fatty acids (VFAs). This paper discussed in detail, the fermentation of primary sludge to produce volatile fatty acids (VFA). The factors affecting fermentation and different types of procedures used in the fermentation process were presented. Primary sludge was also characterized, and the production and applications of VFAs was discussed. The fermentation of primary sludge is a process conducted in anaerobic digestion; this process was examined, including the four major stages of the digestion method. The first two stages were discussed further, because it completes the production of VFAs through fermentation. These two stages were hydrolysis and acidogenesis, based on research and conducted experiments, it was realized that the major factors that greatly affect the production of volatile fatty acids through fermentation processes include: solids and hydraulic retention time; pH and temperature (Pullen, 2015, Stephenson & Stuetz, 2009).

Different fermentation configurations were also identified, it was also noted that the process chosen was dependent on the intensity of the wastewater and fermentation located in the sewage system (Atherton, 1995, p. 1). Different pre-treatment methods were also presented with more focus on chemical pre-treatment, Free Nitrous Acid (FNA) pre-treatment was also discussed. The three major compounds (carbohydrates, proteins and lipids) involved in the fermentation process were discussed as well, with further detail on the hydrolysis and fermentation of each compound. Finally, the factors affecting VFAs and the importance of the production of VFAs were explored. Volatile fatty acids can be utilized for the generation of renewable energy and other valuable resources and the removal of biological nutrients in wastewater. The use of primary sludge for the fermentation process is vital because it contains a significant amount of readily biodegradable organic matter, which aids in an efficient and timely production of volatile fatty acids (Yuan, 2012, p. 40).

A study was conducted to investigate the effect of the FNA pre-treatment on a lab scale, by utilizing a semi continuous (fed batch) process. Four different concentrations of FNA were used: 0.35, 0.7, 1.4 and 2.8 mg N/L, along with the raw sludge and a pH controlled sample. The experiment was done for 24 hours at a constant pH of 5.5 and a temperature of 25$^{o}$C. The variation in the characteristics of the TWAS due to the FNA pre-treatment were analyzed and conversed. The following conclusions were made, based on the results of the experiment:

- It was observed that as the doses of FNA increased, the concentration of SCOD increased, with the highest values noticed at an FNA concentration of 0.7 mg N/L for both experiments.
- For Volatile Solids, a decreasing trend was observed as the doses of FNA increased, with the highest value being when the concentration of FNA was 2.8 mg N/L.
- For Carbohydrate and Protein solubilization, there seemed to be an increase in the concentration, as the concentration of the pre-treatment increased, although there also appeared to be a variation in the values, which cause the conclusion to be unclear.
- Following the nitrogen species experiments, it appeared that there was also an increase in concentration as the FNA doses increased, for TN, the highest value was identified at a FNA concentration of 0.35 mg N/L for 3 HRT and 0 for 2 HRT. For SN (Soluble Nitrogen),

the highest value was found at a FNA concentration of 0.7 mg N/L for 3 HRT and 0.35 mg N/L for 2 HRT.

- The results from alkalinity, ammonia and total phosphorus tests showed similar trends, where the increase in FNA concentrations yielded the increase in the concentration for all three of the experiments. The highest values and their corresponding FNA concentration for alkalinity was 1.4 mg N/L, 0.7 mg N/L for ammonia and 0.7 mg N/L.
- From the tests conducted above, it is safe to conclude that the concentration of FNA that yielded high concentrations in the study was 0.7 mg N/L.

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