Effect of Hydrothermal Pre-treatment on Production of Volatile Fatty Acids from Thickened Waste Activated Sludge using Semi-continuous Fermentation

by
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A Major Research Project
presented to
Ryerson University

in partial fulfilment of the
requirements for the degree of Master of Engineering
in the program of Civil Engineering

Toronto, Ontario, Canada, 2020

Declaration

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Acknowledgement

Thank you, Almighty for giving me the zeal to acquire knowledge, ability, perseverance and opportunity to successfully finish my research study. Taking a moment I express my acknowledgment towards my grandparents (**Murlidhar and ShyamPyari Purohit**) & (**Santosh k. and Kanta Vyas**), without your blessings, this achievement would not have been possible.

I would like to express my deepest sense of gratitude to my research advisor **Dr. Elsayed Elbeshbishy**. His guidance and patience throughout my master's was resolute. He would always be available for my queries and steered me to the right direction. He encouraged me at all the stages of my research project and highly motivated me while I was compiling my work. I would like to thank him for all the opportunities he gave me allowing me to grow constantly.

During my master's, the biggest support I received was from **Dr. Farokh Laqa Kakar**, my fellow colleague in this research project and friend. She has been a great inspiration in my life apart from the project work as well. Expressing the amount of effort she put into her work is inexpressible.

I would like to thank the laboratory of Environmental Engineering at Ryerson University and ER3 group for providing assistance and for their constructive suggestions. I would like to thank Dr. Frances Steve Okoye, Dr. Amir Bazyar, Dr. Ehsaan Hosseini Koupaie, Ahmed El. Sayed and Dr, Salomeh Chegini for always having positive attitude towards me. Special thanks to Dr. Anahita Rabi for her caring attitude and motivation to help me write my research work.

Above all, I am indebted to my family, whose value to me only grows with my age. I express my sincere thanks to my parents (**Anil Purohit and Saroj Purohit**) who gave me the opportunity to explore the opportunities in my way. While growing up I never realized that how unknowingly my father had made me a capable person with his teachings and morals.

I would like to thank my sister and my brother-in-law (**Dr. Pratibha Vyas and Dr. Bhuvnesh Vyas**) for always being a constant support. Without them I would not have been able to achieve this.

Finally, a huge thanks to my amazing friends who were always a call away to help me in all times. **Lakshmi Suthar, Manisha Chaudhary, Laxmi Prajapat and Manvendra Singh**. Thank you for your unconditional support and time. **Farah Samouh and Shubbham Bohra** for always providing me with whatever I needed.

Lastly, a special thanks to **Kamal Purohit, Veena Purohit, Rohan Purohit** for trusting in me and providing me with their blessing throughout this journey.

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Abstract

The research project focuses on the effect of hydro-thermal pre-treatment of the thickened waste activated sludge (TWAS). Reducing the amount of waste generated by the process called anaerobic digestion (AD) includes the benefits of low cost and supplies source of renewable energy. Fermentation is the first part of AD where volatile fatty acids (VFAs) are produced. In this study hydro-thermal pre-treatment was conducted before the fermentation process to enhance the production of VFAs from TWAS. The study is a comparison between the pre-treated sludge and raw sludge using Semi-continuous fermentation for 40 days with hydraulic retention time of 3 days. TWAS was pre-treated at 170°C for 30 min at the pressure 3 bar before the semi-continuous fermentation process, and compared with the raw sample performance. It was found that the amount of VFAs produced from the pre-treated sludge were 26 % higher than that one from the raw sludge. Also, the chemical oxygen demand (COD) solubilisation for the hydrothermally pre-treated sample was higher than that of raw sludge.

Table of Contents

Declaration ii

Acknowledgement iii

Abstract iv

LIST OF FIGURES: vi

LIST OF TABLES vii

Abbreviations viii

INTRODUCTION 1

LITERATURE REVIEW 5

- Anaerobic Digestion Process: 6
- Parameters Affecting AD 10
- Pre-Treatment Methods 13
  - Types of Pre- Treatment Methods 15
  - COMBINATION OF VARIOUS PRE-TREATMENTS 27
- Dark Fermentation for production of Volatile Fatty Acids (VFA's) 29
- Applications of VFAs: 31

MATERIALS AND METHODS 34

  - Substrate and Inoculum 35
  - Hydrothermal Pre-treatment 35
  - Pre-treated Cycle and Collection 36
  - Acidification (Fermentation) Experiment 36
- Sample Analysis 40

RESULTS AND DISCUSSIONS 43

  - Initial Characteristics: 44
  - VSS Reduction: 46
  - VFAs Production: 48

Concluding Remarks: 52

REFERENCES 53

LIST OF FIGURES:

Figure 1 Process Flow diagram of AD Source: (Ramraj, 2014) 6
Figure 2 Parameters Affecting AD Process 10
Figure 3 Effect of Temperature on time of Fermentation source: Dieter 2008 11
Figure 4 Graph depicting Retention Time v/s Biogas production table (Dieter 2008) 12
Figure 5 Types of Pr-Treatment Methods 15
Figure 6 Mechanical Pre-Treatment Technology Applied on Wastes Source: (Javkhlan Ariunbaatar, 2014) 17
Figure 7 Biogas Variations in Temperature when Pre-Treatments are Applied Source: (Alexandre Valo, 2004) 19
Figure 8 Types of Chemical Pre-Treatment 21
Figure 9 Anaerobic 5-L Reactor Source: (L.A. Fdez.-Güelfoa . C.-G., 2011) 24
Figure 10 Cobalt-Gamma Rays Pre-Treatments Source: (Yulin Xiang, 2016) 27
Figure 11 Microbial Fuel Cell 32
Figure 12 Biological Nutrient Removal Process (Carla, 2016) 33
Figure 13 HTP Instrument (Sarwar 2015) 36
Figure 14 Experimental Design (Kakar et. al., 2019) 37
Figure 15 TCOD components: (a) TCOD RAW (b) TCOD HTP 38
Figure 16 AD bottle showing effluent and influent valves 39
Figure 17 Experimental Design Set-up 40
Figure 18 SCOD Values for RAW and Pre-Treated Samples 46
Figure 19 TSS (a) and VSS (b) Reduction in TWAS and HTP TWAS Influent and Effluents 47
Figure 20 VFA production in TWAS and HTP TWAS Influent and Effluents 48
Figure 21 RAW and HTP filtered Effluents colour differences 49
Figure 22 Graph for pH (a) and Ammonia (b) in TWAS and HTP TWAS Influent and Effluents 49

LIST OF TABLES

Table 1 Routes for Waste treatment (Jain et al. 2015b) 3
Table 2 Environmental conditions for different AD mechanisms. 9
Table 3 Time of Regeneration for different bacteria (Dieter 2008) 12
Table 4 Types of Wastes and Pre-Treatments Findings on them (L.A. Fdez.-Güelfoa . C.-G., 2011) 14
Table 5 Different Advanced Mechanical Processes for Pre-Treatment 18
Table 6 Characteristics of Sewage Sludge, Before and After Pre-Treatment 20
Table 7 Types of Nutrient and their Inhibition Levels 22
Table 8 Effect of pre-treatment methods on VFA production 30
Table 9 Main parameter affecting the VFAs production listed in Table below (Lee et al. 2014, Sans et al. 1995, Pereira et al. 30
Table 10 Specification of Experimental Design 40
Table 11 Initial parameters of TWAS and SEED 44
Table 12 Characteristics of TWAS Before and After HTP 45

Abbreviations

TWAS: Thickened Waste Activated sludge

HTP: Hydro thermal pretreatment

AD: Anaerobic Digestion

COD: Chemical Oxygen Demand

CSTR: Continuously Stirred Reactor

FW: Food Waste

HHW: Household Waste

HMF: Hydroxy Methyl Furfural

HPH: High Pressure Homogenizer

HRT: Hydraulic Retention Time

MSW: Municipal Solid Waste

OFMSW: Organic Fraction of Municipal Solid Waste

OLR: Organic Loading Rate

OM: Operational and Maintenance

OSW: Organic Solid Waste

PEF: Pulsed Electric Field

SRT: Solid Retention Time

SS: Sewage Sludge

THP: Thermal Hydrolysis Process

TPAD: Temperature Phased Anaerobic Digestion

WWTP: Wastewater Treatment Plant

TS: Total Solid

UASB: Up Flow Anaerobic Sludge Blanket

VS: Volatile Solid

VFAs: Volatile Fatty Acids

WAS: Waste Activated Sludge

Chapter 1

INTRODUCTION

Introduction

The two of the most argued problem of the world are; reduction in fossil fuels and, an increase in the organic waste. The environmental issues encompassing various concerns such as waste management, waste handling, waste treatment, waste reduction and recycling, and, waste prevention have now become a part of legislative and political matters. The traditional methods of disposal of waste including landfilling and incineration are highly discarded because of the fact that they cause pollution and greenhouse gas emissions.

According to (Appels et al. 2008), disposing of the sludge or the residual after the treatment of waste water is more than 50% of the operating cost of the plant. Hence treating the sludge by Anaerobic Digestion is general practice now a days. The final aim is to achieve lesser solids and reducing the number of pathogens along with removal of dreadful odour problems accompanying the effluent of the treatment plant. It is important to note that the microorganisms in the AD are of variety, having different working conditions. Since, AD consists of four steps, to enhance the workability of the process,pre-treatment can be done which increases the biogas production. Pre-treatment improves the rate-limiting step. By doing so the sludge cells decomposes into solubilized intracellular material and biodegradable polymers can be obtained from the organic fraction.

The City of Toronto treats1.4 million cubic metres of waste water every day and approximately 2600 tonnes of dry solids generated every year according to ((City of Toronto 2018)). the treatment of sludge reduce the moisture content, transform the decomposing material to inert organics, and condition the raw sludge to dispose according to the Environmental Laws.

Generally the purification of water involves a pre-treatment process that eliminates suspended solids upto 50-60%. The residual sludge has water content ranging between 9799% as the organic fraction is separated by the pre-treatment (Franklin L. Burton and George Tchobanoglous 2003). Pre-treatment is nothing but a Biological process that helps in removal of total suspended solids and BOD with help of aerobic microbes. The primary sludge and the secondary sludge (from secondary clarifier) usually combines when a pre-treatment takes place to undergo further thickening at WWTP's. There are many routes of disposal after treating the raw sludge; combined primary and secondary sludge, (Appels et al. 2008). Dry solids content in raw sludge is up-to 1-2% by weight.One third reduction of volume happens in the first step of sludge thickening. this can be achieved either by floatation, gravity, or by belt filtering. After this step, usually AD is done in order to achieve Syngas (60-70% methane) by converting organic matter to reduce solids present in the sludge.

The term anaerobic digestion is self-explanatory, digestion of waste material in anaerobic or without air conditions, it is carried out in sealed or airtight tanks. The exceptional materials that can't be digested are woody materials or any lingo-cellulosic material, which the microbes are unable to digest. Although, the achieved calorific value of biogas is high, AD still has many limitations:

- there is only partial degradation of putrescible matter
- high cost of digester
- reaction rate is much slower than expected
- presence of other gases such as carbon dioxide, hydrogen sulphide and moisture requires purification
- Concentration of heavy metals and industrial toxins in residual sludge that shows presence of non-biodegradable material untouched.

Combination of various steps takes place in order to treat solid waste from different generation sites. Table 1 shows different routes and their suggested treatment procedure.

Table 1 Routes for Waste treatment (Jain et al. 2015b)

Route	Outlets	Required operations
1	Agriculture (land application)	T, R
2	Agriculture	T, MD, R
3	Agriculture	T, AD, R
4	Agriculture	T, AD, MD, R
5	Landfill	T, MD, R
6	Landfill	T, AD, MD, R
7	Solid fuel	T, MD, ID, R
8	Solid fuel	T, AD, MD,ID
9	Ash	T, MD, ID, I
10	Ash	T, AD, MD, ID, I

Where,

T: thickening to 5–6 wt% of Dry solids

AD: anaerobic digestion to produce biogas;

R: road transport;
MD: mechanical dewatering to 25–35 wt% Dry Solids;

ID: indirect drying to 85–95 wt% Dry Solids;
I: incineration.

Objectives:

The main objectives of this study were to evaluate the effect of the hydrothermal pretreatment (HTP) on VFAs production from thickened waste activated sludge under semicontinuous fermentation.

Chapter 2

LITERATURE REVIEW

Anaerobic Digestion Process:

The process flow of the anaerobic digestion is shown in the Figure 1.

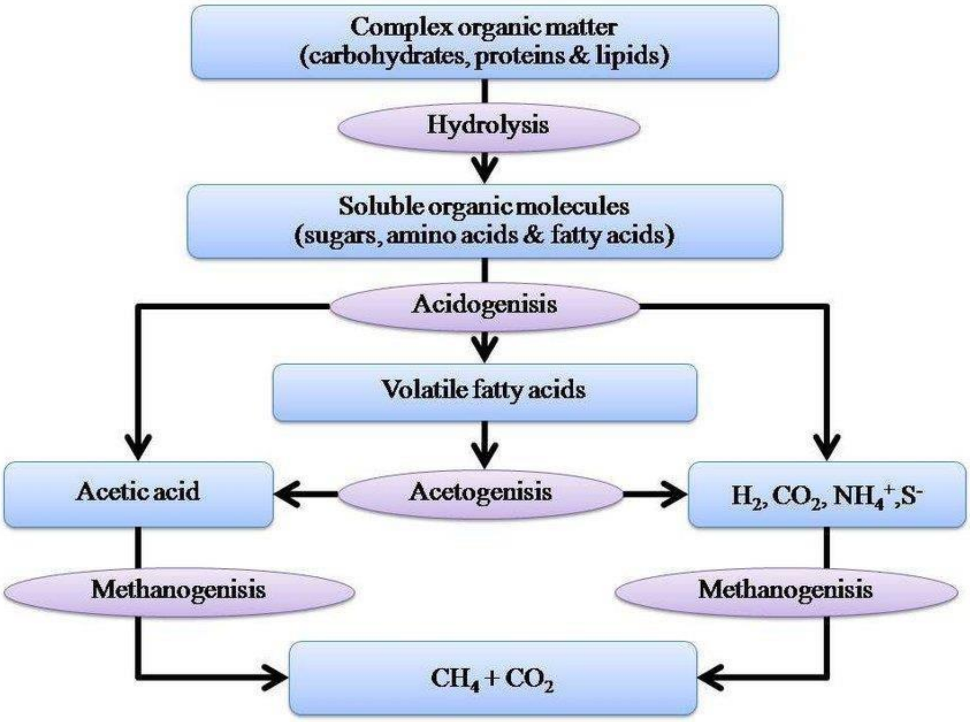


Figure 1 Process Flow diagram of AD Source: (Ramraj, 2014)

As shown in Figure 1, there are mainly four major steps in AD process:

- Hydrolysis
- Acidogenesis
- Acetogenesis
- Methanogensis

AD requires a precise Oxidation Reduction Potential of 200mV to carry out the digestion and a meticulously maintained physical parameters, that governs the microbial activity, such as pH and temperature to produce maximum CO_2 and CH_4 (Dieter 2008). As is the case with any process the rate limiting step is decided on the basis that which step undergoes for maximum time and has the most complex reactions. In AD the rate limiting step is the hydrolysis step because of the conversion of insoluble organic matter and compounds such as polysaccharides, proteins, lipids into simpler amino acids and fatty acids. Though the acids undergo further deformation, however, VFA's are formed during the acidogenesis step or during the fermentation. Aceto-genesis process further converts the acetic acid formed in acidogenesis step into CO_2 and H_2. The ultimate step is methanogenesis, carried out by methanogenic bacteria to breakdown Acetate into methane and carbon dioxide.

Bio-Reactions: In addition to first and second stage, the third and fourth stages are concurrent. Thus, one can categorize them in two processes (Dieter 2008). A great concern is that both categories must have same rate of degradation. Excess CO_2 is formed if the first stages are fast (acid concentration increase lowering the pH) and if the next stages are faster it would refer that there are still many active bacteria in the substrate that must be inoculated. (Dieter 2008)

Hydrolysis

The insoluble compounds like cellulose, proteins and fats are broken down into monomers or basically soluble components. this breakdown is done by exoenzymes of facultative anaerobic bacteria. Carbohydrates on one hand hydrolyse within a few hours, whereas it takes few days for proteins and lipids to hydrolyse. Hydrolysis of Lignocellulose and lignin is difficult and is incomplete even after a few days as there decomposition is where slow. the role of facultative microbes is to remove the dissolved oxygen and create low Redox
Potential.

Acidogenic

The acidogenic stage, gives the short – chain organic acids like butyric, propionic, acetic acid, alcohols, hydrogen and acetate. this is achieved by converting the monomers obtained in the hydrolytic phase and are degraded by the facultative anaerobic micro-organisms. the kind of products that are formed during fermentation is based on the concentration of hydrogen ions formed. Fewer acetate compounds are formed if the hydrogen ions are less.

As explained in Dieter's book, extravagantly explained about the biology of degradation of all the compounds (Dieter 2008):

1. Carbohydrates: propionic acid formed by propioni bacterium, butyric acid by clostridium.
2. Fatty acids: These are degraded e.g. from acetobacter by β - oxidation. Therefore the fatty acid is bound on Coenzyme A and then oxidizes stepwise, as with each step two C atoms are separated, which are set free as acetate.
3. Amino acids: These are degraded by the Stickland reaction by Clostridium botulinum taking two amino acids at the same time – one as hydrogen donor, the other as acceptor – in coupling to acetate, ammonia, and CO2. During splitting of cysteine, hydrogen sulfide is released.

Acetogenic phase:

Bacteria present in this step, degrades the products obtained from the previous acid-o-genic phase. The reaction taking place here though is endo-thermic/endergonic. Formation of acetate by oxidising long chain fatty acids is possible only with low hydrogen concentration. However, literature showed that survival of acetogenic bacteria is only possible with low H_2level. In contrast to which, methaneogenic bacteria require higher hydrogen concentration. However, methanogenic bacteria removes acetogenic substrates making the H_2 pressure level suitable for both conditions. At lower Hydrogen concentration, acetogenic bacteria releases carbon dioxide and acetate as its products but formation of butyric acid, propionic acid, and ethanol also takes place at higher H_2 concentration. Dieter et al., (2008) stated that methane production for about 30% is confined to carbon dioxide and hydrogen reduction.

Methanogenesis

It is the ultimate step of AD where methanogenic bacteria, decomposes acetate into CO_2 and H_2. The amount of methane formed by acetate decomposition is approximately 70% of the total methane production. Jain et al. (2015a) reported that methanogensis is rate limiting step when simple degrading substances occur contrary to complex substances where hydrolysis is the rate – limiting step in fermentation process. Anaerobic Digestion is complex process with rigorous conditions of different microorganism. Achieving constant and optimal rate of degradation Acidogenesis and methanogensis process must remain in equilibrium so as to have maximum efficiency in biogas production (Pavlostathis and Giraldo-Gomez 1991).

Literature showed that methanogenesis process is sensitive towards acidic changes. for fermentation process a pH range of 6.5 – 8 is suitable. However, in cases of faster organic acid formation than the population methanogenic bacteria may result in pH drop, an unfavourable condition to methane producing bacteria. hence there are many parameters that govern the AD such as, population of bacteria, seed, temperature, and pH etc. (Davidsson et al. 2008)

Usually, favourable temperature ranges for AD microorganisms is:

- Meso-philic: works in the range of 35^0 – 37^0 C.
- Thermophilic: works in the range of 55^0 - 57^0 C.

Table 2 Environmental conditions for different AD mechanisms.

Parameter	Hydrolysis/acidogenesis	Methane formation
Temperature	25 – 35 ° C	Mesophilic: 32 – 42 ° C Thermophilic: 50 – 58 ° C
pH value	5.2 – 6.3	6.7 – 7.5
C:N ratio	10 – 45	20 – 30
DM content	< 40% DM	< 30% DM
Redox potential	+400 to − 300 mV	< − 250 mV
Required C:N:P:S ratio	500 : 15 : 5 : 3	600 : 15 : 5 : 3
Trace elements	No special requirements	Essential: Ni, Co, Mo, S

Source: (Dieter 2008)

Rate Limiting Step:

It is the hydrolysis stage of the anaerobic digestion processes of liquid wastes. Literature shows that the primary sludge and the complex organic substrates undergoes anaerobic digestion the process of hydrolysis of organic matter to soluble substrate is the rate-limiting step for solid waste degradation (P. Chulhwan, 2005). The pre-treatments methods such as, physical, chemical or biological pre-treatments methods (or their combination), therefore, are required, to decrease the rate of limiting step.

Pavlostathis et al., (1998) found that the accumulation of products from hydrolysis in the reactor was nearly negligible which draw him to close that the when the cellulosic matter is converted to soluble products that step is rate-limiting step in the overall process (S.G. Pavlostathis, 1998). In the process of fermentation a low concentration of soluble compounds was observed which lead them to consider the hydrolytic stage as the limiting stage of the process as well (D. LEE, 1984). Galisteo suggested that for the complex waste from slaughter house effluents, assessment of activities of soluble and insoluble fraction formulated the results that the hydrolysis of organic-complex material in the degradation process was also a limiting stage. Hence, lignocellulose material that are complex substrates requires to be pretreated (M. Galisteo, 1998). Pre-treatment causes a deep alteration in the structure of complex material. This results in loss of degree of polymerization which weakens the molecular bonds present between lignin and carbohydrates. Wastes such as municipal solid waste (MSW), food waste (FW), and slaughterhouse wastes must be sterilized before AD. Following this directive substrates undergoes pre-treatment methods to obtain a greater energy recovery along-with reduced extra cost for sterilization (Hendriks ATWM, 2009) Although, pretreatment methods enhance the performance of AD, it is still considered unsustainable with respect to environmental footprints (Carballa M D. C., 2011).

Parameters Affecting AD

TWAS typically consist of 10% carbs, 50% proteins, 10% lipids, and 30% other inorganics. All the components together determine the parameter of the sludge characteristics (Park et al. 2005). Figure 2 shows different parameters that affect the AD process.

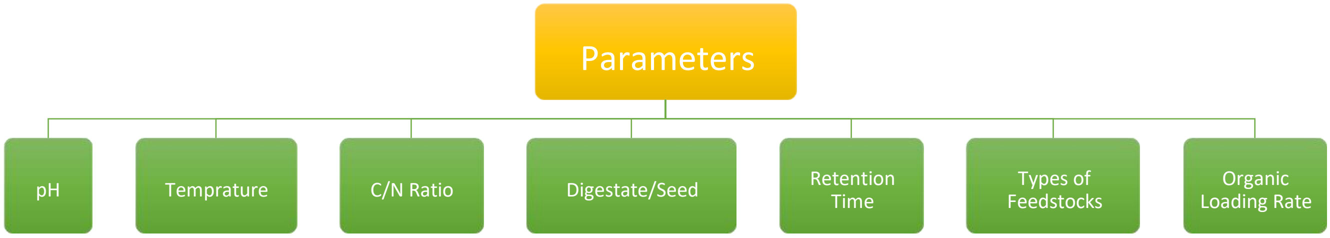


Figure 2 Parameters Affecting AD Process

1. pH:

pH of the substrate changes at all levels of anaerobic digestion. At the acidogenesis stage, pH is expected to be lower than 6 giving away CO_2. In the fermentation process, after 2-3 weeks pH tends to increase as the formation of methane occurs. However, fluctuating pH may result in lower efficiency and less decomposition of the waste substrate. Thus, pH of 6.5-7.5 is maintained by adding buffer solution as and when required. This pH range let the microorganisms remain active (Appels et al. 2008). However studies showed that added of any external substance to the slurry causes imbalance in the population of bacteria (Jain et al. 2015a).

2. Temperature:

Temperature changes affect the growth rate and metabolic rate of microorganisms (Hilkiah Igoni et al. 2008). Methanogens degrading substrates like butyrate and propionate are susceptible to temperature above 70^{o}C. Temperature influences the kinetics of H_2 in digesters and the breakdown of propionates into acetate, CO_2, and H_2 become exothermic reaction at higher temperatures but the methanogenesis reaction that are already exothermic are unfavourable. However, there are a few benefits of having high temperatures:

- Solubility of organic compounds increases
- Chemical reaction rate increases, Reduction in pathogens.

Whereas, the drawbacks of having higher temperatures are:

- Alkalinity and presence of free ammonia increases,
- Controlling the overall AD process becomes very subtle.

Temperature fluctuations more than 1^0 C/day may result in process failure since methane producing bacteria's are most sensitive to such changes (Turovskiy and Mathai 2006).

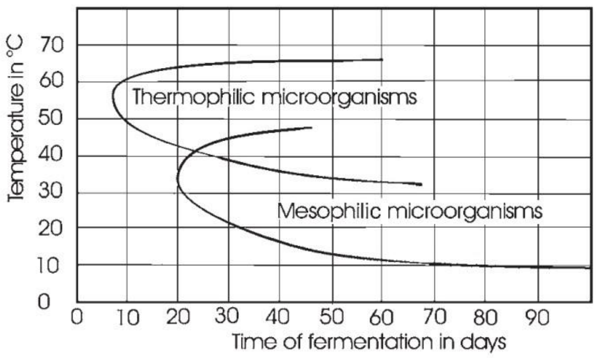


Figure 3 Effect of Temperature on time of Fermentation source: Dieter 2008

3. C/N Ratio

Studies recommend that C/N ratio must be from 25:1 to 30:1 for good bacterial growth. C/N ratio represents the nutrient level in the substrates. If this ratio is not accurate then the increase in Ammonia Nitrogen or VFA accumulation starts which decreases methane formation (Parkin Gene F. and Owen William F. 1986) .With different feedstock, C/N ratio also changes. When Co-digestion of waste activated sludge was done with corn stove, C/N of 15-17:1 was found out to be best suitable but the AD failed when C/N 21 or higher was selected. It was due to the fact that pH decreased suddenly. (Jain et al. 2015a)

Table 3 Time of Regeneration for different bacteria (Dieter 2008)

Anaerobic microorganism	**Time of regeneration**
Acidogenic bacteria	
Bacterioids	< 24 h
Clostridia	24 – 36 h
Acetogenic bacteria	80 – 90 h
Methanogenic bacteria	
Methanosarcina barkeri	5 – 16 d
Methanococcus	Ca. 10 d
Aerobic microorganism	
Escherichia coli	20 min
Active sludge bacteria	2 h
Bacteria living on earth	1 – 5 h

4. Retention Time: Solid and Hydraulic (SRT & HRT)

The time extent to which the substrates remains in the digester system is known as hydraulic retention time. It varies with type of feedstock subjected to AD and the temperature governing the process of decomposition. Methanogenic bacteria has a tendency to double in nature in period of 2-4 days. Hence, retention time should be optimally be 3 days. SRT is the time period of retention of solids in the digester. Whereas, HRT is average time that the liquid sludge is kept in the digester. For a CSTR, every time sludge removal happens the bacterial population is drawn out in some proportion ensuring balance state and avoid failure. Turovskiy and Mathai (2006) studied retention time and obtained a relationship between gas production and HRT. They found that:

- SRT less than 5 days are unsuitable for stable digestion. VFA concentration rises.
- For SRT 5-9 days breakdown of lipids is difficult
- SRT 8-10 days or greater all the sludge components were decomposed.

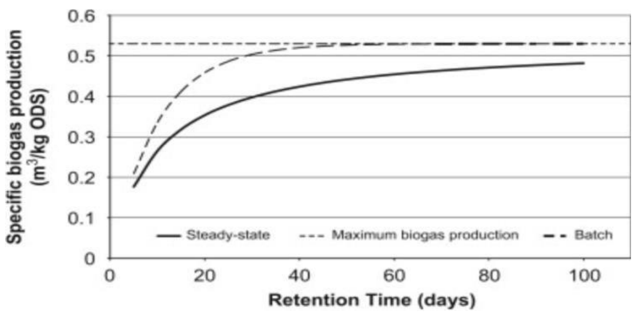


Figure 4 Graph depicting Retention Time v/s Biogas production table (Dieter 2008)

5. Type of Substrate:

Substrate is that component of AD process which determines the rate of degradation, type of technology to be taken and process operation to be opted. Since it is the substrate that needs to be degraded into simpler compounds for energy extraction, absence of any compounds such as carbohydrates, proteins, or lipids may change the metabolism of the microbes. Depending upon what the substrate is made up of intermediate complexes may inhibit or limit the degradation. As described in the literature protein decomposition leads to formation of Ammonia which restrict methane formation. (Jain et al. 2015a)

6. Organic Loading Rate

(Lee et al. 2014) OLR is account of volatile solids that are daily fed into a digester made for continuous feeding. Though, mathematically as OLR increases, the biogas yield also increases to an extent. However, it is seen that the overall digestion process with highest OLR reduces the productivity of methane. It takes about 10 – 15 days for the digester to acclimatize to everyday feeding in the fermentation process and reach steady state. the bacteria in its initial stages of AD, due to high OLR have higher hydrolysis/acidogenesis process rather than methanogenesis which results in production of higher VFAs.

Furthermore, studies have been done to find ideal OLR's. According to Gou et al. (2014), 5 g VS/L/D is suitable for MSW comprising of WAS and FW and. 9.2kg VS/m^3/d is ideal for primary and secondary sludges under mesophilic conditions.

Before explaining the significance of VFAs it is important to understand the types of Pretreatment processes.

Pre-Treatment Methods

Pre-treatment methods highly effect the substrate characteristics depending on which type of method is applied. Based on different literature, it was found that which pretreatments method was suitable for which substrate is illustrated in the Table 4.

Michael Bjerg-Neilsen (2018) states that making organic matter (OM), recalcitrant prior to biogas production is main reason for pre-treating substrates i.e. OM is more readily available to the microorganisms, consequently increasing CH4 and CO2 production, whilst reducing the final digestate volume (Michael Bjerg-Nielsen, 2018).

Table 4 Types of Wastes and Pre-Treatments Findings on them (L.A. Fdez.-Güelfoa . C.-G., 2011)

Substrate	Pre-treatment methods	Important findings
OFMSW	All pre-treatments methods	Physical pre-treatments are applied whereas other methods are not spread at industrial level. (Cesaro A, Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions., 2014)
All organic substrates	All pre-treatments methods	Pre-treatment methods commonly used are thermal and ultrasonic for WWTP sludge, chemical for lignocellulose substrates, and mechanical for OFMSW. (Hartmann H A. I., 2000)
Lignocellulosic substrates	Thermal, thermo-chemical, chemical	The digestibility of lignocellulose substrates improves. (Lopez Torres M, 2008)
Pulp & paper sludge	Thermal, thermo-chemical, chemical	Reduced HRT, elevated methane production, and reduced sludge size (Hendriks ATWM, 2009)
WWTP sludge	Ultrasound, chemical, thermal, and microwave	Enhanced biogas production (30–50%) (Bordeleau ÉL, 2011)
WWTP sludge	Thermal, thermo-chemical, and chemical	Pre-treatments gives a better digestate with high recoverable nutrients. (Carrere H D. C., 2010)

Types of Pre- Treatment Methods

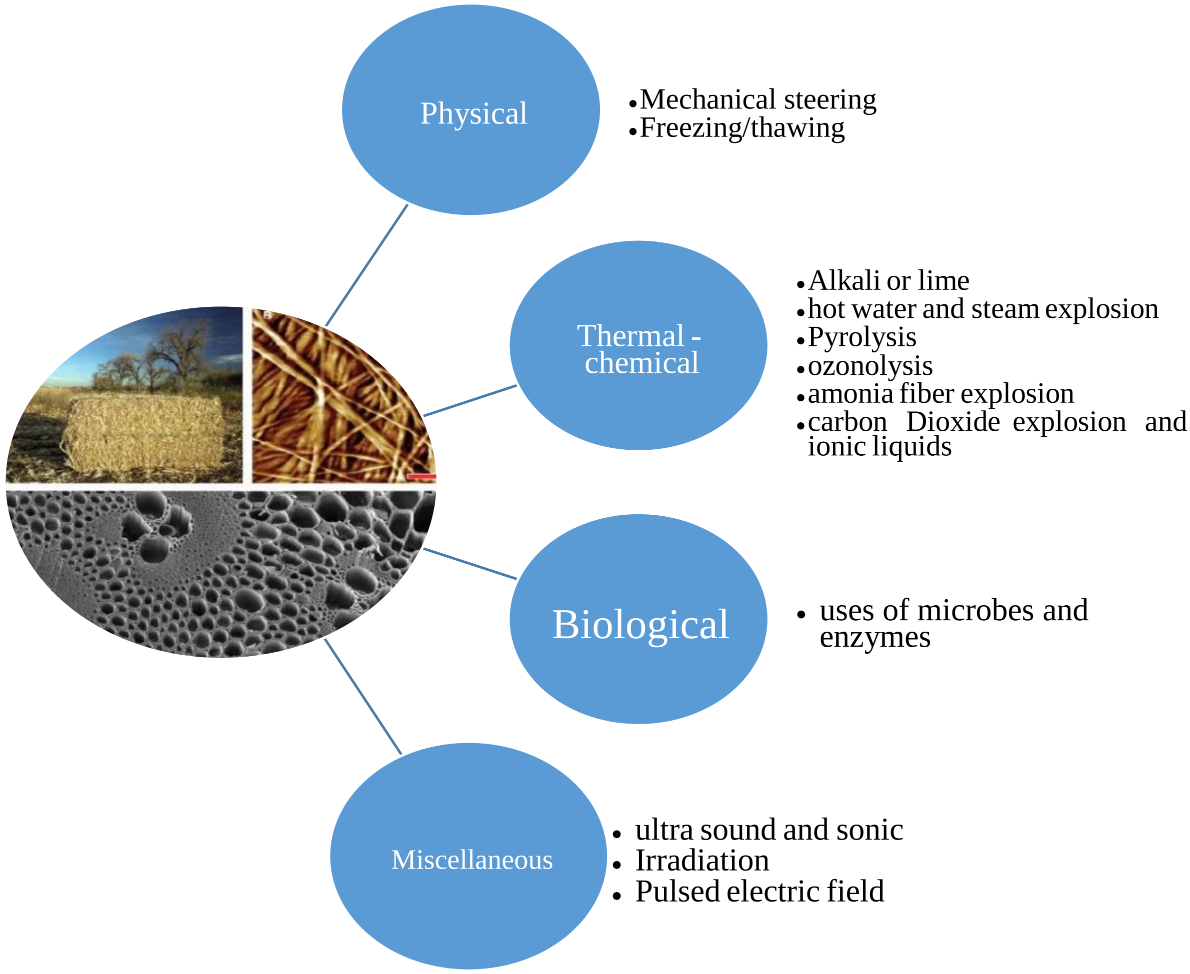


Figure 5 Types of Pr-Treatment Methods

MECHANICAL PRE-TREATMENT
Process Description and Mode of Action

The process involves splintering or grinding of heavy solid particles in the substrates which helps in increasing the specific surface area of the particle. The higher the contact between the microbes and substrate the better the efficiency of AD process(Skiadas IV, 2005). Esposito et al. referred that chemical oxygen demand (COD) degradation depend on particle size. A larger particle radius results in lower decomposition and a lower methane production rate (Esposito G, 2011). Similarly, Kim et al. demonstrated that the relation between particle size and the maximum substrate utilization rate are inversely proportional (Kim IS, 2000). Therefore, before sending the substrate to the AD reactors the particle sizes are to be reduced of the substrates. Various mechanical pre-treatments are applied such as sonication, shear, collision, homogenizer at high-pressure, maceration, and liquefaction.

All the above mentioned methods may also result in some impounding effects. Hartmann et al. proved shearing has more impact than cutting of fibres when maceration technique is applied(Hartmann H A. I., 2000). Likewise, sonication pre-treatments mechanically distorts the complex cell structure. The function of homogenizer is, under high pressure (HPH), to blend substrates under depressurization (Mata-Alvarez J M. S., 2000).

Although the pre-treatment methods are efficient these are uncommon for OFMSW, instead they are more prevalent with substrates such as lignocellulose materials, livestock's and WWTP sludge. For OFMSW, treatment for size reduction by shredder, beads mill, and liquefaction pre-treatments is been under research at lab scale, in contrast to which the rotary drum, and disc screen shredder, disposer and piston press treatment are extensively used at industrial level. (TA, 2006).

Advantages of using Mechanical Pre-treatment (Toreci I, 2009):

- no odour generation,
- elementary implementation,
- Better removal of water from the ultimate anaerobic left over or residue
- A moderate energy consumption.

However, Elvira et al., (2006) stated that the disadvantages involves that the reactors and equipment to have problem of clogging and that there is no significant removal of pathogens and hence the nitrogen phosphorus nutrient content remain same (Perez-Elvira SI, 2006).

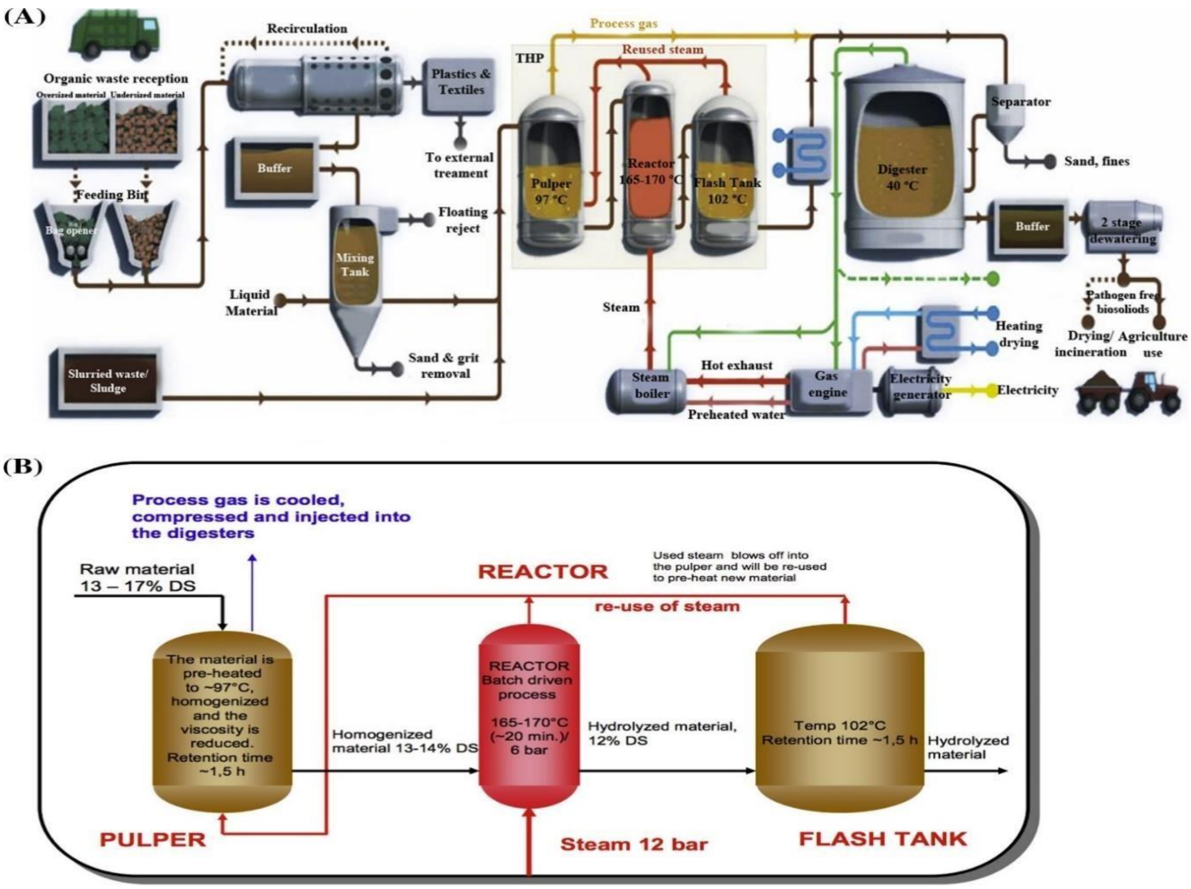


Figure 6 Mechanical Pre-Treatment Technology Applied on Wastes Source: (Javkhlan Ariunbaatar, 2014)

Mechanical Pre-Treatment of OFMSW

Methods such as screen press and rotary drum are commonly used as an effective way of separating and pre-treating OFMSW before the anaerobic digestion. Pre-treating the substrate usually is done to maximize the biogas production. For mechanical methods, the biogas manufacturing rises by 18–36%(Zhu B, 2009). Davidson et al. stated fewer disparities for methane yield/g VS and content of methane in biogas when comparing the results from pre-treated fraction of organic waste with different methods such as disc screen holder, homogenizer, and screw press(Zhang Y, 2013). Although, Zhang and Banks did not find any significant appraisal when mechanical pre-treatments methods were applied(Hansen TL, 2007). Hansen et al. deliberated the outcomes the above mentioned pre-treatment technologies, for qualitative and quantitative analysis on the OFMSW that was sorted at source. Their main findings concluded that when pre-treatment technologies are separated from the impurities at source, the biogas production yield was higher (10-13%), while using a shredder or screw press with magnetic separation (Bernstad A, 2013). Bio-methanation is controlled by the particle size, Izumi et al., and it was observed that reducing size through a beads mill gave higher COD solubilisation, leading to 28% higher biogas yield. Excess size reduction also caused accumulation of VFA when particle size was less than 0.7 mm (Izumi K, 2010). The bacteria involved in the methano-genesis are the methanogens which are sensitive towards intermediate that are acidic (Li Y, 2011), resulting in lower AD process performance. Researchers show that when methods such as electroporation, liquefaction, and high frequency sonication are taken under consideration they resulted in higher performance for OFMSW.

Table 5 Different Advanced Mechanical Processes for Pre-Treatment

S.NO	Pre-treatment method	Increase in Biogas production
1	Electroporation	20-40% ,(Carrere H D. C., 2010)
2	Liquefaction	15-26%, (Carlsson M A. K., 2008)
3	Sonication	16-18%

THERMAL PRE-TREATMENTS

Process Description and Mode of Action

Mechanical treatments are somewhat effective but thermal treatment is most widely studied pre-treatment methods, which is always and successfully applied at industrial scale (Cesaro A, 2014).

Advantages of Thermal pre-treatment include; (Val del Rio A, 2011)

- removal of pathogens,
- Dewatering workability improves
- reduces viscosity of the digestate,
- Enhancement handling of digestate.

Although to increase biogas yield various temperature ranges (50–250 C) are taken to inflate the AD of different untreated OSW (mainly including WWTP sludge), but no meticulous research for OFMSW has been studied. The principle of the thermal pre-treatment lies in disintegrate the cell membranes for solubilisation of organic compounds (Ferrer I, 2008)(Bien JB, 2004).

There is a correlation between solubilizing COD and temperature effects. It was seen that for longer treatment times the COD solubilisation was high given that the temperature was low. Mottet et al. (2009) reported that when comparing different thermal pre-treatment methods such as steam and electric heating, he did not find any notable differences among them, whereas biopolymers were solubilised more when microwave heating was done
(Mottet A, 2009). This is due to the fact that that higher rate of solubilisation is achieved by polarization of macromolecules.

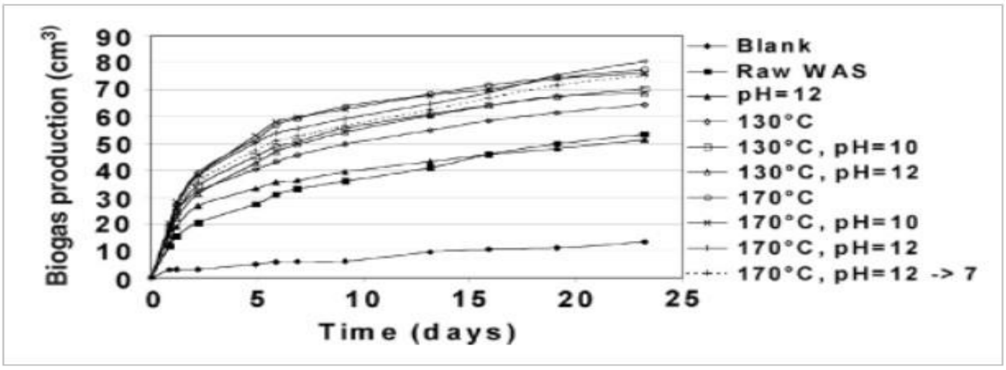


Figure 7 Biogas Variations in Temperature when Pre-Treatments are Applied Source: (Alexandre Valo, 2004)

Intermediate Thermal Hydrolysis Process (ITHP)

- As described by Nielson et al. (2018) the thermal hydrolysis treatments usually performed in a reactor with desired volume, at high temperature and pressure bench and accompanied with electrical heating.
- Data inputs for temperature and pressure are monitored with an error range of within ±1.5 C of the required temperature.
- Using industrial methodology, we know that electrical heating and steam heating has no significant difference in their effect (Mottet et al., 2009).
- TS and VS are measured in accordance with APHA 1999 standards (APHA-AWWAWEF, 1999).
- Heating from 20 degree C to target lasts for 30–35 min.

In his report Neumann et al. (2016) strongly provided the evidence for post ITHP substrates that the odor and colour are changed due to change in the chemical composition. He also found that there was increase in homogeneity (Neumann, 2016). An important chemical founding was that carbohydrate monomer concentrations in untreated samples are usually low, whilst those in treated samples, chemical compound such as glucose, xylose and mannose appear in increased (still small) quantity in all treated samples.

Table 6 Characteristics of Sewage Sludge, Before and After Pre-Treatment

Item	Unit	Untreated sewage sludge	Pre-treated sewage sludge(under optimal conditions)
TCOD	g/L	169.0 ± 1.9	159.1 ± 0.3
SCOD	g/L	1.7 ± 0.1	47.3 ± 0.1
TVFAs	g/L	0.8 ± 0.0	8.2 ± 0.1
T-N	g/L	1.1 ± 0.1	1.0 ± 0.0
S-N	g/L	0.1 ± 0.0	0.2 ± 0.0
T-P	g/L	0.8 ± 0.0	0.8 ± 8.6
S-P	g/L	0.1 ± 0.0	0.3 ± 0.0
T-protein	mg/L	536.7 ± 11.4	483.7 ± 8.4
S-protein	mg/L	232.4 ± 12.7	324.4 ± 5.3
T-carbohydrate	mg/L	2,347.1 ± 81.4	2,147.1 ± 34.3
S-carbohydrate	mg/L	102.7 ± 4.6	343.8 ± 12.6

Source: (Jae-Min Choia, 2018)

CHEMICAL PRE-TREATMENTS

Process Description and Mode of Action

Chemical pre-treatments as the name suggest uses chemicals to destroy or degrade the organic compounds using strong acids, alkalis or oxidants. It's a well-known fact that AD it pH sensitive due to the presence of microbes and after the acido-genesis process to increase alkalinity, alkali pre-treatments is the preferred as chemical pre-treatment method(Li H, 2012)

Some other methods such as acidic pre-treatments and oxidative methods including ozonation are also in preferred for enhancing the biogas production yield and raise the hydrolysis rate. Selecting which chemical pre-treatments to be adopted depends upon which pre-treatment method is applied and the composition of the substrates, that help determine the effect of the technology selected. For the substrates that contains high amount of carbohydrates, degradation take place at accelerated rate causing accumulation of VFA and this causes delay in methanogenesis step. Hence, chemical pre-treatments is not suitable for solid wastes that are easily biodegradable substrates however, substrates that are high in lignin content must undergo chemical pre-treatment for higher yield. (Wang L, 2011)(Fernandes TV, 2009)

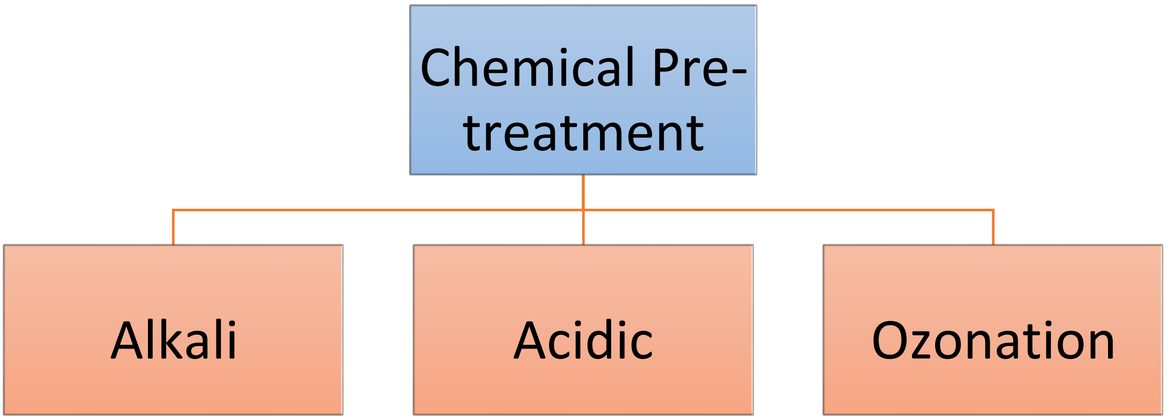


Figure 8 Types of Chemical Pre-Treatment

ALKALI PRE-TREATMENT

Alkali pre-treatments, have two initial reactions that occur are solvation and saphonication, which causes swelling of solids (Carlsson M L. A.-S., 2012). These reaction help increasing the specific surface area and let the anaerobic microbes easily access the substrates(Hendriks ATWM, 2009)(Modenbach AA, 2012;)(Lopez Torres M, 2008). These reaction (saponification of esters and acids or neutralization) also increases COD solubilisation (Lopez Torres M, 2008). It is interesting to note that the biomass itself has some alkaline nature, hence to obtain a greater AD performance, substrates are pre-treated with higher alkali reagents including sodium hydroxide, calcium hydroxide, potassium hydroxide, aqueous ammonia, ammonia hydroxide, and sodium hydroxide with hydrogen peroxide.

ACID PRE-TREATMENT

For lignocellulose substrates acid pre-treatments is more suitable as it not only breaks down the lignin component, but the hydrolytic microbes are more adapted to acidic conditions (Mussoline W, Anaerobic digestion of rice straw: a review. , 2012.). The acid pretreatments has main aim to hydrolyse hemicellulose into monosaccharaides, and precipitate the lignin part (Hendriks ATWM, 2009)(Mata-Alvarez J e. , 2003.). It is usual practise to avoid strong acidic reagents because they may result in formation of by-product compounds such as furfural and hydroxymethylfurfural (HMF) (Modenbach AA, 2012;)(Mussoline W, 2012.) Hence, coupling diluted acids pre-treatments with thermal method is done.

Disadvantages of the acid pre-treatments:

- high degradation of complex substrate leads to loss of fermentable sugar
- Expensive acids and then neutralizing the acidic condition before AD adds additional cost to process (Modenbach AA, 2012;)(Taherzadeh MJ, 2008) (Kumar D, 2011)

Effects of Accompanying Cations Present in the Acid/Alkaline Reagents

Acid and alkali have their separate effect on AD, they may cause hindrance in AD with their reagent cations which includes sodium, potassium, magnesium, calcium.Thus considering the concentration of these cations (Carrere H D. C., 2010)(Appels L, 2008). Kim et al. (2000), learnt that sodium ion concentration presence in the thermophilic AD of FW, resulted in lower biogas production when the substrate had more than 5 g/L of sodium (Kim IS, 2000). The significance of sodium can be justified by the fact that it causes more harm to propionic acid utilizing bacteria rather than other VFAs (Soto M, 1993).

Table 7 Types of Nutrient and their Inhibition Levels

S.No.	Cation	Inhibition level	Ref.
1	Sodium, Na	5g/l	38
2	Potassium, K	8g/L	82
3	Calcium, Ca	200mg/L	84
4	Magnesium, Mg	720mg/L	85

Harmful Effects of Chemical Pre-treatment:

- Calcium ion in excess quantity produces precipitates of carbonates and phosphates that can lead to further scaling in boilers; the buffer capacity is lowered by reduction in methanogenic activity (Zhang B, 2005).
- When Mg ion has high concentrations (>100 mM) it disintegrate methanogens (Schmidt JE, 1993;).
- Trace metals such as cobalt (Co), molybdenum (Mo), selenium (Se), and iron (Fe), tungsten (W), copper (Cu) and nickel (Ni), also enhances AD. Facchin et al. reported a higher biogas production (45-65%) (Facchin V, 2013).

- But AD plants treating solid wastes should not undergo supplemented trace metal chemical pre-treatments, no matter it would produce higher methane but would reduce dewaterability.

OZONATION

Ozonation, is a chemical pre-treatment which neither cause an increase in salt concentration nor have any chemical (Carrere H D. C., 2010). A greater advantage is that it helps in disinfection from pathogens which can be helpful in sludge pre-treatments. (Weemaes M, 2000)(Kianmehr P, 2010). A strong oxidant that is ozone, undergoes into radical decomposition of itself to react with other organic substrates (Sri Bala Kameswari K, 2011). This can takes place in two ways: either directly or indirectly. In the direct reaction structure of the reactant is dependable, whereas the indirect reaction is based on the hydroxyl radicals. That is why recalcitrant compounds are easily biodegraded and approachable to anaerobic bacteria.(Carballa M M. G., 2007).

As we know from the reaction perspective and different other factors like reagents, and substrate chemical pretreatments are widely applied on wastewater sludge and lignocellulose substrates (Modenbach AA, 2012;), but usually not opted for OFMSW. and according to literature ozonation pre-treatments was performed on WWTP. ozone dose ranges from 0.05 to 0.5 gO3/gTS for enhancing AD(Yoem I, 2002). Cesaro and Belgiorno were few of the researchers that proposed OFSW requires 0.16 gO3/gTS for waste separated at source. It resulted in a 37% higher methane production (Cesaro A, Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. , 2013). Addition to which Lopez-Torres and Llorens reported that alkaline pre-treatments results in 11.5% increase in methane production (Lopez Torres M, 2008). The lower lignin content in Patil’s study showed the effect of alkaline pre-treatments of wastewater, stating that it was less effect than mechanical technologies (Patil JH, 2011). Therefore, using acidic and alkaline pre-treatments method is not advisable with a low lignin content substrates.

BIOLOGICAL PRE-TREATMENTS

This pre-treatments procedure includes addition of enzymes such as peptidase, lipase and can occur in both anaerobic and aerobic condition. Although these methods are not very prevalent among substrate which have OFMSW but are well suited for WWTP sludge and OSW. The process steps in a two-phase AD process, the hydrolytic-acidogenic step (first step) marks as a biological pre-treatments method by several researchers(Ge H, 2010). According to literature a higher methane production yield is obtained when separating the acidogens from the methanogens physically. It also resulted in better COD removal efficiency achieved at a shorter hydraulic retention time (HRT)(Hartmann H A. B., 2006). The reason behind this was explained by Parawira et al. (2005) that stimulating acidogenic microbes to secrete enzymes byimprovising the first hydrolysis stage and helping degradation of substrates (Parawira W, 2005). And that this report analyse the first step of the two-phase AD systems are considered as a pre-treatments method.

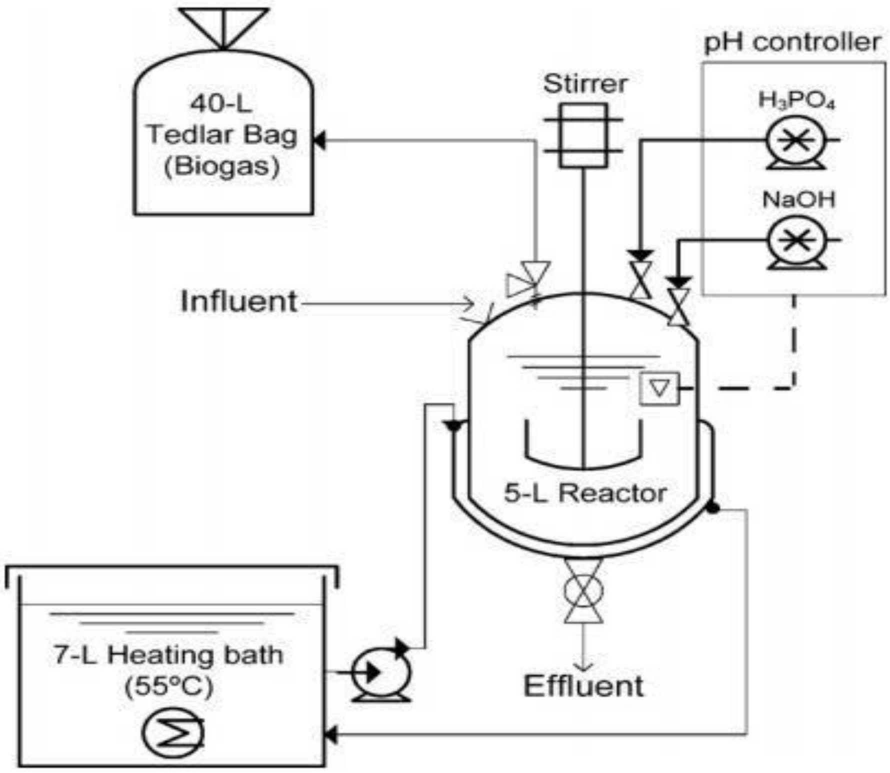


Figure 9 Anaerobic 5-L Reactor Source: (L.A. Fdez.-Güelfoa . C.-G., 2011)

Conventional Biological Pre-Treatments

These include an effective aerobic pre-treatments such as composting or microaeration which increases specific microbial growth producing hydrolytic enzymes. It is done prior to AD(Lim JW, 2013). Fdez-Guelfo et al. (2011) testified that composting results in increased microbial growth rate from 16-205% than achieved by any thermo chemical technologies for pre-treatments (when compared with untreated OFMSW) (Fdez-Guelfo LA, 2011). According to Lim and Wang the augmented activities of hydrolytic and acidogenic bacteria results in formation of VFA at a higher rate when undergone aerobic pre-treatments (Lim JW, 2013). In contrast to which, Brummeler and Koster's study, obtained a negative result showing a 19.5% VS loss when a pre-composting treatment of OFMSW is performed(Brummeler E ten, 1990). Not only Koster, but even Mshandate et al. also spotted a decrease in potential methane yieldfor a sisal pulp waste when kept for longer aerobic pretreatments (Mshandete A, 2005).

Miah et al. did his investigation anaerobic thermophilic bacteria subjected to SS pretreatment and showed that they were closely linked to Geobacillus thermodenitrificans

(Miah MS, 2005). In his study report he mentioned that at 65 C, he achieved biogas (70ml/gVS) having the highest methane content 80-90%. Melamane et al. (2007) deliberated the effect of fungus Trametes pubescens treatment for wine distillery wastewater, which gave result in favour of total COD removal efficiency for the whole process (99.5%) and a rate of 53.3% COD removal efficiency was registered for pre-treatments process.(Melamane X, 2007).

Muthangya et al. (2009) experimented with pure cultures of the fungus Trichoderma reseei and pretreated sisal leaf decortication aerobically and found that the incubated waste material for 4 days gave biogas accumulation upto 30-40% with methane content in 50-60% (Muthangya M, 2009). Likewise Romano et al. did his research on two types of enzymes which were to hydrolyse plant cell walls and increase the biomethanation of wheat grass. Although, this study did not achieve a significant biogas yield or noticed any VS reduction, but the step of hydrolysing was speed up.

Two-Stage AD:

As stated earlier two-phase AD system comprises of a separate methanogenic stage after the hydrolytic-acidogenic stage. However unlike the conventional pre-treatments method, it has both the steps undergo anaerobic condition and no aeration is provided externally.

Advantages of this process includes:

- Stabilize digestate with better pH control;
- Organic loading rate is higher;
- High methane yield resulting from increased specific activity of methanogens;
- Vs reduction is enhanced and
- Pathogen removal has capacity (Agency., 2006)

The disadvantages include:

- Acid forming bacteria result in inhibition;
- Elimination of possible interdependent nutrient requirements for the methane forming bacteria;
- Complex technology and
- Operational and maintenance costs are high (Wang X, 2009)

For vegetable wastes Verrier et al. made a comparison for two-stage methanization reactor with mesophilic and thermophilic based reactors (CSTR). Since vegetable wastes are easily bio-degraded, using two-stage reactor he was able to find thatwaste was 90% converted to biogas, and surpass the reactors- mesophilic and thermophilic- withstanding the higher organic loading rates. Similarly, Zhang et al. suggested the pH control in hydrolysis step to improvise total solid (TS) loading rate and increases biogas potential while can out his research on the two phase AD process for FW and effect of pH(Zhang B, 2005).

Temperature Phased Anaerobic Digestion (TPAD):

Temperature phased anaerobic digestion has recently been a topic for research (TPAD). To explain the process in nutshell it consist of both thermophilic (primary digester) and mesophilic digester as a secondary digester, one followed by other respectively. The advantages of TPAD is to remove pathogen from digestate which has high nutrient content along with higher methane production yields. And that this process as stated by Riau et al. is preferred for the environment using soil conditioner as digestate which is required to be pathogen free(Schmit KH, 2001). Also, Schmitt and Ellis studied the TPAD performance for OFMSW source separated and reported that this process is better than the conventional AD processes. investigating FW in his report Lee et al. used sludge at 70 C in the primary reactor and different temperature ranges for secondary reactor (35 , 65 , 55 C) (Lee M, 2009). As expected he got the best results for the reactor with 70 C temperature and having solid Retention time as 4 days.

For two different temperature for TPAD (80 C and 55 C in the thermophilic reactor and followed with mesophilic reactor) and the conventional thermphilic digestion, are compared by Wang et al. treated FW with polylactide. The above mentioned reactors gave a COD solubilisation of 82% for 80C two stage AD, 85.2% for 55 C for 1st stage thermophilic reactor and 63.5% for conventional type digester. The organic conversion to biogas was 82.9%, 80.8%, and 70.1% respectively as well(Wang F, 2011). comparing the pathogen removal efficiency and biogas yield Song et al. took samples of WAS and treated them with TPAD and compared it to single stage mesophilic and thermophilic digester and results showed thata higher VS reduction upto 12-15% is yielded in TPAD giving stable digestate like mesophilic reactors but the pathogen removal rate is as high as it is in thermophilic digester.

Biohythane Production

Biohythane is a mixture of bio-hydrogen and bio-methane obtained when the two phase AD is optimized from primary reactor and secondary reactor respectively. Studies done on this optimization by Liu et al. acquired from household waste are 43 mlH2/gVS and 500 mlCH4/gVS for thermophilic and mesophilic reactors(Liu D, 2006) whereas Wang et al. obtained 65 mlH2/gVS and 546 mlCH4/gVS from FW (Wang X, 2009).

Chu et al. (2008) reported for the pH range 5.5-6 in thermophilic AD we receive peak hydrogen production. Although he found that there was no methane production in first stage, the bio hydrogen content was 52%, but the methane content increased to 70–80% in the secondary reactor. For the study mentioned by Chu et al. the mass balance showed that approximately 9.3% of COD conversion take place to hydrogen and 76.5% turns into methane. A study conducted by Escamilla-Alvarado et al. on the optimizing of two-stage AD of OFMSW and obtained the same results as Chu et al., having 3-10% hydrogen and getting biogas from secondary reactor as 25-61% (Chu C-F, 2008).

IRRADIATION PRE-TREATMENT

Usually carried on the lignocellulose containing wastes, in this process the use of radiation energy (microwave, ultrasound etc.) increases the biodegradability. The way to carry out the pre-treatment is to load the substrate into restraint which has a specific power of radiation for a particular estimated time. The disruptive effect of radial waves breakdowns the lignocellulose and make it surface area large and accessible for microbial activity and thus sometimes it also decreases polymerization of different structures. (Elsayed Elbeshbishy, 2017)

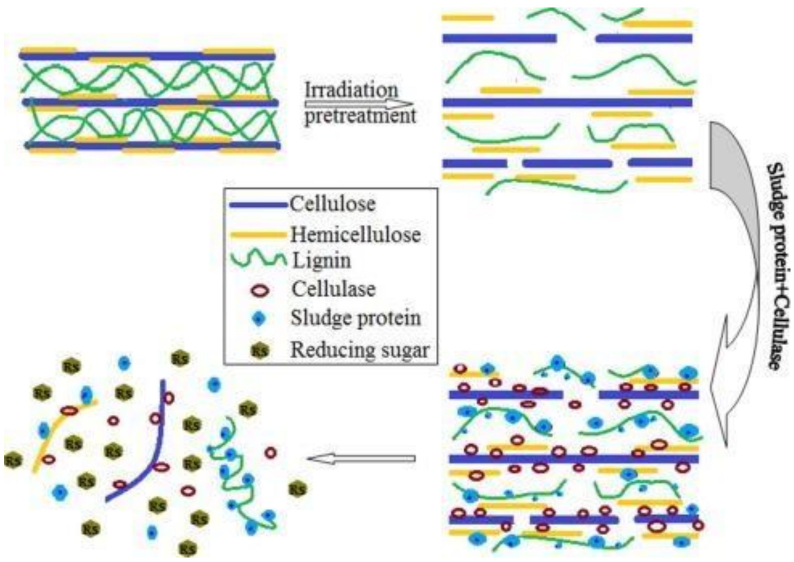


Figure 10 Cobalt-Gamma Rays Pre-Treatments Source: (Yulin Xiang, 2016)

COMBINATION OF VARIOUS PRE-TREATMENTS

Thermo-Chemical Pre-Treatments

To solubilize the particulate organic matter we are constantly depended on the different pre-treatments methods and their various mechanisms (Valo A, 2004). Thus for better performance we can combine two different pre-treatments methods to obtain maximum biogas production and lower HRT and faster AD kinetics.

Another study investigated the AD of OFMSW with combining high temperature microwaves and hydrogen peroxide (chemical) pre-treatments (Kim S, 2012). The combination of microwaves at high temperature (145 C) with chemical pre-treatment helped in lowering the biogas production and increasing the per gCOD. Similarly, as stated in the research of one of the literatures when pig manure was pre-treated with lime and high temperatures (>110C) there was lower biogas production. (Carrere H S. B., 2009). This trend could be justified by seeing the complex polymers (such as melanoidins) formed when the proteins and carbohydrate are hydrolysed at higher rate in the chemical pre-treatment and the amino acids and sugars are heated together with the hydrolysed.

However, thermal pre-treatments with alkaline pre-treatments resulted in increased biogas formation (78%) of with methane content raised to 60% even at lower temperature rather than higher temperature(28% biogas and 50% methane content) (>100 C). reduction in hemicellulose increases AD process.(Carrere H S. B., 2009).\

Thermo-Mechanical Pre-Treatments

When thermal pre-treatments is coupled with mechanical treatment this treatment was not researched much for OFMSW. Zhang et al. found the highest increase in biogas production (17%) when mechanical technology- grinding (up to 10 mm) for rice straw and then applying heatup to 110 C (Melamane X, 2007). comparing the hydrolysis yield when sludge is pretreated with a ultrasonic and alkaline pre-treatments the highest rate of hydrolysis was achieved of 211 mg/l min (Elliot A, 2012;). Wett et al. (2010) did his study on the sludge disintegration when pre-treated at 19–21 bar pressure and 160–180 C for 1 h. this study gave results in increased 75% biogas production with greater dewatering characteristics, this reduced the disposal cost by 25%. However, ammonia concentration was increased to 64% in the reactor due to hydrolysis of protein that leads to instability of AD process(Wett B, 2010).

The work of Schieder et al.(2000) comprisedthe temperature and pressurecatalysed (160– 200 C at 40 bar for 60 min) hydrolysis that had 70% higher biogas recover in a period of 5 days for the AD of SS(Schieder D, 2000).

Various Pre-Treatments Combined with a Two-Stage AD:

For a two stage AD process for biological pre-treatments the three stage classification includes a combined pre-treatments process. Kim et al. deliberated that 95% COD removal takes place when a semi-anaerobic CSTRs is combined with up flow anaerobic sludge blanket (UASB) reactors when treating FW, and a biogas production yield generated is 500 mg/gVS at HRT of 16 days. Theyalso reported that increasing the temperature for acidogenic stage a lower HRT of 10-12 days for the same amount of biogas with a higher methane content (67.4%) (Kim JK, 2006). Kvesitadze et al. (2012) also studied thermophilliccodigestion for the two-stage in OFMSW and pre-treating by freeze explosion of corn stalk. For a pH of 9 resultedin 104 mlH2/gVS and 520 mlCH4/gVS when alkaline pre-hydrolysis was carried out, that generated heat and electricity production to increase by 23% and 26%, respectively (Kvesitadze G, 2012).

Kim et al. (2012) also investigated the biohythane production from hydrogen and methane using a two-phase AD system fed for FW when thermally pretreated;it was found while experimenting that at least 3.4 days would be required for hydrogen production from FW (Kim S, 2012). 48% of hydrogen production was hyped when the methanogenic effluent in the hydro genesis step was applied to reduce the water usage. (Kim S, 2012).

Dark Fermentation for production of Volatile Fatty Acids (VFA’s)

Fatty acids that comprises of short-chain acids of six or less carbon atoms that are later distilled at atmospheric pressure are known as Volatile Fatty Acids. Application of VFA ranges from producing bio-plastics, bioenergy to biological nutrient removal from wastewater (BNR) (Zheng, Chen, and Liu 2010). However, producing them commercially is still a challenge and done by chemical methods. The main carbon source for biological VFAs production is sugars (glucose and sucrose) present mostly in food wastes, organic-rich sludge from wastewater treatment plants. This help in reducing the waste accumulation.

VFA’s are acids produced in hydrolysis and acidogenesis/acidic fermentation. (Sarwar 2015)However, it is well evident that hydrolysis is a rate limiting step, and thus to increase hydrolysis and production of VFA, the municipal wastewater sludge is pre-treated. Many studies have been done comparing different pre-treatment methods. VFAs consists of acetic, propionic, and butyric acids fermented from hydrolysis by-products. Hydrolysis and acidogenesis are consideredas a one process in a single anaerobic reactor Huang et al.

(2015a) stated application of VFA's when simplified into polyhydroxyalkanotes (PHA) or used in Phosphorus removal in wastewater treatment plant. The increase in VFAs results in decrease of pH in the fermentation process.

Pre-treatment of solid wastes for enhancing VFAs production

Hydrolysis involves breaking up of complex substances of waste into simpler ones. Material such as fats, lipids, proteins present in OFMSW hinders the biodegradation rate. Hence, pretreatment methods are adopted. Following table shows the type of pre-treatment method, their relevant study, and amount of VFAs enhancement (Lee 2014).

Pre-treatment can also be done by combining two or more methods but it is important to consider how much waste needs to be treated and the project cost so as to analyse solubilisation extent. However the most effective one proved to be combination of ultrasound and thermal pre-treatment (Dhar et. al).

Table 8 shows the effect of PT on production of VFAs.

Table 8 Effect of pre-treatment methods on VFA production

S.NO	**Pre-treatment Method**	Type of Wastes	Condition for pre-treatment	Effect of Pre-treatment	References
1	Acid	WAS	HCl, pH = 1 for 24 hr.	Increase in SCOD 4 times	(Devlin et al. 2011)
2	Microwave	WAS	2450 MHz waves, for min, 10°C to boiling	14.5 times increase in SCOD	(KIM, PARK, and KIM 2003)
3	Alkaline	WAS	NaOH, pH= 12	Increase in TCOD/SCOD = 5 times	(C.Bougrier et al. 2006)
4	Thermal	WAS	190 °C, 1 h	COD solubilisation of 48%	(C.Bougrier et al. 2006)
5	Ultrasonic	WAS	Frequency 20 kHz, specific energy 9350 kJ/kg initial TS	COD solubilisation of 15%	(C.Bougrier et al. 2006)
6	Biological	WAS	Enzyme used Cellulomonas uda and C. biazotea at temperature 30 °C, HRT 3 d	SCOD increased by 2.9 times	(Park et al. 2005)

Table 9 Main parameter affecting the VFAs production listed in Table below (Lee et al. 2014, Sans et al. 1995, Pereira et al.

pH

- Acidogens can't survive in extreme acidic or alkaline surrounding
- pH range 5.25 - 6.5 for waster water sludge gives higher VFA production rate

Hydraulic Retention Time

- higher HRT produces more VFA, microbes get more time to interact, afterawhile becomes stagnant.
- For OFMSW, HRT usually ranges from 2-6 days

Temprature

- the effect of temprature is minimal, depending on the microbial species observed.
- temprature range between 45° C to 70° C have no effect on VFA production

Organic Loading Rate

- At higher OLR concentration of VFA may increase but the substrate becomes highly viscous.

Applications of VFAs:

VFAs derived from the fermentation process can be used as an energy resource or used to produce electric energy or in the process of biological nutrient removal or lipids as biodiesel, or producing hydrogen. The below discussed applications are a brief summary to introduce where can the VFA be useful.

Polyhydroxyalkanoates (PHA)

(Akaraonye, Keshavarz, and Roy 2010)Biodegradable polymer families that emits carbon dioxide and water vapour into the environment. The released CO_2 is absorbed back during the biological degradation of the waste. Although suitable for the environment its application is inhibited by the higher cost to develop these polymers. Excess care needs to be taken for the parameters ammonia and phosphorous. (Bengtsson et al. 2008) showed that monitoring nitrogen and phosphorous can increase the yield of PHA. Literature showed that a yield of 40-70% PHA can be achieved by the fermented TWAS.

Electricity

A bio-electro-chemical method is developed using micro-organisms to collect chemical properties of the organic waste and use it as a source of electricity. this system is called Microbial fuel cell (MFC). Figure 11 shows the two chamber microbial fuel cell illustrating the working mechanisms of a MFC using VFAs from the fermented waste as organic substrate (Du, Li, and Gu 2007). The two chambers are separated by a proton membrane and cathodic part of the cell is aerobic. Lee et al. (2014) reported that the highest weight molecules of VFAs give higher MFC current (efficient upto 93%).

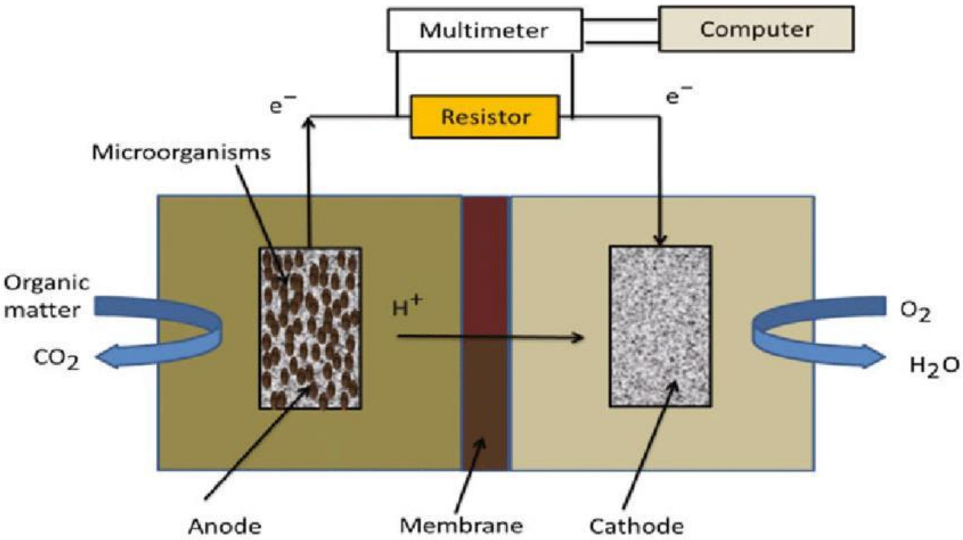


Figure 11 Microbial Fuel Cell

BNR: Biological Nutrient Removal

For nitrogen removal VFAs proves to be an important carbon substrate. the process flow is first aerobic nitrification and then anoxic denitrification. Also, phosphorous removal can be achieved with this process. the C/N ratio for this process ranges from 5 – 8 mg COD/mgN (Lee et al. 2014). Higher removal efficiencies for Nitrogen and phosphorous removal is present when waste derived VFAs are used rather than synthetic.

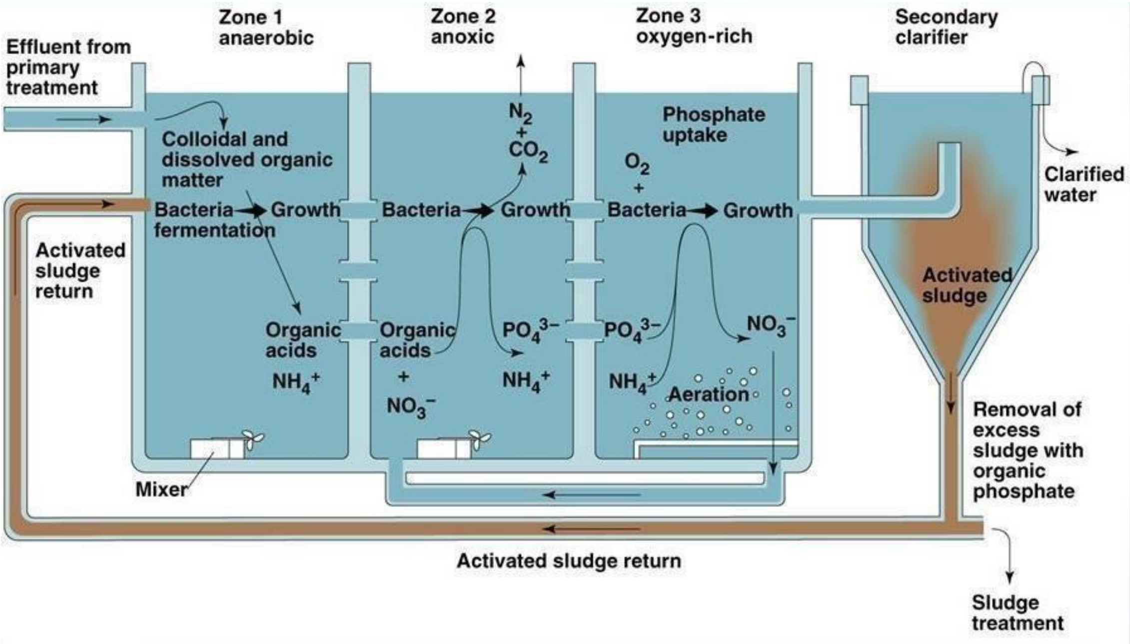


Figure 12 Biological Nutrient Removal Process (Carla, 2016)

Chapter 3

MATERIALS AND METHODS

Materials and Methods

Substrate and Inoculum

Thickened Waste Activated sludge was used in this study as feed. The Hydrothermal Pretreatment of the sludge was done at 170° C with retention time 30 min at a pressure of 3 bars. The sludge was transported from Ash-bridge Waste water Treatment facility, Toronto (Ontario) every two weeks. Average flow rate at the facility in March 2018 was reported to be 563.7 ML/Day serving population of 1,603,700. (City of Toronto 2018). For this study the main purpose was to compare the VFAs production and COD solubilisation on HTP TWAS. Hence, two digester namely RAW and HTP (or System 1 and System 2) were run for 36 days. Substrates were fed to the digesters daily and effluent obtained from them were analysed. The anaerobic digestion tanks at Ashbridge Wastewater Treatment Facility operates at mesophilic temperature range (34–38◦C) and HRT of18days for the sludge. the Organic loading rate of the digester is approximately 1.1 kg TVS/m3 (TVS: Total Volatile Solids) relative to digesters capacity per day.

To produce higher VFA's inoculum is generally thermally pretreated to enhance VFA producing microbes restrict the activity of methanogens. Collected seed for fermentation is heated at 70° C while stirring continuously at 60rpm. For the next 30 min the inoculum is incubated at same temperature (continuing the stirring). Lastly, the seed is cooled down before starting the fermentation (i.e. mixing with TWAS and HTP TWAS).

Hydrothermal Pre-treatment

The current study is done in association with the literature extension provided by Kakar et. al., (2019) determining the optimum temperature for HTP for producing maximum VFA and methane. Kakar et. al., (2019) suggested optimum temperature of 170^0 C. TWAS underwent high pressure thermal pre-treatment before fermentation process. It was performed in mini pressure reactor (Parr® Model 4848) having working volume of 2L. As mentioned earlier, HTP was done at temperature 170° C with retention time 30 min at a pressure of 3 bars. The first 30 minutes were required to reach the temperature, for the next 30 min the pre-treatment was carried out and finally the cooling down phase required approximately 45 minutes before opening the vessel. This mean that rate of heating was 3° C/min and cooling was 2° C/min.

Reactor vessel is attached to a thermocouple both connected to a monitor displaying the temperature of the TWAS inside. Mixing motor of variable speed is built-in which continuously stirs the sample, this avoids overheating and ensures uniform mixing. Tap water is recycled near the vessel containing sample after 30 minutes of operation, with help of flexible tubing attached on the head of the reactor. The hydro thermal reactor was operated by aspec View Parr4848 controller which has auto-tuning for temperature control and retention time.

Pre-treated Cycle and Collection

The pre-treatment of TWAS was carried out as a batch, 4 times pre-treatment would give approximately 3.2L of HTP TWAS. This sample was stored in refrigerator at 4°C and would last for 8 days feed. Laboratory analysis were done for both in influent HTP and the Effluent HTP, consisting of TCOD, TSS/VSS, Alkalinity, SCOD, VFA, pH, and Ammonia. These tests were done in triplicates.

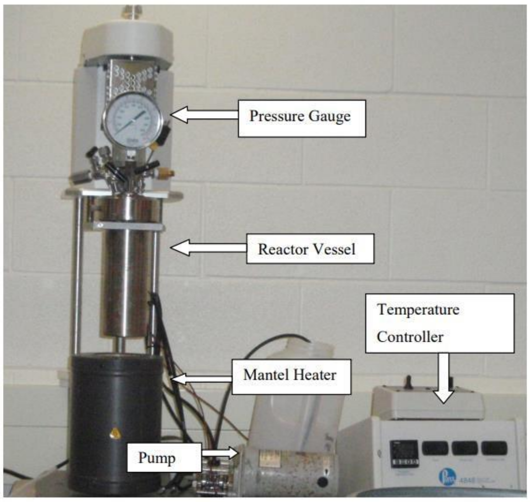


Figure 13 HTP Instrument (Sarwar 2015)

Acidification (Fermentation) Experiment

The fermentation experiment was in continuous mode under mesophilic condition. All thermally pretreated and raw samples were fermented by reactor with capacity of 1.7 liters. The food to micro-organism (F/M) ratio of 1 gTCOD/gVSS is chosen for this test. The pH was adjusted to the 5.50 by adding adequate amount of HCL or NaOH. In two reactors of 2L each, samples were added, one with raw TWAS (received from Ashbridge Treatment plant) and other one with HTP TWAS (pre-treated sample). Both the sample were fermented before starting the digesters.

To calculate the volumes of substrates and inoculums using the F/M ratio of 1 g-TCOD/gVSS following equation was used:

$$\frac{F}{M} = \frac{TCOD(TWAS)\ X\ V1}{VSS\ (SEED) X\ V2}$$

Where, V1 and V2 represent the volumes of substrate and seed respectively. Nitrogen gas was purged into the digesters and then it was sealed making sure that the anaerobic condition is present.

A process flow diagram shows the experimental design of the system.

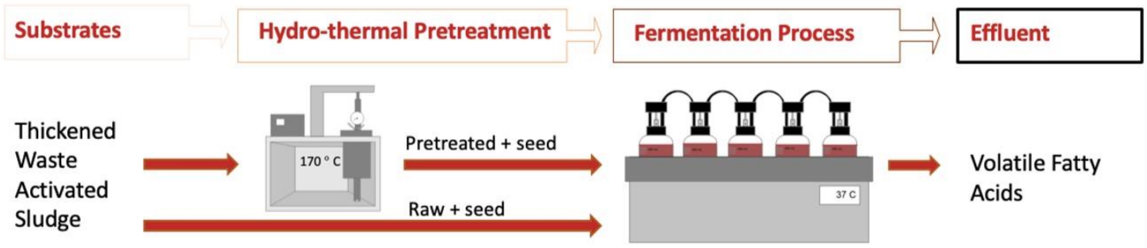


Figure 14 Experimental Design (Kakar et. al., 2019)

Significance of Solubilisation and VSS Reduction in Fermentation:

While changing the parameters (TSS/ VSS) physical properties of the TWAS gets altered. VSS solubilisation can be calculated by the equation:

$$\text{VSS Solubilisation} = \frac{VSS\ i - VSS\ f}{VSSi} \text{ x } 100$$

Where,

VSS_i and VSS_f are the VSS concentration before and after pre-treatment of TWAS respectively. In a similar study, (Burger and Parker 2013) found on pre-treating WAS at 150°C for 30 minutes 56% solubilisation of VSS occurred. Morgan et al. (2010) achieved 2030% TSS reduction with higher COD solubilisation. Hence, conversion from VSS to SCOD by HTP makes biodegradable matter easily available to microorganisms in the digestion process. The particulate matter breakdown to simple organic matter and represented as biodegradability of TWAS. COD solubilisation is the core indicator of how much HTP has on wastewater sludge that can be calculated by:

$$\text{Solubilization percentage (\%)} = (SCOD_{HTP} - SCOD_{Raw})/PCOD_{Raw} \times 100$$

$$PCOD_{Raw/HTP} = TCOD_{Raw/HTP} - SCOD_{Raw/HTP}$$

$$\text{VSS Reduction (\%)} = VSS_{Raw} - VSS_{HTP}/VSS_{Raw} \times 100$$

$$\text{COD (\%)} = \frac{SCODf - SCODi}{PCOD} \text{ x}100$$

$$\text{Mass of } SCOD_F = SCOD_F \times (V_{Sub} + V_{Seed}) - SCOD_{Seed} \times V_{Seed}$$

Where, SCODf and SCODi are SCOD after and before the HTP of TWAS. and PCOD is the particulate COD after HTP. (Burger and Parker 2013) showed that 41% of COD solubilisation occurred at 150°C HTP for 30 minutes.

Where,

$SCOD_{HTP}$: Soluble COD concentration in TWAS after HTP

$SCOD_{Raw}$: Soluble COD concentration in raw sample

$PCOD_{Raw}$: Particulate COD before feeding reactor, of the raw substrate

$TCOD_{Raw}$: Total COD of the raw sample

VSS_{Raw}: Volatile suspended solids (VSS) concentration of the raw sample

VSS_{HTP}: Pre-treated sample Volatile suspended solids (VSS) concentration of the

SCODF: SCOD mass after acidification

VSub: Substrate Volume fed to acidification reactor

SCODSeed: Soluble COD of the inoculum

VSeed: Inoculum Volume fed to both acidification reactor.

To understand the concept of COD and the portion of PCOD and SCOD, observe the following graphs:

(a) (b)

Figure 15 TCOD components: (a) TCOD RAW (b) TCOD HTP

The graph (a) shows the TCOD disintegration proportion of RAW TWAS. it contains SCOD and PCOD in much equal proportion or approximately same amount. However, after the pretreatment SCOD increases and is higher than the PCOD. The inorganic are also depicted to represent their contribution which is nevertheless, very minimal. As for the graph (B) solubilisation is increased by the hydro thermal pre-treatment and thus even the parts of heavier molecules also is converted to smaller compounds such

as proteins. However, lipids take more time and efficient techniques to be converted to SCOD, and thus still corresponds to PCOD in the effluent. Also,
TCOD = PCOD + SCOD

TCOD = 1.42 * VSS + SCOD

- ⇨ PCOD = 1.42 * VSS
- ⇨ 1.42 = $COD_{equivalent}$ of biomass/seed

This gives a clear indication of the relation between decrease in VSS results in increase in SCOD.

Operation of Digesters

Start-up of Digesters

Both anaerobic digesters (HTP and RAW) were operated from day 0 to 36 as a semi continuous fermentation process. The bench scale digesters were fed daily at the same time using influent valve. The effluent was taken out daily for the analysis. The attached gas bags showed the gas formation during fermentation process. However, gas was not measured. at the beginning of the test, the digesters were washed with nitrogen and gas bags were partially filled with nitrogen gas so as to not let any air inside the digesters.

Figure 16 AD bottle showing effluent and influent valves

On the First day, working volume of digesters was 1.8L. 1.2L of inoculum was added to 400 ml of TWAS and/or HTP TWAS, and then the digester were placed in fermentation water bath maintained at temperature of 37°C, connected with a power source. Each day the influent was added to the digesters (400ml). Effluent collected in the start-up phase, day 1 to day 13, were analysed every alternative day. Whereas, in Steady-phase the effluent was analysed every day. The amount fed was same for bothsystems. To achieve desired HRT of 3 days the working volumes of the digesters were kept same.

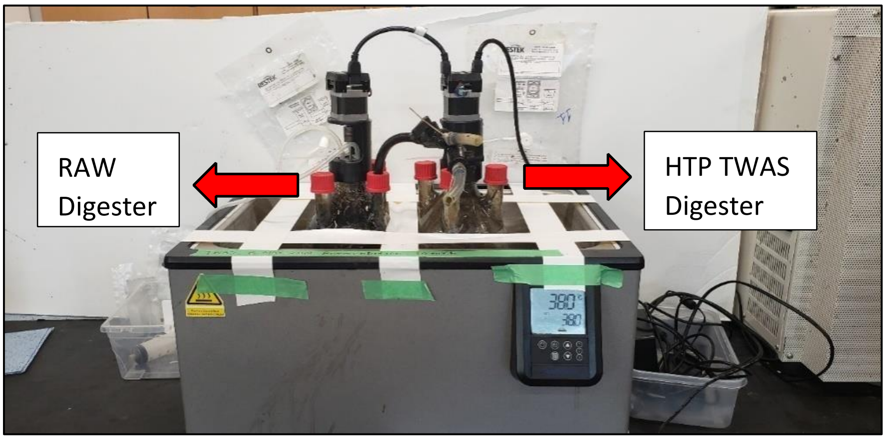


Figure 17 Experimental Design Set-up

Table 10 Specification of Experimental Design

S.NO.	Specifications	Value
1	pH	5.5
2	HRT (retention time)	3 days
3	OLR (Loading rate)	0.4L/day
4	Time	>30 days

Anaerobic Digester Monitoring

This test is basically a fermentation process, an acidogenic phase of the anaerobic treatment. the effluent collected every day had to be measured for pH range and if any value greater or smaller than pH = 5.5, then either 3.5M HCl or NaOH was added to the running digesters. The effluent was characterized by other analysis as well.

Sample Analysis

All the physical and biochemical properties listed were analysed as described in sections of Standard Methods for the Examination of Water and Wastewater (APHA 2017).

Suspended Solids

As stated in Standard Methods 2540 D and E, total suspended solids (TSS), and volatile suspended solids (VSS) were measured. All the sample must pass through Whatman Glass Microfibre filter 934 with a pore size of 1.5µm that had been previously dried at 550ºC in the oven. Aluminium dish must be the carrier for filtered samples and was then dried to be at

105ºC for at least one hour. The aluminium dish with the dried filter paper must be weighed prior to placing samples in it. After oven drying measure the weight of the sample. TSS is represented by the increase in weight. Finally, the filter paper was once again heated at 550ºC for at least 45 minutes. The weight loss represents the VSS. If the sample is to be transported to, it is important to cover the aluminium dish to prevent any foreign particle to enter.

TCOD: Total Chemical Oxygen Demand

This experiment is performed in accordance with Standard Method 5220 D (APHA, 2017). Range for HACH vials is 0-1500 mg COD /L. For measuring chemical oxygen demand (COD). Effluent was diluted prior to analysis with various dilution factors and a calibration curve was obtained in order to determine suitable dilution factor. The sample was then diluted, after that 2 ml of which was added to each vial. To minimize errors, experiment was done in triplicates along-with a blank vial(2ml of distilled water) having a COD value of 1000mg/L. After placing the sample in each vial it is incubated for 2hours at 150°C in COD reactor. The samples are allowed to cool down measured using HACH DR/2000 Spectrophotometer.

SCOD: Soluble Chemical Oxygen Demand

14 ml of sample was collected and centrifuge for 30 minutes. To perform this test use a NonSterile membrane filter, Cat. No. CA28148-584 Pall® Life Sciences, VWR, Canada, of pore size 0.45 µm membrane filter supernatant was filtered. It was then diluted and added to COD vials. After 2 hours of incubation, sample was measured in HACH Spectrophotometer.

Ammonia Standard

To measure ammonia HACH vials with a range of 0-50 ml NH3-N, use centrifuged and filtered samples similar to COD vials. Add volume of 0.1 ml of filtered sample into the vial, Ammonia Salicylate and Ammonia Cyanurate Reagents also added together. Rigorously shake the sample and keep for 20minutes. Prepare a blank sample as well. At the completion of the analysis note the reading in HACH DR/2000 Spectrophotometer.

VFAs:

For Volatile fatty acids determination there was no dilution performed. Volatile Acid TNT Reagent Set is used. To perform the analysis procedure as mentioned on the kit. Sample is collected in a glass or plastic bottle and caped tightly. Refrigerate the sample below 6^0 C if immediate analysis is not possible. Prevent agitation or exposure to air of stored sample. Use centrifuged sample passed through 0.45µm filter. Keep the pH of the sample in the range 3-9. Add 0.4 ml of Solution A (present in the kit), and add 0.4 ml of sample (centrifuged). Keep the vials in DRB200 reactor at 100^0 C for 10 min. After 10 min. sremove and cool the vials. Then, add 0.4ml of Solution B, and 0.4 ml solution C. Add 2ml of solution D. Close the cap and shake the bottle 2-3 times. Keep the sample for 3 minutes to have reaction time. Read the VFAs in the Hach machine DR/2000 Spectrophotometer.

Chapter 4

RESULTS AND DISCUSSIONS

Results and Discussions

In this section discussion mainly focused on the characteristics of the raw sludge, and the Hydro-thermal pre-treated sludge. Furthermore, the effect of HTP after the digestion is alsocompared with the initial characteristics

Initial Characteristics:

Table 11 represents the physical and biochemical properties of TWAS and SEED. this would provide a platform to compare results with the effluent obtained after the fermentation. All the performed Lab analysis are explained in the previous section and the values evaluated from them are summarized in this section. the components of characterizing the sample were TCOD, SCOD, Alkalinity, VFA, TSS/VSS, Carbohydrates, Proteins, Ammonia, and pH. The results represents average values of the various samples.

Table 11 Initial parameters of TWAS and SEED

Parameters (mg/L)	TWAS	Seed
TCOD	56850 ± 1000	23150 ± 150
SCOD	1570 ± 140	700 ± 30
VFAs	120 ± 15	70 ± 5
TSS	50000 ± 5	20 ± 2
VSS	3300 ± 5	11 ± 2
Carbohydrates	350 ± 50	NA
Proteins	400 ± 30	NA
Alkalinity	500 ± 10	900 ± 100
Ammonia	70 ± 5	
pH	7 ± 0.2	7 ± 0.1
VSS/TSS ratio	66%	50%
SCOD/TCOD	2.8%	3.02%

*Analysis were conducted in triplicates.

Table 12 Characteristics of TWAS Before and After HTP

Parameters	RAW TWAS	After HTP
TCOD	56850	40000
SCOD	1570	15700
VFAs	120	2300
TSS	50	35
VSS	35	22
Alkalinity	500	750
Ammonia	70	430
pH	7	6.1
VSS/TSS ratio	66%	62.7%
SCOD/TCOD	2.8%	40%

The TCOD is in accordance with the literature (Davidsson et al. 2008) and (Sarwar 2015). VSS/TSS ratio is 66% for TWAS and 62.7% for HTP sludge and is as expected. The SCOD/TCOD for HTP is significantly higher than the RAW TWAS. The SCOD concentration, however, observed in HTP was increased which would ensure breakdown of complex materials into simpler organics. Also, beyond a value of 3000mg/l, ammonia tends to inhibit anaerobic digestion systems (Sarwar 2015). The increase in pH justifies the presence of ammonia level, indicating finish of fermentation process. Around the end of fermentation process the pH was noted to be 6.5 ~ 7.

The effect of HTP on solubilisation/SCOD:

The acid digester shows the significance of the acid forming bacteria and are 10 times faster growing as compared to methanogens. The methane production in the acidogenic phase would lower the TCOD values. However, the reduction in TCOD values seen in the above table suggest insignificant reduction.

The following graph shows average the SCOD (of the fermentation process). As already mentioned HTP increase the solubilisation mechanism of the microbes and having a larger portion in the TCOD. A value of 40% solubilisation was achieved indicating liquidation of carbs, proteins and lipids into lower weighted compounds.(Park et al. 2005) suggested that pre-treatment for 170° C for 60min however resulted in higher COD solubilisation, and thus production of CH_4 was increased greatly. Lastly, HTP also increases metabolic activity of the sludge. Sarwar (2015) reported that sCOD solubilisation increased by 28-45% after the pretreatment. Nevertheless, the efficiency highly depend on the type of sludge, properties of sludge, chemical composition of sludge and microbes, mode of carrying out the pretreatment.

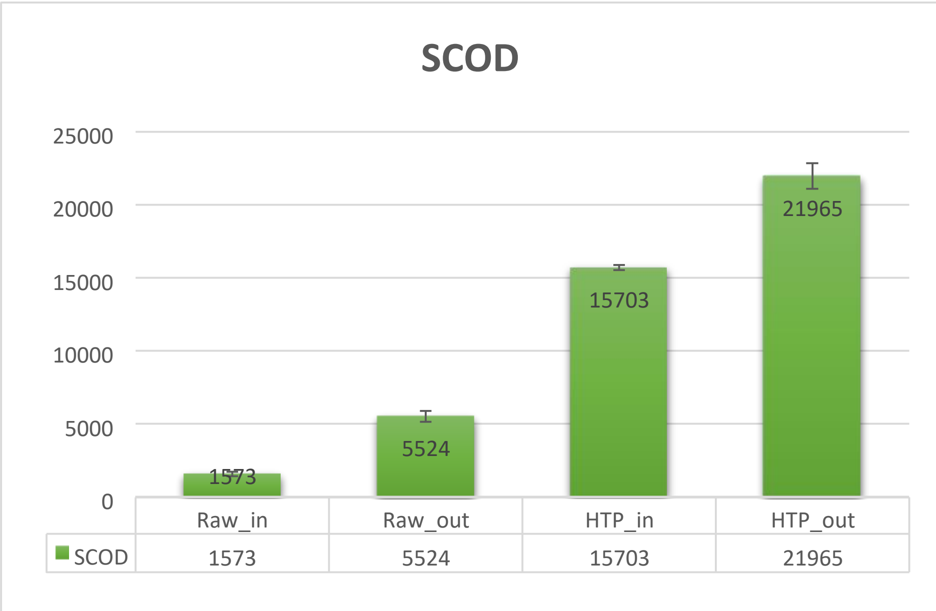


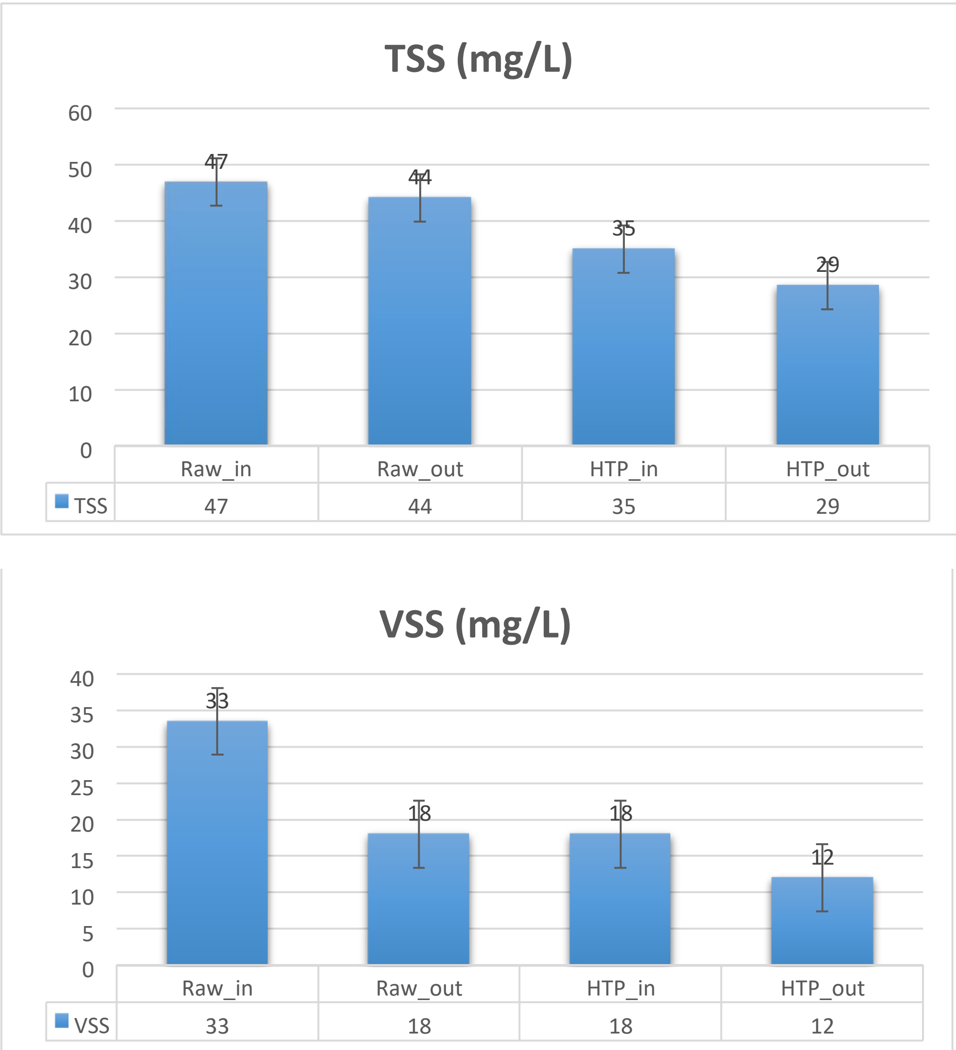
Figure 18 SCOD Values for RAW and Pre-Treated Samples

It is important to conserve the TCOD even if the SCOD increases, as it shows that all organic matter is digested. Other researchers stated that a small variance (in this study observed to be 15%) is acceptable in the TCOD value (which could be due to presence of external experimental errors or instrumental errors) (Burger and Parker 2013) (Kakar et al. 2019). Overall, the TCOD was conserved during the pre-treatment process.

VSS Reduction:

The impact of pre-treatment on particulates is usually quantified by calculating suspended solids (total and volatile) the graphs below shows the average TSS, VSS. A reduction TSS value of TWAS and HTP is seen but is not that significant. However, the TSS of RAW effluent was decreased by the fermentation process which is expected. This would suggest that the inorganic portion remained the same.

The VSS however can be seen to greatly reduce from Raw to HTP the reduction of 17% and 44% in the values of TSS and VSS were observed respectively, which comparative to the literature is approximately in the range. The TSS reduced from 47000mg/L to 44000mg/L in the TWAS effluent which shows removal efficiency to be 6.5%, for HTP, reduction is 17% and is statistically inconsequential. Similarly, For VSS the removal efficiency is slightly higher around 45% for TWAS and for HTP (18000 mg/L to 12000mg/L) is observed to 33% which is desirable.



(a)

(b)

Figure 19 TSS (a) and VSS (b) Reduction in TWAS and HTP TWAS Influent and Effluents

The % reduction is calculated by : TSS (%) = $(TSS_{RAW} – TSS_{HTP})/TSS_{RAW} * 100$

VFAs Production:

The by-products of the hydrolysis phase are consumed in the fermentation process and converted to simple sugars, amino acids and volatile fatty acids that in the methane-o-genesis would result in the CH_4 production. It is deemed to have higher VFA production to achieve higher CH_4 after the HTP to the RAW sludge. The graph below shows the significant VFA production seen after fermentation and also after HTP. Comparing RAW and HTP influents The effect of HTP on RAW sludge can be seen by the value hiking from 122mgCOD/L to 2309mgCOD/L. the increase in the values in about 18 times higher after HTP, true to the literature studied by (Sarwar 2015)(Huang et al. 2015b).Although nominal difference of 35% in the effluent VFA production is observed, it is important to analyse this, as it will contribute to methane production in the later stages. Another study showed that Acetic acid and Butyric Acids are the main components that degrade and produce methane (Hwang, Lee, and Yang 2001).

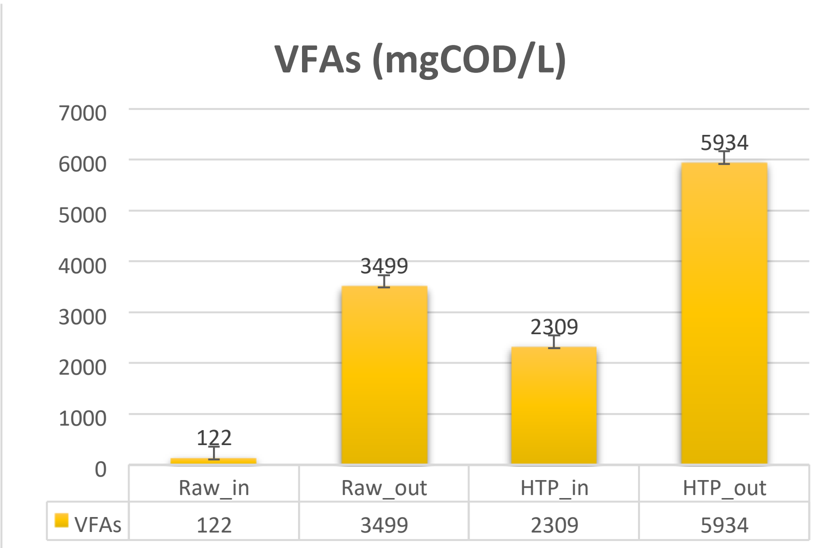


Figure 20 VFA production in TWAS and HTP TWAS Influent and Effluents

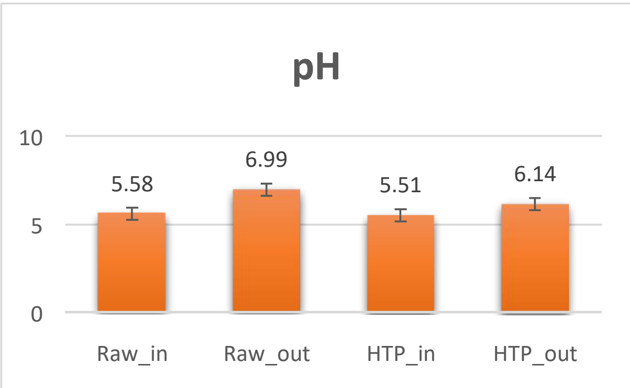
The Figure below shows the colour effect of HTP sample after centrifuging and filtering on 0.45 μm filter paper. RAW TWAS has much pale colour than HTP TWAS after the fermentation which is observed to have dark yellow colour.

Figure 21 RAW and HTP filtered Effluents colour differences

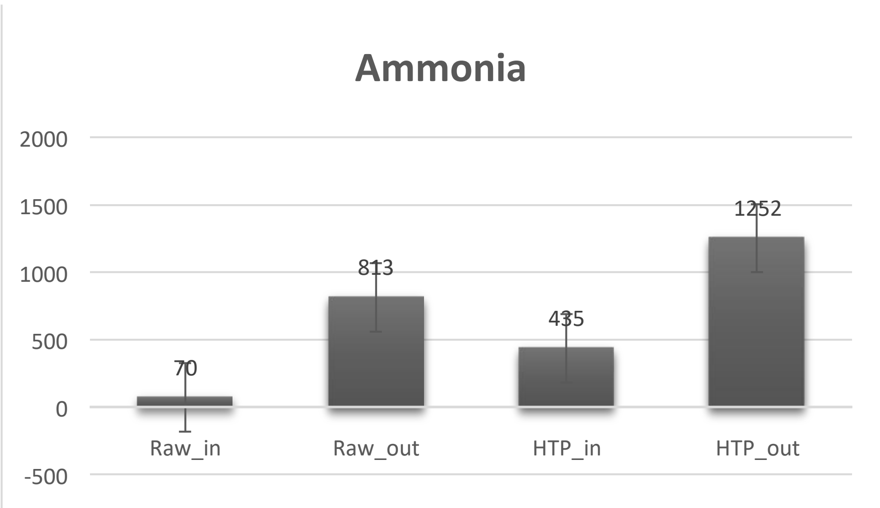
pH and Ammonia:

the pH was continuously monitored at 5.5 and any changes were adjusted by adding diluted HCL and 3.5 molar NaOH to balance the pH. However, at the completion of fermentation process all the microbes had come been emitted out and pH comes to the range of 6.5-7. This shows the beginning of methanogenesis process and forming of methane. In both the cases this was seen however, in the HTP the pH at the end of experimental process was found to 6.14

Figure 22 Graph for pH (a) and Ammonia (b) in TWAS and HTP TWAS Influent and Effluents



(a)



(b)

Ammonia was also increased from 70 mgN/L to 813 mgN/L which shows hydrolysis of proteins. According to Sarwar et al., 2015, the increase in HRT increases ammonia. Although, the increase in both system was observed to be same which implies that for the ammonia producing microorganisms the fermentation rate is similar and is not dependent on the pre-treatment mechanisms.

Chapter 5

CONCLUDING REMARKS

Concluding Remarks:

The research suggested that, thermal pre-treatment before the anaerobic digestion achieved higher Volatile Fatty Acids production and more solubilisation. After the fermentation/acidogensis process of TWAS the substrate is rich of carbon substrate and can be applied to BNR removal mechanisms. However, it is crucial to consider the sludge characteristics before deciding the thermal pre-treatment temperature and retention time. The increase in the SCOD and ammonia values was much appreciated but parameters such as proteins and lipids, need to be determined.

The study showed a positive result of the effect of thermal pre-treatment on VFA production, which is a much need bioenergy source and easily available. It would be better to conclude and recommend that a varied range of parameter still requires to be addressed such as viscosity, particle size distribution, fractional VFA (to look which all VFA, acetic, butyric, propionic etc. are present in the fermented substrate) and proteins. This would allow us to have a broader picture about the composition of the sludge and determine the efficiency of the fermentation process.

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