

**Effect of Thermal Hydrolysis Pretreatment on Solubilization of Primary Sludge and
Thickened Waste Activated Sludge (TWAS) During Dark Fermentation Process**

By

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Abstract

Effect of Thermal Hydrolysis Pretreatment on Solubilization of Primary Sludge and Thickened Waste Activated Sludge (TWAS) During Dark Fermentation Process

Master of Engineering, 2019

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Civil Engineering

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The objective of this research was to evaluate the effect of thermal hydrolysis pre-treatment on the solubilization of primary sludge (PS) and thickened waste activated sludge (TWAS) through the semi-continuous fermentation process under the mesophilic conditions. For this measure, the inoculum (anaerobic digestate), Primary Sludge (PS) and Thickened waste activated Sludge (TWAS) was subjected to the pre-treatment condition. The pre-treatment temperature ranged from 20°C to 170°C. Then both raw and pre-treated sample was introduced to the semi-continuous reactors for the fermentation process. The degree of solubilization was achieved 18% for raw (unpretreated sample) and 38% for the pre-treated sample. Moreover, the volatile suspended solids (VSS) reduction rate for the raw and pre-treated sample was 24% and 50% respectively. Additionally, the soluble COD production yield for the raw and pre-treated sample was obtained 247 mg COD/g VSS and 544 mg COD/g VSS correspondingly.

Keywords: Fermentation process, Anaerobic digestion, Thermal hydrolysis pretreatment.

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Dedication

To my supportive parents, and my brother and best friend, Ardalan.

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1. Introduction

Each year approximately 150 billion kilograms of industrial and domestic waste is generated in the United States. Among these amounts of waste, it is estimated that almost 100 billion kilograms are biodegradable (Edward A Bayer, 2007).

The majority of Municipal solids waste (MSW) which is deposited in the landfills and subjected to anaerobic processes includes primarily of cellulose in a variety of forms such as newspaper, wood, and cardboard (Edward A Bayer, 2007). Figure 1 illustrates this procedure.

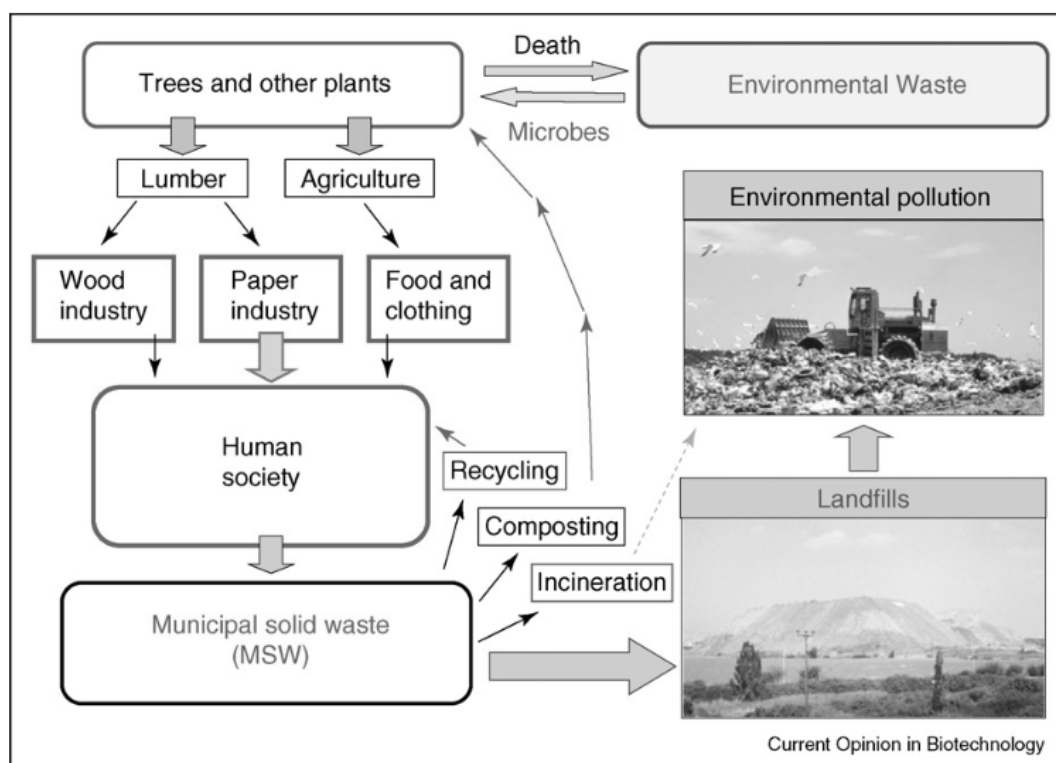


Figure 1 The fate of cellulose in the environment (Edward A Bayer, 2007)

In these anaerobic processes, a coalition of microorganism is responsible for the break down the polymeric substance. However, nowadays regarding the uncontrollable landfill environment, the population of these microorganisms has a drastic increase which results in ineffective anaerobic digestion in the landfills. On the other hand, generally landfills contaminate groundwater, and their capacity is filled rapidly. Therefore, there are two main alternatives to dispose of the MSW which is either leave the landfill waste in place or find a way to manage and recuse them as a new product

in the industry (Edward A Bayer, 2007).

Biomass is known as the domestic, sustainable and renewable energy sources which result in producing a variety of energy carriers such as methane and hydrogen. One of the low-cost technologies that generate such products through this procedure is called fermentation (Edward A Bayer, 2007).

In the following section, the process and different stages of anaerobic digestion are introduced.

2. Anaerobic digestion process

2.1. Overview

Series of biological process in which organic materials are braked down into biogas (mainly methane and carbon dioxide) and the combination of solid and liquid effluents (digestate), by various cultures of microorganisms in the absence of oxygen, is defined as anaerobic digestion (AD). Dairy manure, food processing waste, plant residues, municipal wastewater are the primary sources of organic matters (Bajpai, 2017), (Cleverson Vitorio Andreoli, 2007) (Lida Chen, 2014). Figure 2 represents the different stages of the AD and anaerobic pathways.

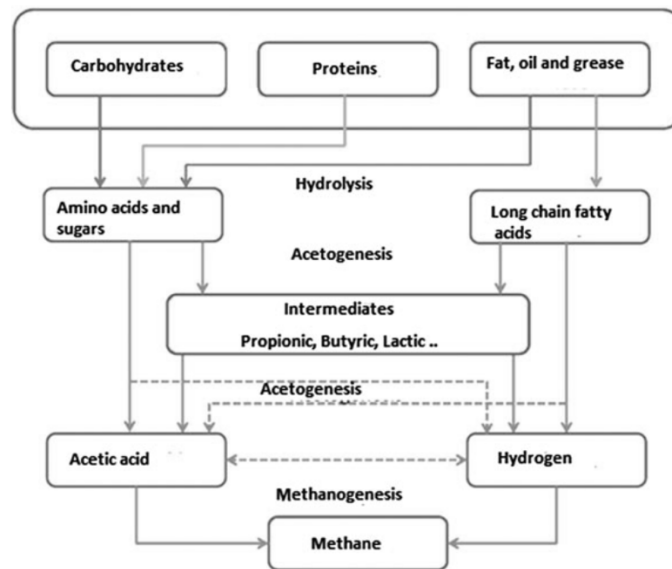


Figure 2 Anaerobic pathway (Bajpai, 2017)

2.2. Stages of AD

2.2.1. Hydrolysis

The first step of anaerobic digestion is called Hydrolysis in which insoluble organic materials and higher molecular mass compounds such as lipids and fats are transformed to soluble organic materials (for instance amino acids) to compounds suitable for the use as a source of energy and cell carbon.

Since in this stage large organic molecules are not in a suitable size to be used as the food source by microorganisms, specific microorganisms produce extracellular enzymes to break down these large molecules into the smaller piece. Therefore, proper energy sources are made, for microorganisms with cutting different types of organic material. There are also some specialized microorganisms, for instance, saccharolytic microorganisms that cut sugars and proteolytic microorganisms that break down proteins. Moreover, the decomposition rate during this stage can be considered as a function of the substrate's nature (Kayode Feyisetan Adekunle, 2015).

2.2.2. Acidogenesis

In this stage, different facultative anaerobic bacteria break down the monomers manufactured in the hydrolytic stage into short chain organic acids (Such as acetic acids and butyric acids), alcohols, hydrogen, and carbon dioxide. The concentration of produced hydrogen in this stage has a profound impact on the outcome of the fermentation process (Kayode Feyisetan Adekunle, 2015).

2.2.3. Acetogenesis

In the third phase, the outcome of the acidogenesis stage is consumed by other microorganisms. Moreover, in this stage, the products that are not able to be transformed into methane are converted to the methanogenic substrate, volatile fatty acids, and alcohols. Further Volatile fatty acid is oxidized to the methanogenic substrate (such as acetate hydrogen and carbon dioxide) and volatile fatty acids with longer than one-unit carbon chain is oxidized to acetate and hydrogen (Kayode Feyisetan Adekunle, 2015).

2.2.4. Methanogenesis

This phase is the slowest biochemical reaction of the process. This feature makes the methanogenesis stage a critical step in the anaerobic digestion process. In this stage, methanogenic microorganisms produce carbon dioxide and methane from primary products under exact anaerobic circumstances (Kayode Feyisetan Adekunle, 2015).

3. Fermentation Process

Fermentation generally refers to the transformation of sugar into an organic acid or alcohol. Since ancient times the fermentation process was intentionally used by humankind to enhance conservation and organoleptic properties of food. However, in the industrial scale fermentation processes use microorganisms to convert solid or liquid substrates into various useful products (Leona Paulová, 2013).

Moreover, modern industrial fermentation processes are described differently. In these processes, bioreactors are usually used. The bioreactors are classified regarding to many features such as types of feeding of the bioreactor (batch, fed-batch, and continuous mode), immobilization of biocatalyst (free or immobilized cells), the characteristic state of matter in the system (submerged or solid substrate fermentation), single strain/mixed culture processes, mixing of the bioreactors (mechanical pneumatic, and hydraulic agitation), or the availability of oxygen (aerobic and anaerobic processes) (Leona Paulová, 2013).

Depend on the advantages and disadvantages of each setup the type of implemented bioreactor or fermentation process is chosen (Leona Paulová, 2013). The factors should be considered in this process are examining the properties and availability of raw materials, necessary investment and operating cost, sustainability, availability of workforce as well as desired productivity and return of the investment (Inui, 2010).

4. Types of the fermentation process

4.1. Submerged Cultivation

Submerged cultivation of microbial cells in bioreactors provides a controlled environment for the efficient production of high-quality end products and to reach optimum productivity. Industrial bioreactors run in batch, fed-batch, or continuous mode are used to culture different types of microorganisms producing a variety of products (Leona Paulová, 2013). These different approaches to submerged cultivation will be discussed in the following sections.

4.1.1. Batch cultivation

In batch cultivation process, the medium, nutrients, and inoculum are introduced to a closed system bioreactor. At the beginning of the cultivation the conditions usually are aseptic (Figure 5-a), and the volume of the medium in the bioreactor remains constant theoretically; however, there may be small differences in the culture volume practically because of a feeding rate of acid solution to maintain the pH at an acceptable level and taking a sample or injecting gas in to the culture. However, compared to the total volume of the bioreactor, these volume changes are too small; therefore, the working culture volume of the bioreactor can be considered constant. (Leona Paulová, 2013)

Typically, before introducing usable cells in the bioreactor, it should be filled with the sterilized medium of nutrients. After injecting cells which is the first step of batch cultivation, the cells follow the four-phased procedure described by (Mond, 1949).

- a) **Lag phase:** In this phase, although the cells are active metabolically, there is no increase in the biomass concentration, using nutrition substrate and manufacturing products, since the cells are adapting to their new environment. The concentration of cells in the inoculum, the composition of inoculation and cultivation media and the size of inoculum are the main factors that affect the length of the lag phase.
- b) **Exponential (Log) phase:** This phase is known by the rapid reproduction of cells. The concentration of biomass in this phase in the exponential function of time. Moreover, the specific growth rate remains constant since there are no growth limitation circumstances. Because of the proliferation, consuming of the carbon and basically, energy sources are

fast in log phase.

- c) **Stationary phase:** By the end of the log phase, the specific growth rate reduces progressively due to the decrease in nutrients. This measure leads the whole procedure to the next phase which is called the stationary phase. In this phase, the growth rate states steady, and the rest of the remaining carbon and energy sources are consumed. This is a significant phase for the fusion of secondary metabolites.
- d) **Death Phase:** In this phase since most of the energy sources are consumed cells are dying in an exponential function of time. The transition time between the sanitary phase and death phase depends on the microorganism and how fast the nutrients are utilized. The fermentation process is usually ended after the log phase or before the death phase (Brian Pumphrey, 1996).

These four phases are represented in Figure 3.

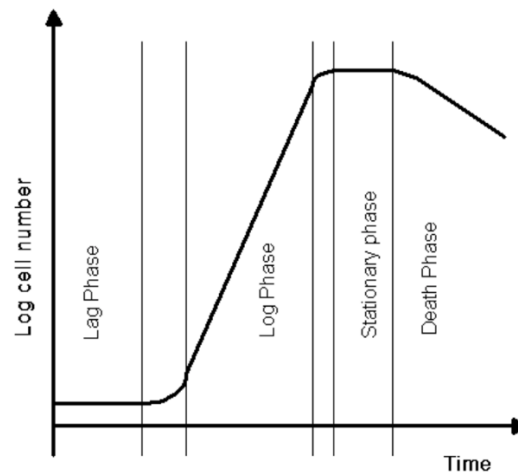


Figure 3 Growth curve of Bacterial culture (Brian Pumphrey, 1996)

4.1.2. Fed batch cultivation

In fed-batch cultivation, process nutrients are added to a semi-open system bioreactor gradually and aseptically while the product is retained inside the reactor (figure5-b). Therefore, unlike the volume of the culture in the batch reactor, the volume of the culture in a fed batch reactor increase during this period (Leona Paulová, 2013).

There are some Features in the fed-batch cultures which makes them work more efficient than the

batch cultures such as the possibility to prolong products synthesis, the ability to reach higher cell densities and thus increase the quantity of the products, the capacity to enhance yield or productivity by controlled sequential addition of nutrients and the feature of prolonged productive cultivation over the unprofitable periods when the bioreactor would typically be prepared for the new batch (Leona Paulová, 2013).

Selecting a proper method to feed the bioreactor can improve the performance of the culture. There are two typical feeding strategies which are discontinues feeding and continues feeding of nutrients. The former method is achieved by regular or irregular pulses of the substrate, and the latter method can be designed regarding the pre-calculated profile represents in Figure 4.

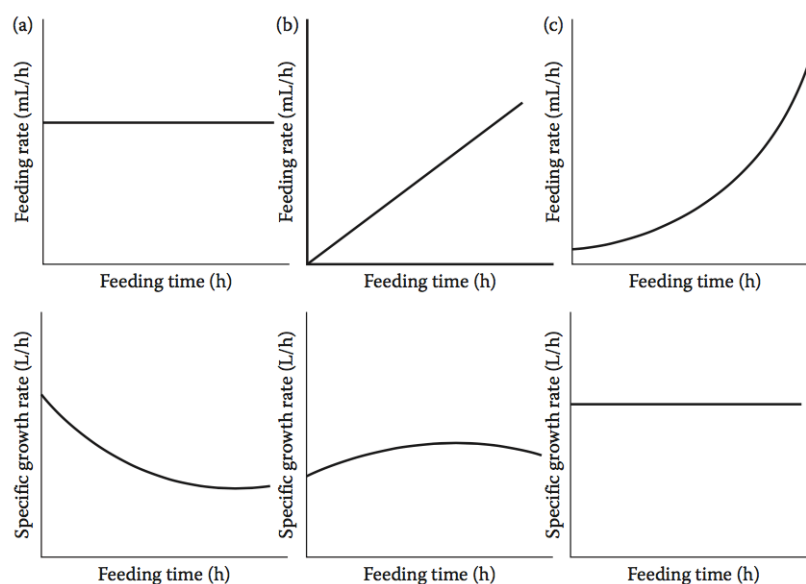


Figure 4 Relationship between the pre-calculated profile of substrate and specific growth rates of culture. (a) constant feeding rate, (b) linearly increasing feeding rate, and (c) exponential feeding rate. (Leona Paulová, 2013)

4.1.3. Continuous cultivation

In continuous cultivation process nutrients inject an open system bioreactor continuously and aseptically and simultaneously the cells and metabolites removed; therefore, the volume of the culture broth remains constant (Figure 5-c). The specific growth rate of cells in continues cultures are steady and are therefore this system are controlled by the availability of the limiting nutrients. The growth rate of cells in this system is equal to the dilution rate (Leona Paulová, 2013).

The main factors which distinguish continuous cultures from fed batch cultures mode are the possibility to set up optimum circumstances for a long-term and maximum product synthesis, the ability to achieve stable product quality and a distinct reduction in the unprofitable period of bioreactor operation (Leona Paulová, 2013). However, continues operation may not be suitable on a large-scale project because of some problems such as increasing risk of contamination due to the pumping of the medium in and out of the bioreactor, the danger of genetic mutations in the production strain in a long-term operation and additional investment may be required for technical facilities (Leona Paulová, 2013).

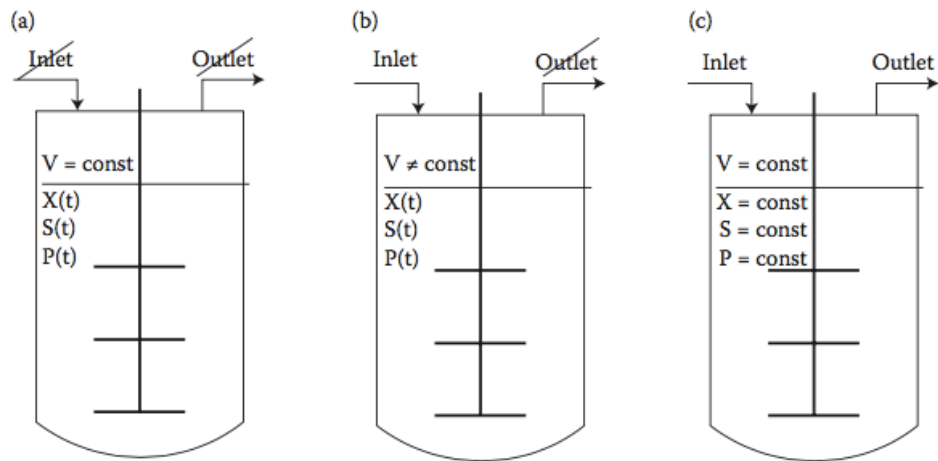


Figure 5 simplified scheme of (a)Batch, (b)Fed batch and (c)continuous cultivation (Leona Paulová, 2013)

4.2. Solid Substrate Fermentation

Solid substrate fermentation refers to a system that includes a low level of water and concentrated water-insoluble substrate that microorganisms are cultured on it. This system mostly was used for the production of traditional food in eastern countries. Nowadays in western countries, it mostly has industrial usage (Leona Paulová, 2013).

4.2.1. Features of Solid Substrate Fermentation

The main characteristics of solid substrate fermentation are mainly different from submerging cultivation since the media of this system contains very low-level water (Udo Holker, 2005). Table 1 illustrates these features.

Table 1 Main distinctions between solid substrate fermentation and submerged cultivation
(Leona Paulová, 2013)

Solid substrate Fermentation	Submerge Cultivation
Low water Content of the cultivation medium (40-80%)	Liquid cultivation medium (-95% water content)
Three-phase system: gas-liquid-solid	Two-phase system: gas-liquid
Complex substrate insoluble in water, high local concentration of nutrients	Nutrients are dissolved in water, concentration of nutrients are lower
Nonhomogeneous system, gradient of nutrients	Homogeneous system
Microorganisms are grown on the surface of the solid substrate	Microorganisms are grown in the Liquid medium
Gas-liquid and liquid-solid oxygen transfer	Gas-liquid oxygen transfer
Limitation in heat, oxygen and nutrient transfer	Transport processes are usually not limited (exception can be oxygen transfer)
Heat is removed by using a stream of air or by placing bioreactor into the temperature-controlled chamber.	Cooling is achieved by bioreactor jacket cooling system
Process monitoring, and control are difficult	Online monitoring and control of the process are common
High concentration of the products	Products are dissolved in the liquid phase

The advantages of this system over classical submerged technology are usage of a concentrated medium, resulting in a smaller reactor volume and lower capital investment costs, the lower risk of contamination with yeasts and bacteria due to low moisture levels and substrate complexity, the higher product yield and easier product recovery and the use of agricultural waste as substrate for specific applications (Ali, 2011) (Barrios-González, 2012).

5. Thermal hydrolysis pretreatment of Sludge

5.1. Introduction

Thermal hydrolysis is a technology that includes the application of heat at above autoclave temperature for a specific period of time for sludge pre-treatment before it uses for the anaerobic digestion process. (Ødeby, 1996)

In this technology live steam injection is used as the heat generator; the injection usually occurred at design temperature and related pressure which is quickly discharged momentarily. (Pereboom, 2014)

Initially, Thermal hydrolysis technology was used to enhance sludge dewaterability; however recently the utilization of the technology is focused on the improvement of the sludge biodegradability. (Wilson, 2009)

Generally, thermal hydrolysis is more efficient for materials containing high concentrations of proteins and carbohydrates, while it has less influence on lipids. This measure clearly explains that thermal hydrolysis is more suited to activated sludge rather than primary sludge (Barber, 2016). Regardless of the sludge, carbohydrates, proteins and lipids respond the same way to thermal hydrolysis and the differences in the efficiency of thermal hydrolysis in various sludges is because of their composition (Wilson, 2009). On the other hand, since the concentration of lipids is higher in primary sludge than activated sludge, much higher levels of VFAs is produced during the thermal hydrolysis treatment to degrade the products of unsaturated lipids (Wilson, 2009).

Increasing the temperature of the sewage sludge includes sequence reactions, which is explained in Figure 6.

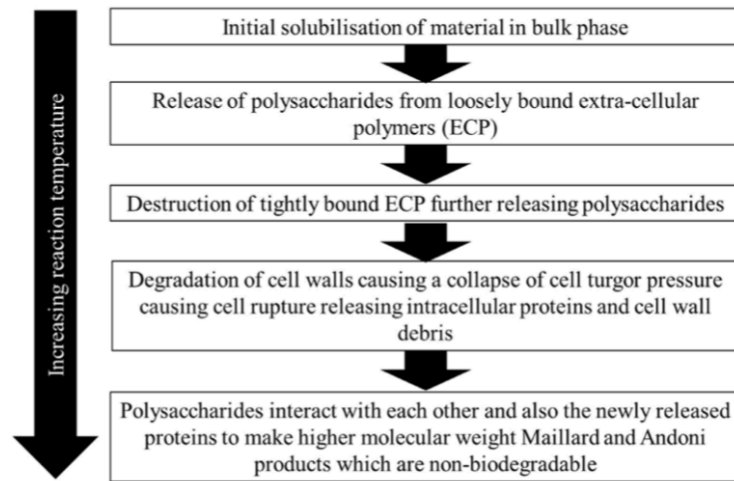


Figure 6 Sequence of reactions due to the increasing temperature of sludge. (Barber, 2016)

If the temperature of the thermal hydrolysis reaction increases up to optimum temperature range, downstream sludge anaerobic digestibility will be improved, apparent viscosity and the average particle size will be reduced, solubility of carbohydrate and proteins and potential for refractory compound formation (COD, Nitrogen, colour) will be increased and it has a negligible impact on the solubility of lipids (Barber, 2016).

Moreover, if the temperature increases beyond the optimal temperature, downstream sludge anaerobic digestibility and viscosity will be reduced, production of refractory material and dewaterability will be enhanced (Barber, 2016).

5.2. Influence of thermal hydrolysis on sludge rheology

Since rheology change due to thermal hydrolysis tolerates higher digester loading rate and aids in dewatering, it can be considered as the most critical consequence of thermal hydrolysis technology on sludge treatment. (Stickland, 2015). Because sludge behaves as non-Newtonian shear thinning thixotropic fluid, the temperature can have a fundamental impact on the sludge. (Eshtiaghi, 2013).

The viscosity will be reduced in many ways as the temperature increases during thermal hydrolysis treatment. First of all, according to the Arrhenius' law in reversible fractions, the viscosity of free water within the fraction of sludge will be decreased (Eshtiaghi, 2013). Secondly, in a partly reversible way, the material within the sludge will be destroyed thermally (the denaturation of proteins or destruction of extracellular polymers) (Farno, 2015). Finally, rheology will be affected

because of the increase of interactions between compounds due to the increase of the temperature (Forster, 1983).

On the other hand, since due to the steam explosion the particle size is reduced, increasing in the viscosity is expected. However, according to the presented rheology data from thermal hydrolysis, the factor mentioned above outweighs the enhancement of viscosity (Pevere, 2006), (Lotito, 2014). To put it in a nutshell, the factors affecting the sludge rheology can be summarized as sludge type, density, solids content, particle size and distribution, settleability, abrasiveness, particle friability, surface charge, liquid phase conductivity, pH and surface chemistry (Forster, 1983).

Moreover, dewaterability of mesophilically digested sludge will be enhanced almost 10 percentages points by thermal hydrolysis. This improvement depends on the composition of sludge and dewaterability test (Barber, 2016).

5.3. Role of ammonia in thermal hydrolysis

The upper pH limits in anaerobic digestion are monitored by unionized ammonia. In addition, methane synthetizing enzymes can be inhibited directly by charged ammonia (Chen J. O., 2014).

During the thermal hydrolysis along with increases in loading rate due to altered rheology, the solubility of proteins and proteins breaking down enhancement, the rate of ammonia and alkalinity is increasing, which lead to pH rise as well. Consequently, the pH of the mesophilic digestion plant is normally from 7.5 to 8 if it is headed by thermal hydrolysis (Barber, 2016).

5.4. Other impacts

As a result of thermal hydrolysis, the production rate of refractory organics can be higher (Barker, 1999). In addition, regarding hydrolysis, it is possible to accelerate the rate of gas production during the anaerobic digestion process which means that the retention time increases (Barber, 2016).

Moreover, thermal hydrolysis can also affect odours and growth of marker organisms, particle size, co-digestion and foam and scum control during the digestion process (Barber, 2016).

6. The Volatile fatty acid production process

6.1. Introduction

VFA are short-chain fatty acids consisting of six or fewer carbon atoms which can be distilled at atmospheric pressure (Wee Shen Lee, 2014). The applications of VFA are varied from the production of bioplastics, bioenergy, and the biological removal of nutrients from wastewater (Mengmeng, 2009) (Uyar, 2009) (Chang, 2011) (Zheng, 2010). Nowadays although mostly the profitable production of VFA is accomplished by chemical routes, the interest in biological routes of VFA production is raised due to increasing of the price of nonrenewable petrochemicals such as oil which is used as the raw materials (Huang, 2002) (Akaraonye, 2010). The main source of carbon in the biological production of VFA is pure sugars such as glucose or sucrose (Zigová, 1999) (Kondo, 1996). However, this measure raise concerns about using food to produce chemicals. Regarding this fact, instead of using food organic-rich waste such as sludge, food waste, organic fraction of municipal solid waste and industrial wastewater are utilized to produce VFA. The transformation of waste to VFA also is an alternative route to decrease and manage the number of wastes (Wee Shen Lee, 2014).

VFA production from the waste in an anaerobic process which includes hydrolysis and acidogenesis or fermentation (or dark fermentation) (Bengtsson S. H., 2008) (Su, Improving hydrogen production from cassava starch by combination of dark and photo fermentation. , 2009).

Figure 7 illustrates these procedures.

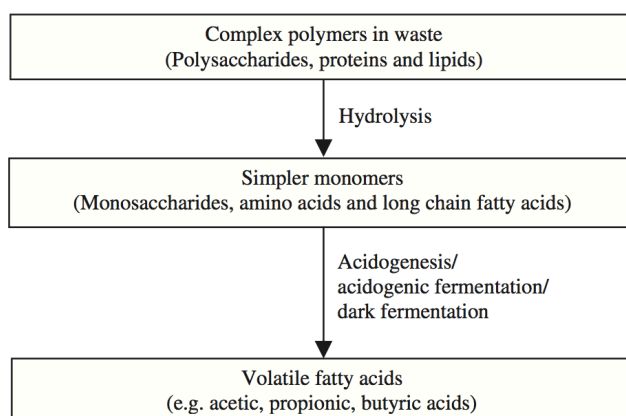


Figure 7 Volatile Fatty Acid production (Wee Shen Lee, 2014)

In hydrolysis, the hydrolytic microorganisms break down the complex organic polymer into simpler organic monomers. In the next stage, these monomers are fermented into VFA such as acetic, propionic and butyric acids are produced by acidogens. Both processes involve a complex consortium of obligate and facultative anaerobes, such as Bacteriocides, Clostridia, Bifidobacteria, Streptococci, and Enterobacteriaceae(Weiland, 2010).

6.2. Anaerobic techniques for VFA production

Attached growth and suspended growth are the two technologies which are usually used for anaerobic VFA production. The types of reactor developing for VFA production depends on the chosen technology. As it can be seen in Figure 8-a packed bed reactors are utilized for attached growth technology. In such reactors, the biomass grows and attach on porous packing material such as alumina-based ceramic cubes and granular activated carbon (Wee Shen Lee, 2014) (Beccari, 2009) (Bertin, 2004).

However, in these technologies, waste including high concentrations of suspended solids may clog the packed bed reactor. Regarding this problem fluidized bed reactor has been created in which the biomass grows will be attached to a small solid medium such as sand that remains in suspension by upward flowing motion of the fluid (Figure 8-b) (Grady Jr, 2011).

Conversely, biomass can grow freely in suspended growth technology. The up-flow anaerobic sludge blanket reactor (UASB) (Figure 8-c) and the continues stirred tank reactor (Figure 8-d) are the reactors developed based on suspended growth technology (Wee Shen Lee, 2014) (Eddy. M, 1991).

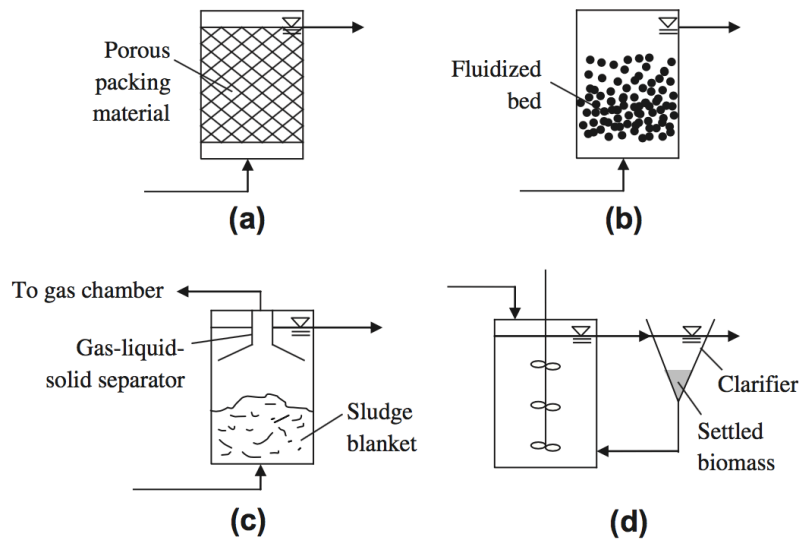


Figure 8 Types of reactor used for anaerobic VFA production (a)packed bed reactor, (b)fluidized bed reactor, (c)up flow anaerobic sludge blanket reactor and (d) continuous stirred tank reactor with recycling of biomass. (Wee Shen Lee, 2014)

6.3. Factors influencing the VFA production

Many factors are affecting the concentration, yield, and concentration of VFA production such as operational pH, temperature, retention time, organic loading rate. These factors will be explained in this section.

6.3.1. pH

Since the acidogens cannot survive in an acid or alkaline environment, the pH value plays a vital role in the VFA production,(Liu H. W., 2012). Typically for VFA production, the optimal pH range is from 5.25 to 11. Table 2 illustrates the specific range of pH for different kinds of waste (Wee Shen Lee, 2014).

For instance, the pH value is from 8 to 11 if the sludge is used. The alkaline condition enhances the hydrolysis of sludge through ionization of the charged groups of the extracellular polymeric substance in the sludge which are mainly carbohydrate and protein (Wingender, 1999). Moreover, the optimal pH value for the hydrolysis and acidogenesis of kitchen waste was considered 7. This value provides the higher solubilizing percentage of carbohydrate, protein, and lipid which results in the better concentration of VFA in comparison with pH value of 5, 9, and 11 (Zhang B. Z., 2005). Conversely, if the VFA is extracted from wastewater, an acidic environment is more

suitable, and pH value should be in the scope of 5.25 to 6.0 (Oktem, 2006) (Bengtsson S. H., 2008). Therefore, for the food waste and wastewater the VFA production should be under acidic circumstances while, for sludge, it is better to be under alkaline condition (Wee Shen Lee, 2014).

Table 2 Optimal pH for VFA production (Wee Shen Lee, 2014)

Type of wastes	pH range studied	Optimal pH (range)	Reactor type and operating conditions	VFA production performance
Primary sludge	3–11	10	Batch reactor, room temp., 5 d	60 mg COD/g VSS/d
Waste activated sludge	4–11	9	Batch reactor, 35 °C, 5 d	298 mg COD/g VSS
		8	Batch reactor, 55 °C, 9 d	368 mg COD/g VSS
	8–12	11	Batch reactor, 25 °C, 4 d	1558 mg COD/L
	8–11	11	Batch reactor, 60 °C, 7 d, 0.02 g SDBS ^a /g VSS	259 mg TOC/g VSS
Kitchen waste	5–11	7	Batch reactor, 35 °C, 4 d	36,000 mg/L
Cheese whey	3.5–6	5.25–5.5	Continuous stirred-tank reactor, 37 °C, HRT 2 d	0.83 ^b
Paper mill effluent	4.9–6	5.5–6	Continuous stirred-tank reactor, 37 °C, HRT 2 d	0.76 ^b
Pharmaceutical wastewater	5–6.3	5.5	Continuous-flow completely mixed reactor, 35 °C, HRT 0.5 d, OLR 13 g COD/L/d	44% ^c

^a Sodium dodecylbenzene sulfonate.

^b (VFA-COD/wastewater influent soluble COD).

^c (mg VFA-COD in the effluent/mg COD in the influent) × 100%.

6.3.2. Temperature

VFA can be produced from waste under different temperature ranges which are psychrophilic (4–20°C), mesophilic (20–50°C), thermophilic (50–60°C), and hyper-thermophilic (60–80°C) conditions (Wee Shen Lee, 2014). VFA produced, VFA production rate and VFA yield will be increased as the temperature increases within the psychrophilic and mesophilic temperature (Maharaj, 2001) (Bouzas, 2002) (Yuan, 2011) (Zhang P. C., 2009). For example, the VFA produced from WAS increases by 300% if the temperature rises from 10 to 35 °C (Zhang P. C., 2009). This measure is because of the presence of a more significant amount of soluble carbohydrate and protein due to the improved sludge hydrolysis at a higher temperature. Likewise, during the fermentation of PS, VFA production increases almost six-fold when the temperature increases between 8 and 25°C (Maharaj, 2001) (Zhang P. C., 2009) (Bouzas, 2002).

VFA production will be still enhanced if the temperature increases from mesophilic condition to thermophilic region and hyper-thermophilic region. In thermophilic temperature, VFA yield is higher than mesophilic temperature due to faster biological acclimatization and more active abiogenesis. Moreover, the production of VFA has a better performance in hyper-thermophilic temperature (Wee Shen Lee, 2014). However, in a study (Yu J. Z., 2013) founded that VFA production was not affected at the thermophilic temperature of 45–70 °C. While according to (Guihua Zhuo, 2012) at the thermophilic temperature the acid forming enzymes activities were

lower than that at mesophilic temperature. These differences may be caused by different microbial species in the studies.

6.3.3. Retention time

The retention time of waste (hydraulic retention time) and microbial cultures (solid retention time) in the anaerobic reactor are two of the main factors for VFA production in the acidogenic fermentation of waste (Wee Shen Lee, 2014).

a. Hydraulic retention time (HRT)

By applying HRT, microorganisms will get the chance to have more time to react with the waste (Ben, 2011) (Bengtsson S. H., 2008) (Sans, 1995). For instance, VFA production from OFMSW improves in the range of 2-6 days with HRT (Sans, 1995). However, extending HRT is not always useful for example by increasing the HRT from 4 to 12 hours the VFA production from dairy wastewater became almost double while if the extension of HRT increases from 16 to 24 hours there will be of 6% improvement in the VFA production (Lim, 2008) (Fang, 2000).

b. Solid retention time (SRT)

The HRS and SRT will both the same in case of using sludge to produce VFA since the waste substrate and mixed culture will be in the same phase. Although SRT should be long enough to promote hydrolysis in sludge, it should not be very long since the growth rate of methanogens is lower than acidogens and lower SRT can avoid the influence of methanogens in the anaerobic reactor (Ferrer, 2010). For instance, in the acidogenic fermentation of WAS when the SRT increases from 4 days to 12 days VFA concentration improve 44% while, more if SRT prolongs more and reach to 16 days the concentration of VFA become lower (Feng, 2009).

6.3.4. Organic loading rate

The amount of waste which can be considered in terms of COD, VS and VSS that is fed to the reactor daily per unit reactor volume is called organic loading rate (OLR). Although the influence of OLR on the production of VFA might seem inconsistent, in some cases by increasing this measure the VFA concentration increases (Wee Shen Lee, 2014). The linear increase of VFA concentration produced from starchy wastewater with OLR ranging between 1g COD/L/d to 32 g

COD/L/d is an example of this issue (Yu J. , 2001).

6.3.5. Additive

In the past few years in order to improve the VFA production from sludge, some additives such as surfactants and enzymes are used. Table 3 represents the effect of such additives on VFA production (Wee Shen Lee, 2014).

Table 3 Effect of adding surfactants and enzymes on VFA production (Wee Shen Lee, 2014)

Additive	Waste	T (°C)	Duration (day)	Additive dosage	Maximum VFA concentration (mg COD/L)
SDBS	Waste activated sludge	21	6	Nil	339
				0.02 g/g dry sludge	2599
	Waste activated sludge + primary sludge	21	6	Nil	118 ^a
SDS	Waste activated sludge	21	6	0.02 g/g TSS	174 ^a
				Nil	191
				0.05 g/g dry sludge	1001
				0.1 g/g dry sludge	1143
α -Amylase + neuter protease	Waste activated sludge	50	5	0.06 g/g dry sludge (amylase:protease = 1:3)	1281
SDS + α -amylase + neuter protease	Waste activated sludge	50	7	SDS = 0.1 g/g dry sludge Enzyme = 0.06 g/g dry sludge (amylase:protease = 1:3)	1457

^a mg COD/g VSS.

6.4. Application of VFA

There are plenty of applications for VFA produced from the acidogenic waste fermentation such as the production of biodegradable plastics, generation of bioenergy and biological nutrient removal (Wee Shen Lee, 2014). In this section, the application of VFA will be discussed.

6.4.1. Poly-Hydroxy-Alkanoates

One of the environmentally friendly biodegradable polymers that can be generated by microorganisms is Poly-Hydroxy-Alkanoates (PHA). PHA has various applications in the industry; however, since in its production well defined expensive carbon substrate is used (almost 31% of the total production cost), the high production cost limits its replacement over petrochemical-based plastic (Philip, 2007) (Choi, 1997). Therefore, using VFA which is a low-cost substrate is a promising option to reduce the cost of PHA production (Wee Shen Lee, 2014). As excessive nutrients would favour the growth of microorganisms and decrease the conservation

of VFA to PHA, regulate the ammonium and phosphorus contents of fermented waste that is rich in VFA is vital before using it in PHA production (Albuquerque M. G., 2007). Moreover, limited nitrogen and phosphorus circumstance increase PHA content and yield (Bengtsson S. W., 2008).

Higher content of PHA can be achieved by using pure microbial culture (Table 4). However, this measure needs a sterile condition which led to spending more energy, equipment, and financial investment. Conversely, if pure microbial culture replaces by mixed culture, there is no need for sterilization; therefore, the cost is reduced (Wee Shen Lee, 2014). In order to enhance the PHA content achieved by mixed culture, the conditions of the cultivation reactor should be optimized by feeding the suitable VFA type and fine-tuning the PHA production circumstances. Therefore, by applying such strategies PHA content in the range of 40 – 77% can be obtained. In addition, it is better for mixed culture to be fed with fermented food waste, fermented activated sludge and fermented paper mill effluent (Jiang Y. M., 2012) (Chua, 2003) (Albuquerque M. G., 2010) (Jiang Y. H., 2011) (Mohan, 2013) (Jiang Y. C., 2009) (Reddy, 2012).

Table 4 PHA production by pure and mixed microbial cultures from VFA (Wee Shen Lee, 2014)

Source of VFA	Pure culture	PHA content ^a (wt%)
Fermented starchy wastewater	<i>Alcaligenes eutrophus</i>	34
Fermented palm oil mill effluent	<i>Ralstonia eutropha</i>	>90
	<i>Comamonas</i> sp. EB 172	86
	Mixed culture	
Fermented palm oil mill effluent	Activated sludge	40
Fermented paper mill wastewater	Activated sludge	48
	Activated sludge	77
Fermented wood mill effluent	Activated sludge	29
Fermented sugar cane molasses	Activated sludge	75
	Activated sludge enriched with glycogen-accumulating organisms	37
Fermented waste activated sludge	Activated sludge	57
	Activated sludge	73
Fermented food waste	Activated sludge	40
Fermented food waste and sewage sludge	Activated sludge	48

^a (PHA/cell dry weight or VSS or TSS) × 100%.

6.4.2. Bioenergy

As the price of fossil fuels increases, it is necessary to find another alternative to generate energy. In this regard wasted derived VFA is a low-cost energy source to produce different energy forms such as electricity, biogas, hydrogen, and biodiesel.

a. Electricity

As it is shown in Figure 9 microbial fuel cell (MFC) is a bio-electrochemical system which

includes an anaerobic anodic chamber and the aerobic cathodic chamber separated by proton exchange membrane that uses microorganisms to turn the biochemical energy to an electricity source (Du, 2007).

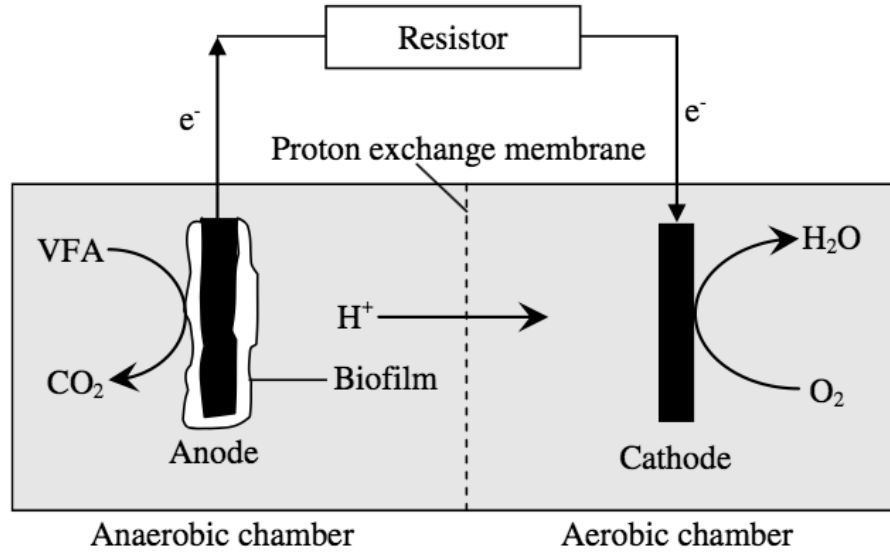
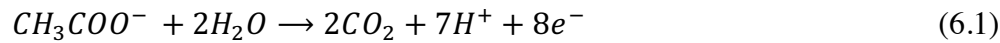


Figure 9 Electricity generation from VFA through the microbial fuel cell (Wee Shen Lee, 2014)

At the anode microorganisms (which are on a formed biofilm) oxidizing VFA to produce electrons, protons and carbon dioxide. The protons pass through the proton exchange membrane to enter the cathodic chamber whereas the electrons flow to the cathode through an external circuit. By combining electrons with protons and oxygen, water is produced which complete the electrical circuit. Reactions 6.1 and 6.2 represent the anodic and cathodic reactions respectively. In these reactions, acetate is used as an organic substrate(Wee Shen Lee, 2014) (Du, 2007).



Two compartment MFC, single compartment MFC the up-flow mode MFC and the stacked MFC are different kinds of MFCs used for electricity generation. Usually, in such procedures, fermented rich VFA waste utilizes without treatment (Chang, 2011) (Mohanakrishna, 2010) (Nam, 2010).

There is different electricity generation performance based on the type of VFA used in MFC (Table

5). For instance, VFA manufactured from acetate fed MFC has the coulombic efficiency (CE) of 93% and it is almost two times higher than molecular weight VFA (Wee Shen Lee, 2014) (Freguia, 2010).

Table 5 Performance of electricity generation from VFA via microbial fuel cell (Wee Shen Lee, 2014)

Performance of electricity generation from VFA via microbial fuel cell.

Volatile fatty acids	Electricity generation performance			Electrodes	
	Current (mA)	Power density (mW/m ²)	Coulombic efficiency (%)	Anode	Cathode
Acetate	28	–	93	Granular graphite	Granular graphite
Propionate	13	–	55		
n-Butyrate	16	–	49		
i-Butyrate	5	–	67		
n-Valerate	12	–	33		
i-Valerate	13	–	34		
Hexanoate	12	–	44		
Mixture of acetate, propionate, butyrate and valerate	21	49 ^a	–		
Acetate	–	64	72	Carbon felt	Perforated titanium plate coated with platinum
Propionate	–	58	36		
Butyrate	–	51	43		
Mixture of acetate, propionate and butyrate	–	1.32 ^a	38		
Fermented food waste	–	15	–	Carbon felt	Carbon paper coated with platinum
Leachate of fermented municipal solid waste	–	5.9 ^a	6	Carbon cloth	Carbon cloth coated with platinum

^a W/m³.

b. Biogas

Biogas is one of the VFA products which is produced from single anaerobic digester under anaerobic condition. Because of its high methane content biogas is mainly used for heat or power generation. The procedure of generating biogas is called one phase anaerobic digestion. Within this process, it is challenging to provide an optimal condition for microorganisms that responsible for acidogenesis and methanogenesis; therefore, the suboptimal performance of the one phase anaerobic digestion is considered (Wee Shen Lee, 2014) (Lv, 2010).

Because of the challenging optimal conditions in one phase anaerobic digestion, two-phase anaerobic digestion is proposed to resolve this problem. In two-phase, anaerobic digestion there are two digesters in which each of them contains one group of microorganisms. The first digester works under the condition of acidic pH and short SRT to cultivate fast-growing acidogenes and the second digester operates under natural pH and higher SRT to cultivate slow-growing methogen (Grady Jr, 2011). The two-phase anaerobic can work at higher OLR in addition better biogas is achieved from two-phase anaerobic digestion than that from one

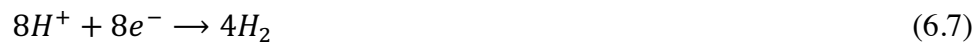
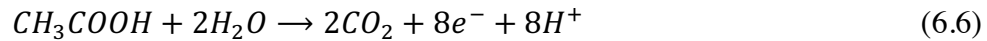
phase anaerobic digestion (Demirer, 2005). Also, two anaerobic digesters can produce methane (from the first digester) and hydrogen (from the second digester) simultaneously (Cavinato, 2011).

c. Hydrogen

Waste derived VFA can produce hydrogen through photo fermentation, electro hydrolysis or micro electrolysis cell (Wee Shen Lee, 2014). In photo fermentation VFA transformed to hydrogen in the presence of light by purple non-sulfur bacteria (Levin, 2004). Since dark fermentation produce hydrogen and VFA, the photo fermentation frequently combines with dark fermentation to improve the overall hydrogen production (Su, Improving hydrogen production from cassava starch by combination of dark and photo fermentation. , 2009) (Chen C. Y., 2008) (Su, Combination of dark-and photo-fermentation to enhance hydrogen production and energy conversion efficiency. , 2009). Another approach to producing hydrogen is electro hydrolysis of VFA. In this procedure, electrons are released from a metal electrode regarding the direct current voltage. Meanwhile, protons are generated from electro hydrolysis of VFA and combine with electrons to produce hydrogen. Electro hydrolysis of VFA can be combined with the anaerobic reactor to produce in situ hydrogen (Tuna, 2009) (Wee Shen Lee, 2014). Equation (6.3) to (6.5) present this reaction:



The microbial electrolysis cell is another electrochemical option. In this procedure, hydrogen is produced through the cathodic reduction of a proton released from the micro-oxidation of VFA at the anode (Liu H. G., 2005) (Wee Shen Lee, 2014). Following equations illustrate this reaction.



d. Lipids for biodiesel

Biodiesel which can be produced from lipids through transesterification process is typically a methyl ester of long chain fatty acid (Wee Shen Lee, 2014) (Wee Shen Lee, 2014). Most of the resources that edible lipid usually obtained are from rapeseed oil, palm oil, and soybeans (Gui, 2008). An alternative for such resources to produce lipids is waste derived VFA (Fei Q. C., 2011). Moreover, both soybean and jatropha oil has the same fatty acid composition as microbial lipid synthesized from VFA(Fei Q. C., 2011).

6.4.3. Biological nutrient removal

One of the important carbon substrates that can contribute to removing nitrogen and phosphorus is VFA. Since the carbon substrate in wastewater is not efficient enough, there must be such additional carbon substrates to stabilize the biological nutrient removal process (BNR). The range of carbon to nitrogen for combined nitrification/denitrification should be between 5 and 10 mg/COD/mg/N (Henze, 1991). This range for 1 mg phosphorus is 7.5 to 10.7 mg/COD/mg/N (Grady Jr, 2011). Lower molecular weight VFA are preferred for nitrogen removal by denitrifying bacteria (Elefsiniotis, 2007). The first VFA which is consumed during this process is acetate followed by propionate and butyrate, and valerate, while, increasing propionic acid content in domestic wastewater is more efficient for long-term phosphorus removal (Elefsiniotis, 2007) (Chen Y. R., 2004) (Wee Shen Lee, 2014).

7. Materials and Methods

This section illustrates the materials and methods which were employed for volatile fatty acid production using semi-continuous fermentation process.

7.1. Substrate

In this research, the inoculum (anaerobic digestate), Primary Sludge (PS) and Thickened waste activated Sludge (TWAS), was taken from the effluent of anaerobic digester located in Ashbridge Bay municipal wastewater treatment plant as the main sewage facility of Toronto which has the working capacity of 818,000 m³/d. To analyze the main factors of the influents, 6 samples were from raw and pre-treated influents were taken and analyzed. The main characteristics of the inoculum are summarized in Table 6.

Table 6 The main Features of the inoculum (Raw and Pre-treated)

Type of influent/ Parameters	Raw	CV _{Raw}	Pre-treated	CV _{Pre-Treated}
pH	5.5 ± 0.2	3.6%	5.5 ± 0.2	3.6%
TS (mg/L)	33038 ± 2049	6.2%	32027 ± 1445	4.5%
VS (mg/L)	25245 ± 1055	4.2%	25929 ± 898	3.5%
TSS (mg/L)	32004 ± 1807	5.6%	21137 ± 1869	8.8%
VSS (mg/L)	25241 ± 1734	6.9%	14852 ± 950	6.4%
TCOD (mg/L)	41989 ± 2087	5%	40522 ± 3686	9.1%
SCOD (mg/L)	2669 ± 1732	65%	14623 ± 325	2.2
Alkalinity (mg/L CaCO ₃)	793 ± 241	30%	659 ± 239	36%
VFA (mg/L as acetic acid)	1476 ± 766	52%	2391 ± 745	31%
Total Carbohydrate (mg/L glucose)	4758 ± 438	9.2%	5363 ± 2575	48%
Soluble Carbohydrate (mg/L glucose)	100 ± 42	42%	1725 ± 448	26%
Ammonia (mg/L NH ₃ -N)	124 ± 86	69%	214 ± 77	36%

7.2. Hydrothermal treatment process

The hydrothermal process which follows the ramp/soak pattern was used to treat the raw sludge at 170°C. As it is shown in Figure 10, regarding this pattern during four steps the temperature of the feedstock were increased from 20 °C to 170 °C. However, a cooling system was provided and enclosed to the hydro-thermal to reduce the temperature of the treated sludge to the room temperature. This significant decrease in temperature is shown in figure11. The thermal set up is shown in figure12.

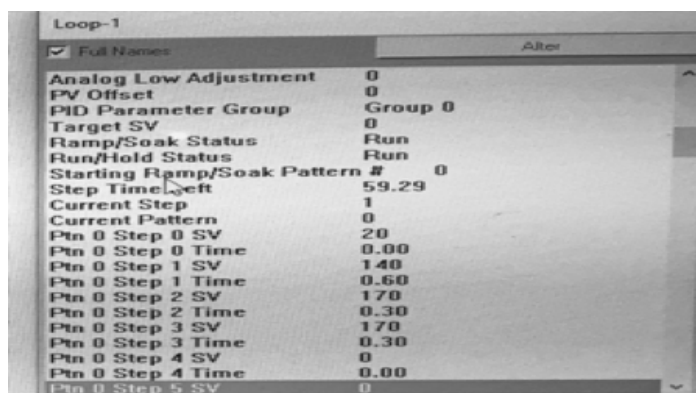


Figure 10 Temperature pattern

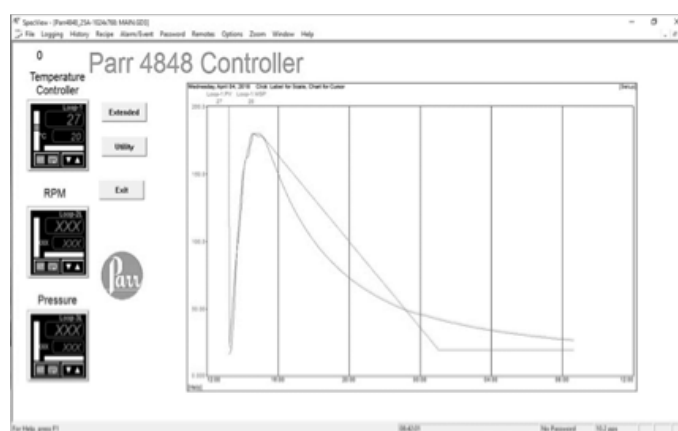


Figure 11 Temperature graph

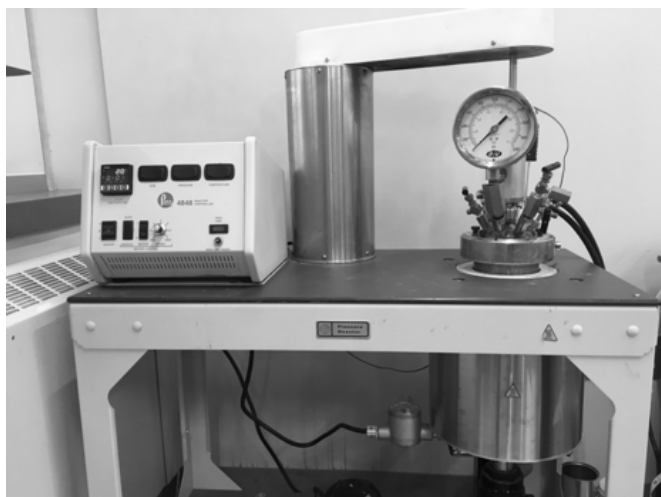


Figure 12 Par 4848 pressure reactor

7.3. Experimental procedure

In order to study the fermentation process under the anaerobic conditions for each feedstock (raw and pre-treated) a reactor with a volume of 1.5 liter was provided (Figure 13). Moreover, to maintain the homogeneity, each reactor was armed with reactor stirrer with a speed of 150 round per minute. These two reactors were placed in a water bathtub and kept in the water with a temperature of 38 °C to satisfy the mesophilic condition. Also, a gas bag was attached to each reactor which monitored the methane production.

To maintain the three days retention time, every day 500 ml of the influents were injected to the reactors through a valve which was provided at the bottom of the reactor; meanwhile, 500ml of the effluents was extracted through another valve provided at the top of the reactor. To satisfy the anaerobic condition, the headspace of the reactors was connected to the nitrogen gasbags. These gasbags also simplified the feeding process by preventing suction effects.

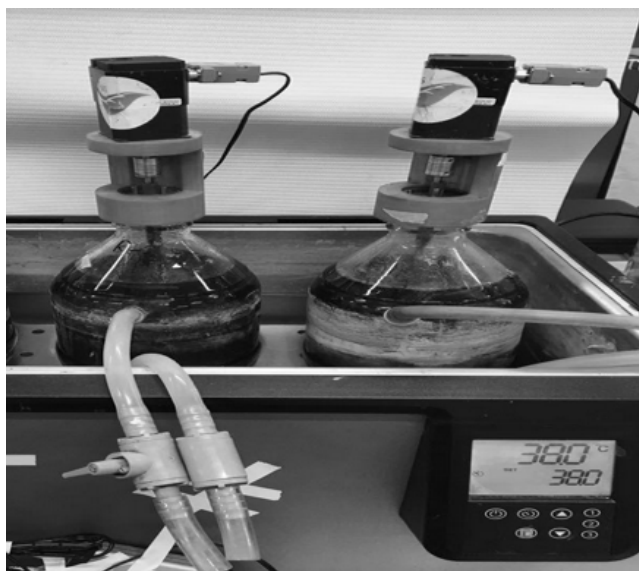


Figure 13 reactors of the fermentation process for raw (left) and pretreated (right) sludge

7.4. Analysis of the main characteristics of influents and effluents

In this experiment the following characteristics of the feedstocks and effluents were analyzed:

1. pH
2. Total and volatile solid content (TS and VS)
3. Total and volatile suspended solid content (TSS and VSS)

4. Total and Soluble chemical oxygen demand (TCOD and SCOD)
5. Alkalinity
6. Volatile Fatty Acid (VFA)
7. Total and Soluble carbohydrate
8. Ammonia

In order to make the final results more accurate, these features were analyzed triplicates.

7.4.1. pH

Since the influents (raw and pre-treated sludge) were kept in the fridge, before testing the pH of influents, first they were put in water to reach the room temperature to be prepared for feeding process. Moreover, the pH of the effluents was measured instantly after the sampling process. To test the pH, the VWR Benchtop pH meter (Model AB15) was used (figure14) which was calibrated with pH reference standard every time before testing.

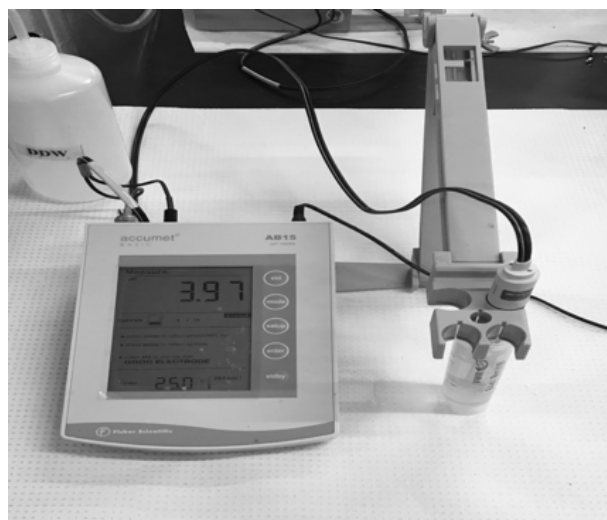


Figure 14 pH meter

7.4.2. Total and Volatile solid content

For these analyses, the standard guidelines of 2540B and 2540E were followed. In order to measure the TS and VS, the aluminum dishes were prepared and weighted. 5ml of each sample were put in each dish and weighted. The samples were dried for 90 minutes at 105°C in the oven and weighted. Regarding the equation 7.1, the percentage of the TS content were measured.

$$\%TS = \left[\frac{(\text{dish+dried sample})_{\text{mg}} - (\text{dish+sample})_{\text{mg}}}{(\text{dish+sample})_{\text{mg}} - (\text{dish})_{\text{mg}}} \right] * 100 \quad (7.1)$$

To test the VS content, the dried samples were put in the furnace at 105°C for 30 minutes and weighted. Using equation 7.2 the percentage of the VS content were measured.

$$\%VS = \left[\frac{(\text{dish+ignited sample})_{\text{mg}} - (\text{dish+dried sample})_{\text{mg}}}{(\text{dish+dried sample})_{\text{mg}} - (\text{dish+sample})_{\text{mg}}} \right] * 100 \quad (7.2)$$

7.4.3. Total and Volatile Suspended Solid Content

For these analyses, the standard guidelines of 2540D and 2540E were followed. To measure TSS and VSS a 1.5 μ m glass microfiber paper filter was put in the aluminum dishes and weighted together. 2ml of each sample was filtered and dried for 90 minutes at 105°C in the oven and weighted. Regarding the equation 7.3, the weight of the TSS content was measured.

$$\text{TSS} = (\text{dish} + \text{dried filtered sample})_{\text{mg}} - (\text{dish} + \text{filter})_{\text{mg}} \quad (7.3)$$

To test the VSS content, the dried sample was put in the furnace at 105°C for 30 minutes and weighted. Using equation 7.4 the weight of the VS content was measured.

$$\text{TSS} = (\text{dish} + \text{ignited filtered sample})_{\text{mg}} - (\text{dish} + \text{dried filtered sample})_{\text{mg}} \quad (7.4)$$

7.4.4. Total and Soluble chemical demand

In order to follow the method 8000, high-range (20-1500 mg/l) COD reagent vials were utilized for TCOD and SCOD analysis.

To test the total COD of the samples, each sample (raw and pre-treated) were diluted in DDW regarding proper dilution factor. For the analysis, 2ml of each diluted sample was added to vials.

However, to test the soluble COD of the samples, first, the samples were centrifuged for 30 minutes at the speed of 9800 round per minute. Afterwards, the centrifuged samples were filtered using 0.45 μ filters. The filtered samples were diluted in DDW with suitable dilution factor. Finally, 2ml of the diluted samples were added to the vials along with 2ml of DDW as a blank to calibrate the measurements.

Both TCOD and SCOD vials were shaken strongly and put in the COD reactors for 120 minutes at 150°C to be digested. The TCOD and SCOD of the samples were measured operating HACH

DR 3900 spectrophotometer. In order to achieve the total and soluble COD of the samples (raw and pre-treated), the measured total and soluble COD of the diluted samples was multiplied by their respected dilution factor.

Figure 15 and 16 represent the centrifuge machine and spectrophotometer used in this analysis.



Figure 15 centrifuge machine



Figure 16 HACH DR 3900 spectrophotometer

7.4.5. Alkalinity test

In order to follow the method 10239, high-range (25-400 mg/l CaCO_3) total alkalinity reagent set were utilized for Alkalinity analysis.

To measure how alkaline samples are, the filtered sample using for SCOD test were used and diluted in DDW with appropriate dilution factors. The diluted samples again were added to the vials along with 2ml of DDW as a blank to calibrate the measurements, and read using HACH DR

3900 spectrophotometer. To achieve the alkaline portion of the samples (raw and pre-treated), the measured alkalinity of the diluted samples was multiplied by their respected dilution factor.

7.4.6. Volatile Fatty Acid test

To follow the standard method 10240, high-range (50-2500 mg/l) esterification method TNT 872 reagent set were utilized for Volatile Fatty Acid analysis.

To measure the concentration of the volatile fatty acid of the samples, the filtered samples using for SCOD test were used and diluted in DDW with suitable dilution factors. The diluted samples were added to the vials along with 2ml of DDW as a blank to calibrate the measurements and measured using HACH DR 3900 spectrophotometer. To achieve the concentration of the volatile fatty acid of the samples (raw and pre-treated), the measured VFA of the diluted samples was multiplied by their respected dilution factor.

7.4.7. Total and Soluble Carbohydrate test

The procedures of this test were the same as the analysis of TCOD and SCOD. However, the data was calibrated regarding a glucose standard calibration curve at the concentration of between 5 mg/L and 1000 mg/L. Moreover, phenol 5% weight by volume, diluted sulfuric acid and standard glucose were employed as the reagent. Finally, deionized water was used as a blank. The protocol for this analysis was the phenol-sulfuric method.

7.4.8. Ammonia test

In order to follow the method 10031, high-range (0.4-50 mg/l CaCO_3) Amver Ammonia reagent set were utilized to analyze the concentration of the ammonia for the samples.

To measure the concentration of the ammonia, the filtered samples using for SCOD test were used and diluted in DDW with appropriate dilution factors. The diluted samples again were added to the vials along with 2ml of DDW as a blank to calibrate the measurements, and read using HACH DR 3900 spectrophotometer. To achieve the ammonia concentration of the samples (raw and pre-treated), the measured ammonia concentration of the diluted samples was multiplied by their respected dilution factor.

At the end of this section Table 7, represents the frequency of feeding/Sampling operation and analyzing the influents and effluents for each specific test.

Table 7 frequency of the feeding, sampling and analysis

Substrate	Influents	effluent
Feeding/Sampling	Every day (including weekends)	Every day (including weekends)
pH	Every day (including weekends)	Every day (including weekends)
TS/VS	Once a week	Twice a week
TSS/VSS	Once a week	Twice a week
TCOD/SCOD	Once a week	Twice a week
Alkalinity	Once a week	Twice a week
VFA	Once a week	Twice a week
Total/Soluble Carbohydrate	Once a week	Twice a week
Ammonia	Once a week	Twice a week

8. Results and Discussion

In this section, the output of the abovementioned analysis for each sample (raw and pre-treated) will be discussed. Moreover, these outputs for both influents and effluents will be compared. In this research, the number of analyzed samples taken from each type of effluent (Raw and Pre-treated) was 8 samples.

8.1. Total and Volatile solids

Figure 17 compares the average concentration of Total and Volatile solid (TS and VS) in raw and pre-treated influents and effluents. As it can be seen the raw influent contained the highest TS concentration (33038 mg/L); however, since the pre-treated influent was more soluble due to the hydrothermal treatment process, it contained less TS concentration than the raw influent which was 32027 mg/L. On the other hand, due to the digestion process, the TS concentration decreased from 33038 mg/L in raw influent to 29199 mg/L in raw effluent and from 32027 mg/L in pre-treated influent to 24820 mg/L in pre-treated effluent.

Moreover, as it can be seen in Figure 17, the behaviour of VS concentration was almost the same as the TS concentration. VS concentration reduced from 25245 mg/L in raw influent to 22323 mg/L in raw effluent, and from 25929 mg/L in pre-treated influent to 18618 mg/L in pre-treated effluent.

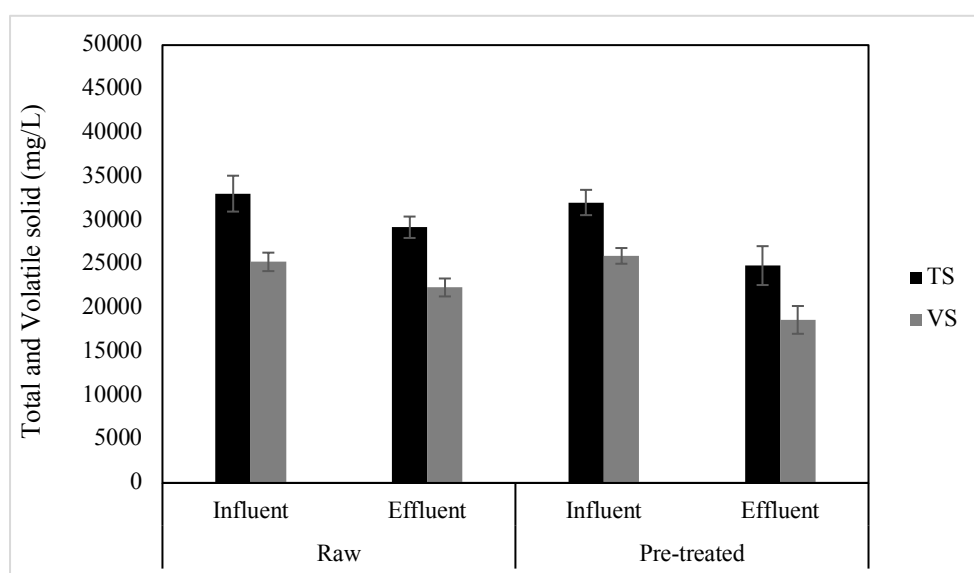


Figure 17 Total and Volatile solid concentration(mg/L)

During the fermentation process, the reduction rate of TS and VS concentration in the raw sample was almost 12%. Conversely, the reduction rate of TS and VS concentration in the pre-treated sample was about 23% and 28% respectively. Regarding this measure, it can be understood that the organic matter braked down more efficiently in the pre-treated sample since it was more soluble due to the hydrothermal pre-treatment.

Furthermore, based on Figure 17 the reduction rate in TS and VS for the combination of hydrothermal pretreated process and fermentation were 25% and 26% respectively.

8.2. Total and Volatile suspended solids

As it can be seen in Figure 18 the concentration of Total suspended solids (TSS) decreased from 32004 mg/L in raw influent to 25517 mg/L in raw effluent, and from 21137 mg/L in pre-treated influent to 18614 mg/L in pre-treated effluent. Moreover, the concentration of Volatile suspended solids (VSS) reduced from 25241 mg/L in raw influent to 19193 mg/L in raw effluent, and from 14852 mg/L in pre-treated influent to 12495 mg/L in pre-treated effluent.

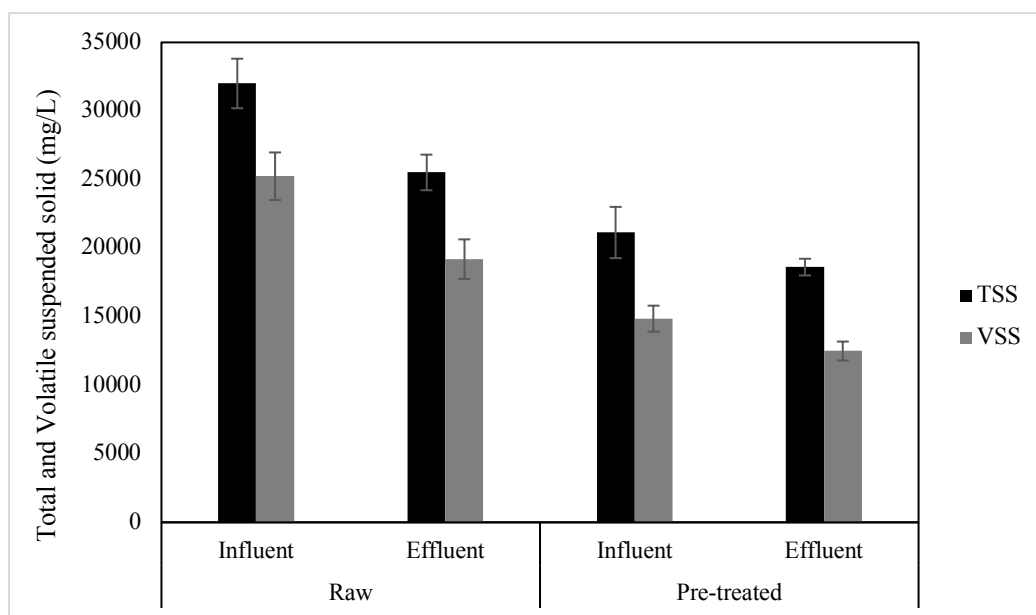


Figure 18 Total and Volatile suspended solid concentration

As it is illustrated in Figure 18, the average concentration of TSS and VSS in raw and pre-treatment influents were more than that in the raw and pre-treated effluents. The reason for this measure was the degradation of organic matters due to the digestion process.

The reduction rate of TSS and VSS concentration in the raw sample was approximately 20% and 24% respectively. However, the reduction rate of TSS and VSS concentration in the pre-treated sample was about 34% and 41% correspondingly. Therefore, it can be understood that the degradation process of the organic materials was more efficient for the pre-treated sample since it was more soluble due to the hydrothermal pre-treatment.

8.3. Suspended solid reduction

As it was discussed, due to the degradation of the organic matter, the concentration of the TSS and VSS will be reduced. Figure 19 illustrates the proportion of these reductions regarding the fermentation process, hydrothermal hydrolysis pre-treatment and the combination of the pretreatment process and the fermentation.

As it can be seen in Figure 19 during the fermentation process, hydrothermal hydrolysis pre-treatment and the sequential hydrothermal hydrolysis pre-treatment and fermentation, the concentration of TSS reduced by 24%, 34% and 42% respectively. However, the VSS concentration decreased by 24%, 41% and 50% correspondingly.

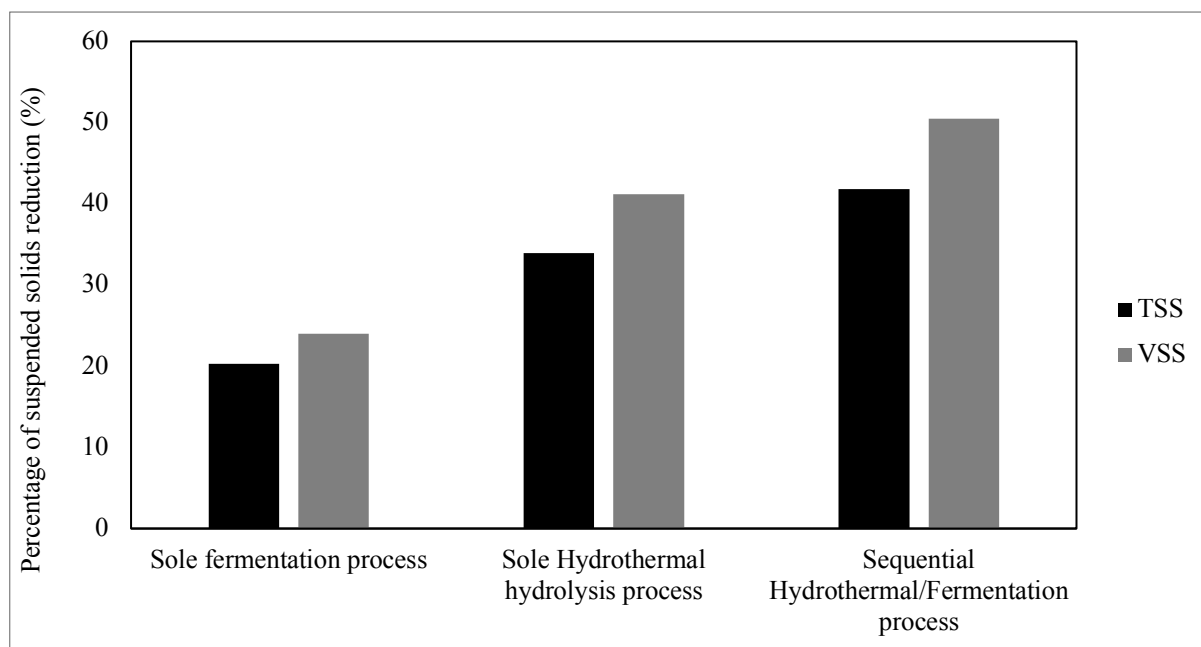


Figure 19 Percentage of suspended solids reduction

Therefore, based on the presented data for VSS reduction, the biodegradation of the organic materials was more efficient when the sample was subjected to pretreatment and then fermented.

This measure is because of the enchantment of solubility due to the hydrothermal hydrolysis pre-treatment before the fermentation.

8.4. Total and Soluble COD

As can be seen in Figure 20, there was almost no change in the value of the total COD (TCOD) in influents and effluents. It can be observed that TCOD values for raw influent and effluent were 41989 mg/L and 41875 mg/L respectively. Also, these values for pre-treated influent and effluent were 40522 mg/L and 42639 mg/L correspondingly. These constant trends demonstrated that in the digestion process only the particular COD converted to soluble COD (SCOD).

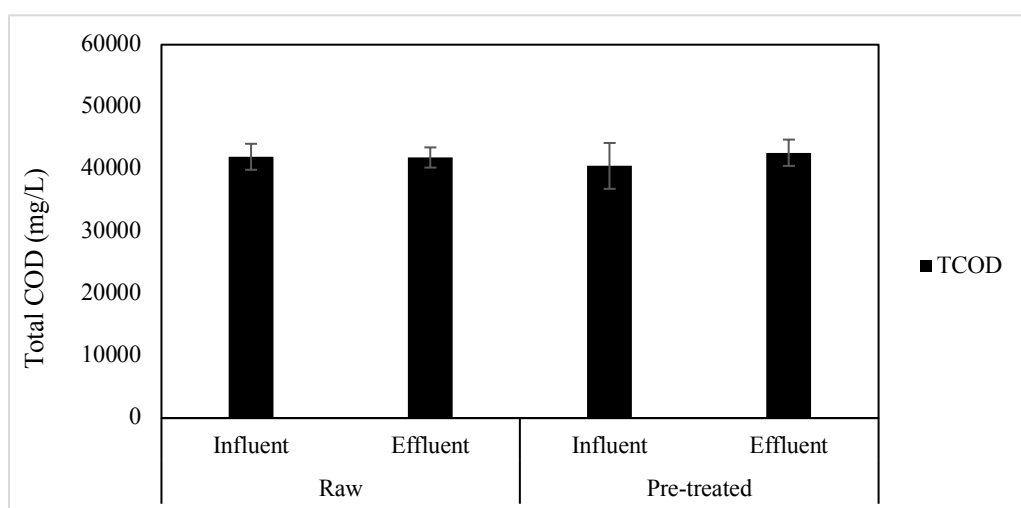


Figure 20 Total chemical oxygen demand

Since hydrothermal process makes the sample more soluble, as shown in Figure 21 there was a significant difference between the SCOD value in raw influent and the SCOD value in pre-treated influent.

Moreover, as mentioned above during the digestion process particle COD is converted to SCOD; therefore, the SCOD value increased from 2669 mg/L in raw influent to 9902 mg/L in raw effluent. Also, the SCOD value increased from 14623 mg/L in pre-treated influent to 16404 mg/L in pre-treated effluent.

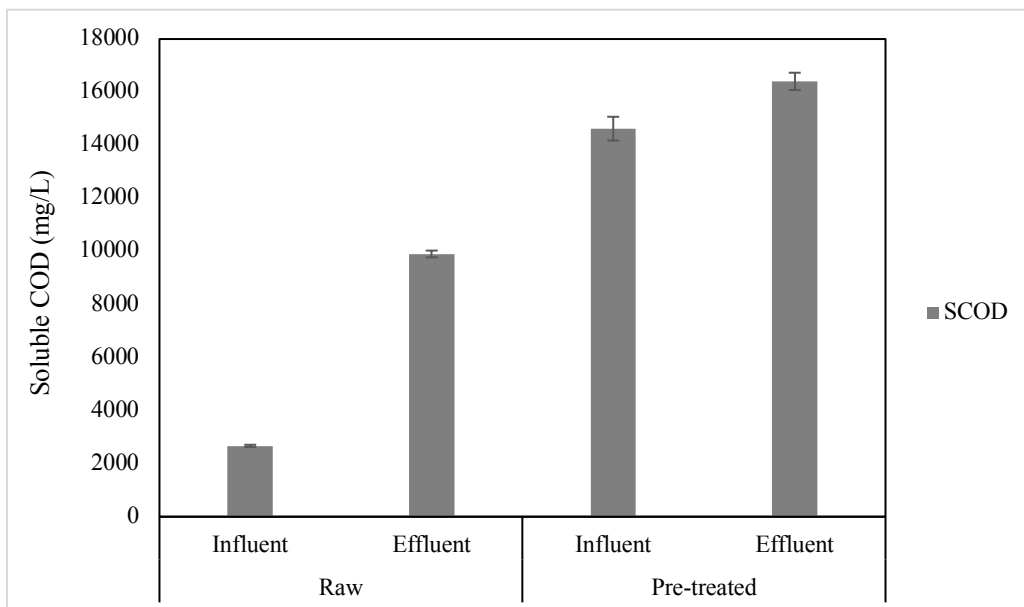


Figure 21 soluble chemical oxygen demand

Based on the presented data in section 8.2 for VSS in the raw and pretreated sample, the COD production yield based on the fermentation process, for the raw sample was 287 mg COD/g VSS and for the pre-treated sample was 112 mg COD/g VSS. However, the COD yield for the combination of hydrothermal pre-treatment and fermentation was 544 mg COD/g VSS. This measure indicates that the overall efficiency of the fermentation process will be increased if the sample first was subjected to pretreatment and then fermented.

8.5. Degree of solubilization

The degree of COD solubilization of each process specifies the ratio of the SCOD produced during the process by the particle COD of the influent substrate.

As shown in Figure 22, the degree of solubilization during the fermentation process, hydrothermal hydrolysis process and the sequential hydrothermal hydrolysis pre-treatment and fermentation, were 18%, 30% and 35% respectively.

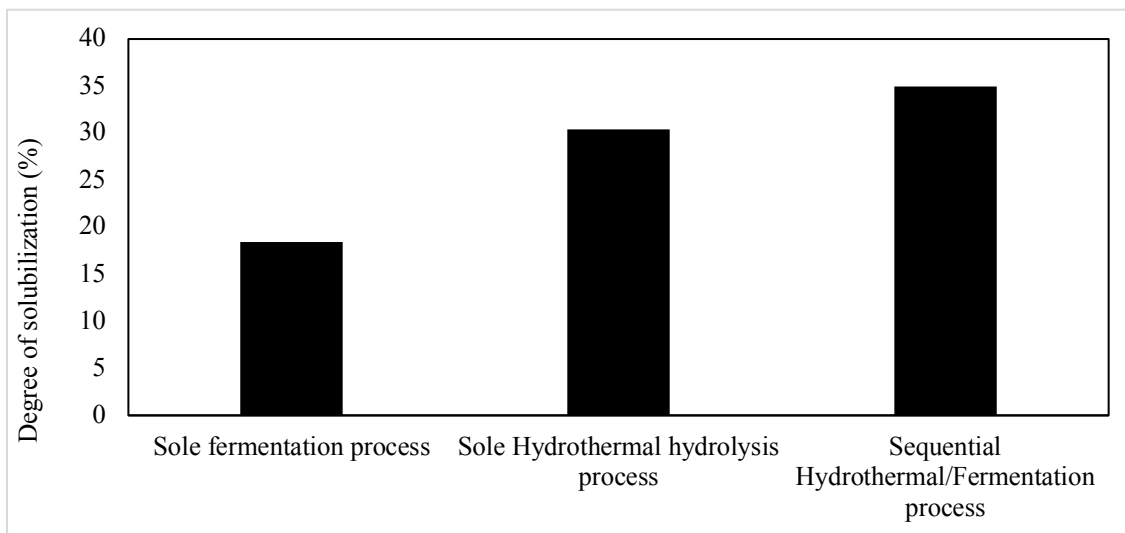


Figure 22 Degree of solubilization

Regarding these ratios, it can be understood that the conversion of the particle COD to SCOD was more efficient when the sample was subjected to pretreatment and then fermented. This measure also supports the reason that the VS and VSS reduction rate in the pre-treated sample was more than that in the raw sample (as it was discussed in section 8.2 and 8.3).

8.6. Alkalinity

Alkalinity is another indicator of biological activities. Increasing in the Alkalinity values shows more biological activity. Since biological activities increased during the digestion process to breakdown the organic matter of substrates, effluents became more alkaline than effluents. As it can be seen in Figure 23 the alkalinity value in raw and pre-treated substrate almost followed the same trend.

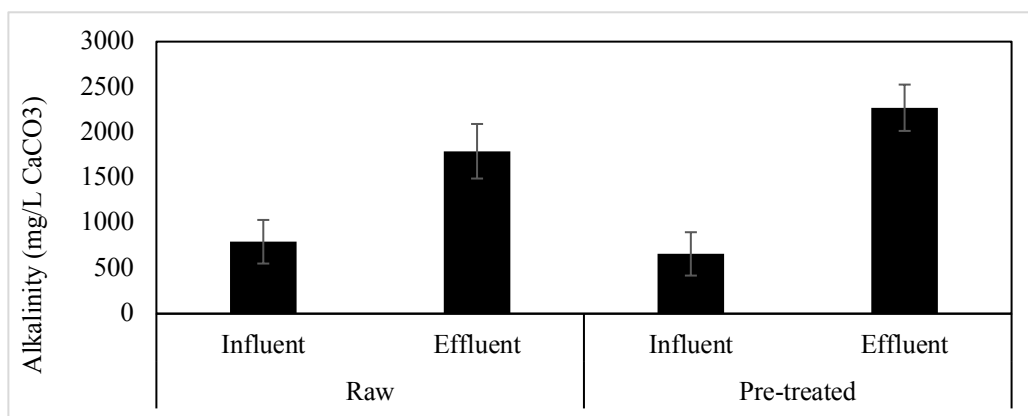


Figure 23 Alkalinity

The Alkalinity value increased from 793 mg/L CaCO_3 in raw influent to 1791 mg/L CaCO_3 in raw effluent. Moreover, the Alkalinity value increased from 659 mg/L CaCO_3 in pre-treated influent to 2270 mg/L CaCO_3 in pre-treated effluent. Since the pre-treated sample was more soluble than the raw sample, the biological activities during its fermentation were more than that in the fermentation of the raw sample. Consequently, the pre-treated effluent was more alkaline than the raw effluent.

8.7. Volatile Fatty Acid (VFA)

VFA is one of the main products of the fermentation process. VFA production is due to the biological activities of acidogen bacteria. As it can be seen in Figure 24, since the biological activities were more in the pre-treated sample rather than the raw sample, it had the highest VFA concentration which was 5659 mg/L as acetic acid.

As it is illustrated in Figure 24, the VFA concentration increased from 1476 mg/L as acetic acid in raw influent to 4563 mg/L as acetic acid in raw effluent. Moreover, the VFA value increased from 2391 mg/L as acetic acid in pre-treated influent to 5659 mg/L as acetic acid in pre-treated effluent.

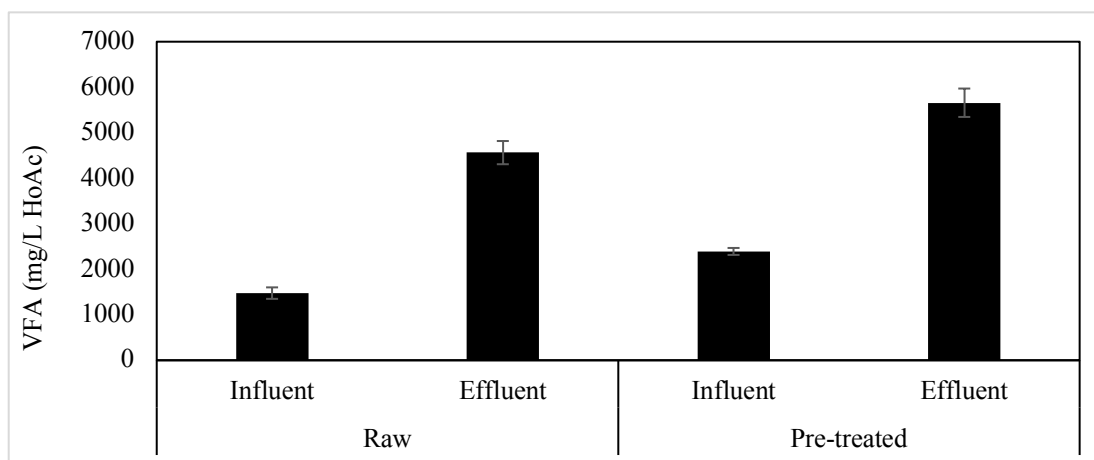


Figure 24 Volatile Fatty Acid

The VFA production during the fermentation process was 3087 mg/L as acetic acid for raw the sample and 3267 mg/L as acetic acid for the pre-treated sample. Moreover, the VFA production yield for the raw and the pre-treated sample was 122 mg VFA / g VSS and 220 mg VFA / g VSS

respectively. The VFA yield was more in the fermentation of raw sample than the VFA yield in the fermentation of pre-treated sample, which showed during the fermentation process the conversion rate of the organic matter into VFA in the pre-treated sample was more than the conversion rate of the organic matter into VFA in the raw sample.

8.8. Total and Soluble Carbohydrates

The reduction in the concentration of total carbohydrates is due to the volatile acid production during the fermentation. Figure 25 shows the concentration of total carbohydrates in raw and pre-treated influents and effluents.

Initially, the average values of total carbohydrate were about 4758 mg/L glucose and 5363 mg/L glucose in raw and pre-treated influents respectively. However, during the fermentation process, these values reduced to 3620 mg/L glucose and 3133 mg/L glucose in raw and pre-treatment effluents respectively.

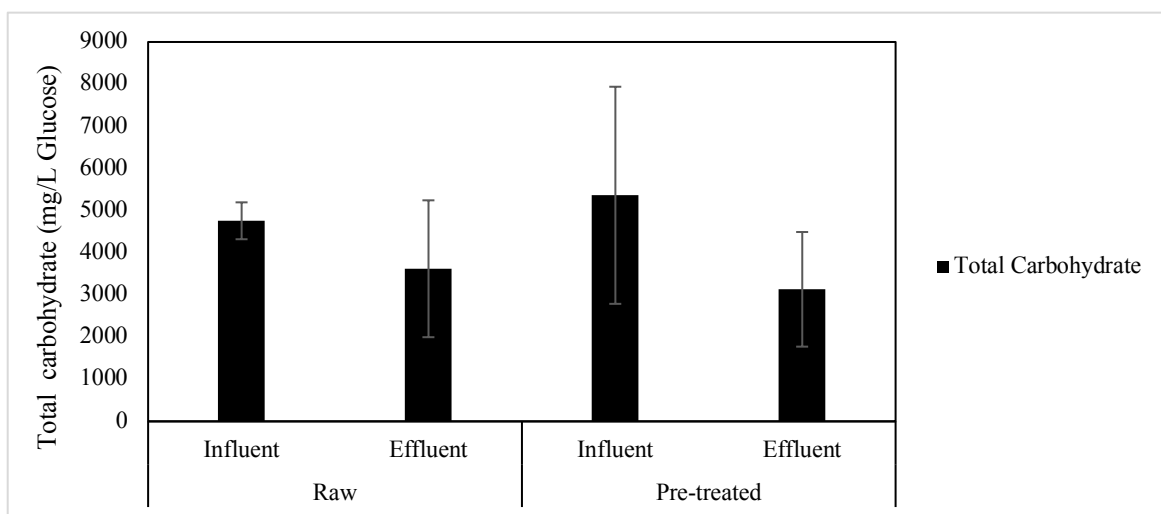


Figure 25 Total Carbohydrate

As it is shown in figure 26, the concentration of soluble carbohydrate in the pre-treated influent was significantly more than that in the raw influent. This measure was because of the hydrolysis pre-treatment of the substrate which results in the more soluble sample.

As it can be seen in Figure 26, the concentration of soluble carbohydrate increased from 100 mg/L glucose in raw influents to 262 mg/L glucose in raw effluent which because of the solubilization during the fermentation process while the concentration of soluble carbohydrate decreased from 1725 mg/L glucose in pre-treated influent to 886 mg/L glucose in pre-treated effluent. This

reduction in the concentration of soluble carbohydrate was because of further degradation of soluble carbohydrates to VFA or Millard reaction of soluble carbohydrates and proteins because of the pre-treated process.

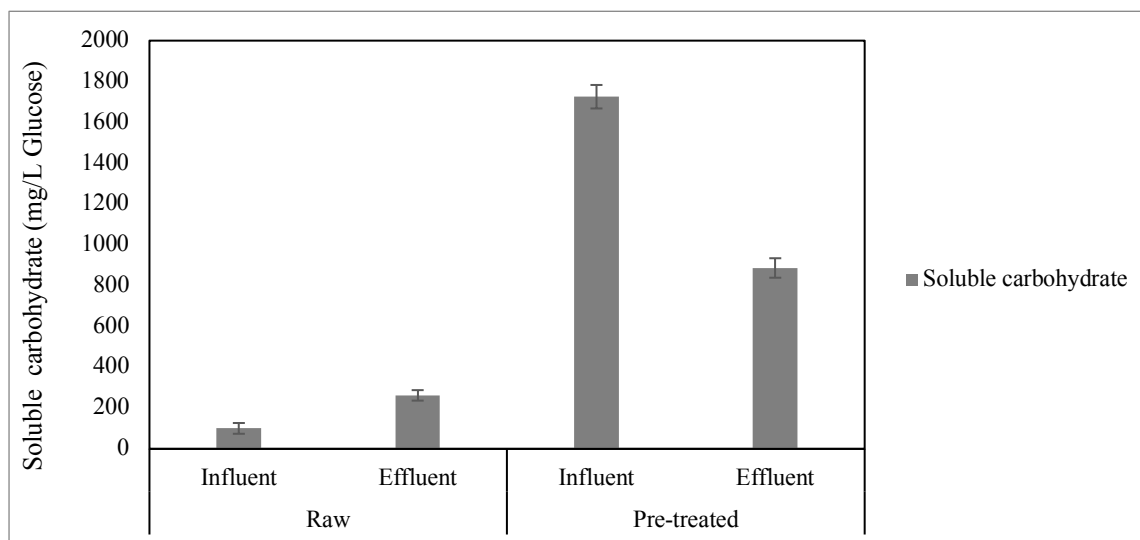


Figure 26 Soluble carbohydrate

8.9. Ammonia

Figure 27 represents the changes in values of ammonia during the fermentation process.

The ammonia concentration increased from 124 mg/L NH_3 -N in raw influent to 684 mg/L NH_3 -N in raw effluent. Moreover, the ammonia concentration increased from 214 mg/L NH_3 -N in pre-treated influent to 948 mg/L NH_3 -N in pre-treated effluent. Increasing the ammonia concentrations was because of the fermentation process.

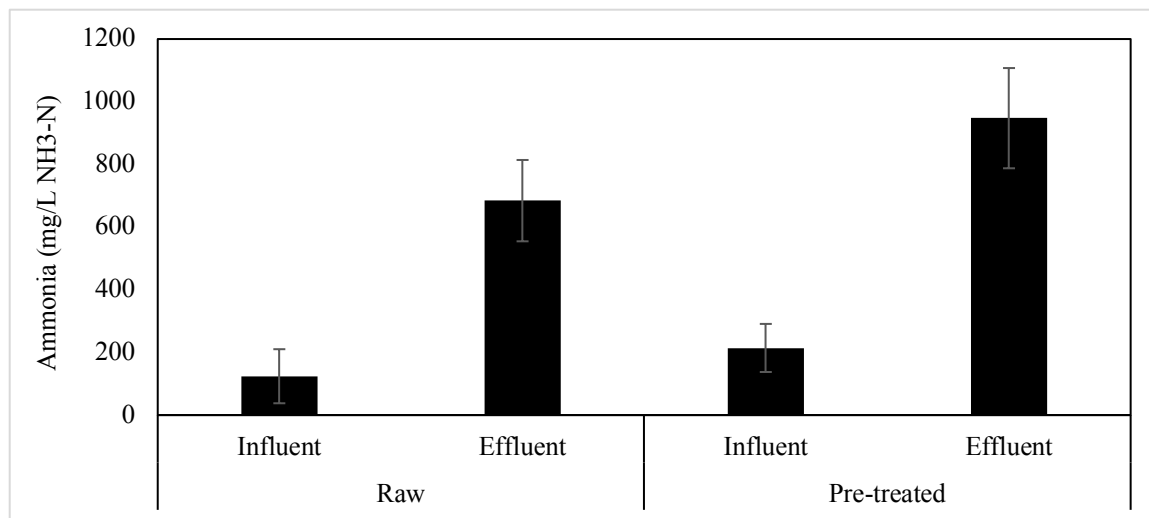


Figure 27 Ammonia

9. Conclusion

The findings of this research revealed that the degree of the solubilization of the inoculum was improved from 18% to 38% when the sample was subjected to pre-treatment and then fermentation rather than being only fermented. Moreover, the VSS reduced by 24% and 50 % during the fermentation process and sequential pre-treatment and fermentation process respectively. Since the pre-treatment made the sample more soluble, the SCOD production yield increased from 247 mg COD/g VSS in the raw sample to 220 mg COD/g VSS in the pre-treated sample. In addition, the VFA production yield enhanced from 122 mg VFA/g VSS to 220 VFA/g VSS for raw and pre-treated sample respectively.

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Glossary

AD – Anaerobic Digestion

COD – Chemical Oxygen Demand

HRT – Hydraulic Retention Time

MFC – Microbial Fuel Cell

MSW – Municipal Solid Waste

OLR – Organic Loading Rate

pH – power of Hydrogen

PHA – Poly-Hydroxy-Alkanoates

PS – Primary Sludge

SCOD – Soluble Chemical Oxygen Demand

SRT – Solid retention time

TCOD – Total Chemical Demand

TS – Total Solid

TSS – Total Suspended Solid

TWAS – Thickened Weight Activated Sludge

VFA – Volatile Fatty Acid

VSS – Volatile Suspended Solid