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Evaluation of Biogas Production Potential of Glycol-Containing Industrial Waste from Kårstø Processing Plant

Master's Thesis

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ABSTRACT

This research has been carried out to investigate the potential for methane gas production from untreated glycol containing industrial waste water from Kåstrø gas refinery, Tysvær Norway. Biogas methane potential (BMP) has been tested using a batch system (AMPTS II) at 35^0C, whereas operating condition and inhibition has analyzed using Continuous Stirrer Tank Reactors (CSTR) at 20-25^0C. The goal of this research is to investigate the main limiting factors for anaerobic digestion (AD) for the particular type of industrial organic waste. Saline waste water gave 50% of BMP with 0.50 ± 9 g COD/ g COD of specific methane yield (SMY) and degradation time was 3 days. 20% BMP was resulted with SMY - 0.22 g COD/g COD and degradation time was 5 days from not-saline waste water. From the CFSTR system, SMY were 0.45 g COD/g COD and 0.20 g COD/g COD for saline and not-saline waste water respectively. Optimal COD loading in 1000 mL CFSTR were 40 gCOD/d and 26 g COD/d for saline and not-saline waste water. About 90% COD mass balance was achieved in RI containing saline waste water with constant yield in 18 days but RII containing not-saline waste water achieved only 19% COD mass balance in 26 days. The experimentally produced methane is lower than the expected methane production. The cause of inhibition in this AD process either by unbiodegradable COD or other long chain hydrocarbon is unknow. The experiment on anaerobic treatment of highly concentrated glycol containing waste need the pretreatment before digestion. The failure in the system concluded that VFA causes the pH reduction and as a result - the shutdown of reactor. Both batch and CFSTR system gives the close values for SMY, so batch reactor is best choice for anaerobic treatment of this waste water which will reduce both economic and operational costs.

Keywords: *Anaerobic Treatment, Glycol Containing Waste Water, High COD, Operational Parameters, Methane Production*

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Abbreviations

AD	Anaerobic Digestion
ACP	Anaerobic Contact Process
AMPTS	Automatic Methane Potential Test System
ATP	Adenosine Triphosphate
BMP	Biochemical Methane Potential
CFSTR	Continuous Flow Stirrer Tank Reactor
CHP	Combined heat and Power
COD	Chemical Oxygen Demand
EGSB	Expanded Granules Sludge Bed Reactor
EG	Ethylene Glycol
FB	Fluidized Bed
HA_C	Hydrochloric Acetic Acid
HRT	Hydraulic Retention Time
LCFA	Long Chain Fatty Acid
NT	Norwegian Technology
OLR	Organic Loading Rate
PG	Polyethylene Glycol
SMY	Specific Methane Yield
SRT	Solid Retention Time
STP	Standard Temperature Pressure
TDS	Total Dissolved Solid
TEG	Tetra Ethylene Glycol
TFS	Total Fixed Solid
TOC	Total Organic Carbon
TS	Total Solid
TSS	Total Suspended Solid
TVS	Total Volatile Solid
UASB	Up- flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid

1. INTRODUCTION

The fermentation of organic materials in the absence of oxygen, there by producing methane and carbon dioxide, is called anaerobic digestion (AD) (Henze, 2008). In the past, anaerobic digestion was used to carry out the treatment of animal manure and slurries using the stabilized treated sludge from waste water plants. From 1970's organic waste was introduced as feed stocks from industries and municipal waste. Later on, 1990's the cultivation crops were introduced as a source of feed stocks (Wellinger, 2013). So, it is clear that the production of biogas has been popular from historical point of view. Anaerobic treatment of wastewater has direct positive effect on net energy production, replacement of fossil fuels sources with biogas production, reduction of greenhouse gases, production of small well stabilized sludge called granular sludge in the bioreactor (Batstone, 2002; Henze, 2008).

Anaerobic degradation is more help for the degradation of higher molecular weight glycol compounds (Dwyer & Tiedje, 1983). Microorganisms play a vital role in the conversion and utilization of organic compound (Tchobanoglus et al., 2003). Microorganisms use glycol as a carbon sources which can be easily degraded in the absence of oxygen through acitogenesis and methanogenesis to produce biogas (Johnson & Taconi, 2007). The presence of hydroxyl group facilitated the biodegradability of ethylene in 1 to 2 weeks of incubation (Battersby & Wilson, 1989). As the fermentation highly based on the growth of specific microorganisms for specific substrate (Gaston & Stadtman, 1963; Amon et al., 2007)., *Clostridium glycolicum* has unique ability to utilize glycol and to produce methane gas. They grow well only in ethylene and propylene glycol in temperature between 22 to 37 ^{0}C and pH 7.4 to 7.6 (Gaston & Stadtman, 1963).

The glycol produces ethanol by the hydrolysis process in the first step followed by acetaldehyde in the second step. The oxidation of ethanol produces acetate and methane which will be more dependent on the low concentration of H_2 (Hydrogen). The presence of aceticlastic methanogens helps in the formation of methane with the consumption of hydrogen produced from the oxidation of ethanol (Dwyer & Tiedje, 1983). Glycol is highly biodegradable so, anaerobic digestion is the best option for energy recovery as methane and pollution control (Yuan & Zhu, 2016). It causes the organic pollution due to its high solubility and biodegradability properties, so such high strength organic waste needs to be treated before

discharging into water sources (Henze, 2008). Removal of COD is highly based on degradation rate than on HRT (Yuan & Zhu, 2016).

Ethylene, diethylene and triethylene glycols were easily biodegradable and produces natural gas methane (CH_4) and Carbon dioxide (CO_2) at 106, 97 and 98% respectively (Baltersby & Wilson, 1989). The molecular weight of ethylene 400, diethylene 1,000 and polyethylene 20,000 and degradability is inversely proportional to the number of ethylene oxide monomers per molecule (Dwyer & Tiedje, 1983).

Tri Ethylene Glycol (TEG) is used in the oil and gas industry in dehydration of gas. The reason for dehydration of natural gas is to prevent the pipelines to freeze due to humidity. As the TEG is placed into contact with gas, it strips the water out of the gas. During regeneration process of TEG, the ethanol is contaminated with dissolved salt from formation water, and other chemicals. There might contain enough benzene regarded as a hazardous waste on exceeding the concentration > 0.5 mg/L. Long exposure to benzene causes the harmful effects on the bone marrow and decreasing the red blood cells causing anemia. So, this become waste water containing glycol which need to be regenerated or treated before using or discharging.

The biochemical methane potential (BMP) is used for the determination of possible methane yield of the selected substrate, anaerobic degradability and rate of degradation whereas continuous fermentation test provides the information on the long-term performance of a substrate in the bioreactor (Wellinger, 2013). High rate anaerobic system like; anaerobic contact process (ACP), anaerobic filters, anaerobic sludge bed reactors (ASBR), Upflow anaerobic sludge bed reactor (UASB), Anaerobic expanded and fluidized bed system (EGSB and FB) are commonly in used for better and effective anaerobic waste water treatment (Henze, 2008). The used of different reactor has its own propose. Limitation on the performance of reactors is not only depend on the degradability of the glycol containing waste but it might also contain various hydrocarbon which can causes the inhibition and operational problem.

1.1 Objectives

The main objective of this work is to test the industrial glycol-containing waste and evaluate the biogas potential, as well as investigate the limiting factors: organic loading, potential sulphate inhibition and alkalinity limitations. Required degree of pre-treatment for the substrate

is to be defined under the experiment too. Evaluation of the aforementioned objectives are experimentally evaluated in two types of reactors; Batch reactor and Continuous flow stirred tank reactor (CFSTR). The experimental data obtained under the experiment of high COD contain waste water can be used further for design and operational strategies for anaerobic treatment.

1.2 Novelty

Anaerobic digestion of glycol is not a new process and a large number of small and large-scale experiments have been reported. However, specific data is needed for evaluation of the biogas production potential from particular industrial organic waste with high COD level and different degree of contamination. For the application in this research it was studied the high COD glycol-containing waste from Kårstø processing plant of North Rogaland.

The novelty of this particular experimental work resides in testing raw industrial glycol-containing waste with high COD level as a potential substrate for biogas production, which at satisfying results, could be considered energy-efficient method for organic waste treatment with positive environmental effect in terms of reducing CO_2 emission, converting waste into energy, as well as getting less dependent on the fossil fuels.

1.3 Project Realization

This research was part of the fulfillment of the author's master's degree under the Environmental Engineering program at the University of Stavanger and was carried out in collaboration with Norwegian Technology AS.

Norwegian Technology is a company which focuses on development of technical solutions for municipal and industrial waste, with special attention to offshore produced waste. However, there is a number of other industries which producing large amount of hazardous organic waste, which potentially can be treated in a more environmentally friendly way. Anaerobic treatment of organic waste with production of biogas as byproduct is a large step towards waste conversion into energy strategy. The company has initiated the research to obtain more information about process of biogas production from raw industrial waste with high COD, as well as potential limitation for the process for future scale-up.

1.4 Thesis Outline

This thesis contains the following sections:

Chapter I gives information on importance of biogas production from industrial waste from point of view of circular economy and environmental aspect.

Chapter II includes the background and theory related to this research.

Chapter III contains information on the materials used and explained the clear methodology which will help to carried out the similar research in the future.

Chapter IV provides all the results from the experiments.

Chapter V provides the interpretation on results.

Chapter VI presents conclusions which have been drawn on the basis of theoretical overview and results from experimental part and also recommendations for further research of the topic.

Appendix Section contains detailed data from experimental works.

2. LITERATURE REVIEW

Biogas treatment plant produced 98% methane, which can be upgraded to biomethane and has the same properties as natural gas. The biogas can be converted to electricity by desulfurization and removing water by drying and cooling. It can be converted to heat in cogeneration unit called CHP (Combined Heat and Power) by burning. Both biogas and biomethane can be store and used as motor fuel. Biogas can be used to replace carbon compound in the plastic product. So, with all these properties of biogas, it can be supplied as sustainable source of renewable energy (Wellinger, 2013).

2.1 Role of Microorganism in Anaerobic Digestion

Role of anaerobic microorganism in the digestion of organic wastewater is to remove and breakdown the organic compounds (Tchobanoglus et al., 2003). The bacteria are similar to those found in the stomach of the ruminants which require the basic condition like; absence of oxygen, uniform temperature, pH and optimum supply of nutrients (Wellinger, 2013). Varieties of anaerobic microorganisms play a crucial role in the digestion of organic materials where the end product includes 60-70 % methane and 30% - 40% carbon dioxide and the rest are the residue of organic compounds (Jain et al., 2015) which contains 2 – 8 % H_2O, O_2 and traces of S_2 (Sulfur), as well as H_2S (Hydrogen Sulfide) (Wellinger, 2013). Batstone et al. (2002) presented the structured model for anaerobic digestion which explains the biochemical and physiochemical processes that takes place inside the reactor. The physiochemical processes describe the association and dissociation, as well as gas- liquid transfer, whereas biochemical steps describe the degradation of complex particulate waste into homogeneous particulates. This homogeneous particulate is converted to carbohydrates, proteins and lipids. The anaerobic digestion model includes reactor with a liquid volume and a sealed gas headspace at atmospheric pressure, as shown in Figure 1.

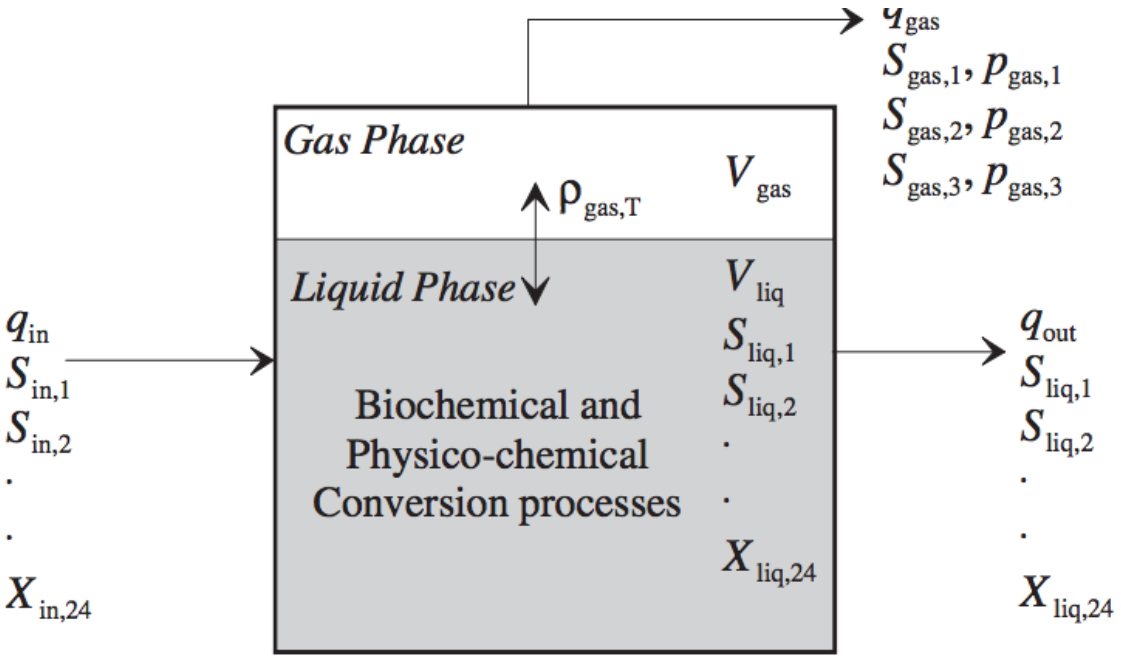

Figure 1 Input and output with constant volume of anaerobic reactor (Batstone et al., 2002)

Microbial metabolism needs energy for the synthesis. The aerobic degradation requires much more energy (Δ G°) than the anaerobic degradation. For example, for glucose, the energy required is -2882 and -428 kJ/mole for aerobic and anaerobic degradation respectively. There are two types of microorganism involved in the anaerobic degradation process; Anaerobic heterotrophic and Methanogenesis bacteria. Anaerobic heterotrophic plays an important role in acid fermentation, iron reduction, sulphate reduction and methanogenesis. the acid fermentation process gives the product of VFA (Volatile fatty acid), acetate, propionate and butyrate whereas methanogenesis bacteria consume organic compound as a source of carbon and converted it into carbon dioxide and methane gas (Tchobanoglus et al., 2003).

The best tools for the calculation of expected CH4% in the biogas production process is the function of COD/TOC ratio if the composition of organic compound is unknown as shown in Figure 2. The amount of CO_2 is less in the biogas due to its high solubility properties as well as some part is chemically bond in the water phase (Henze, 2008). TOC give the information on the total amount of organic carbon in the feed sample but does not give any information on its biodegradability and oxidation state of carbon. So, COD analysis is best for the determination of total organic carbon in the feedstocks (Wellinger, 2013).

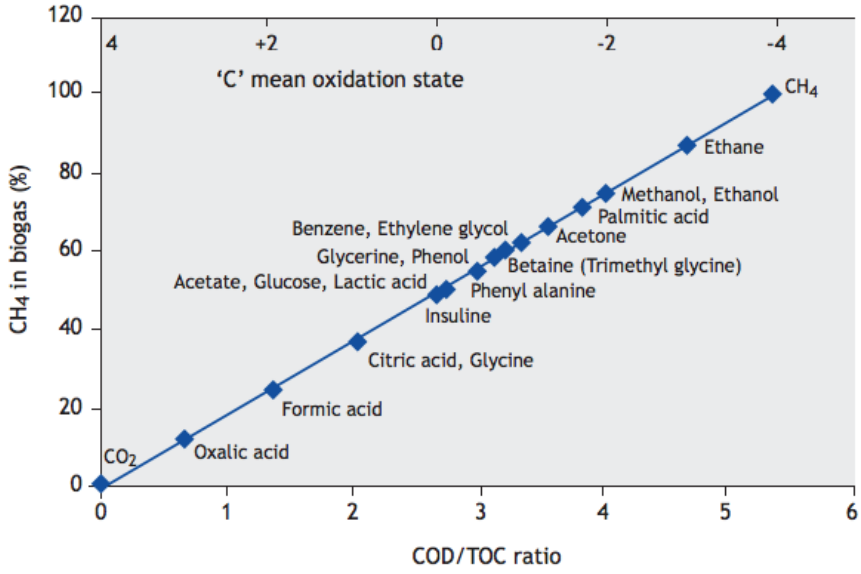

Figure 2 Theoretical estimation of biogas by cod/toc ratio from complete degradation of organic Substrate (adopted from Henze, 2008)

In the biological treatment process particulate and soluble COD is transformed into the acceptable end products. It is further fractionized into different biodegradable and non-biodegradable soluble and particulate fraction where freely biodegradable organic produces the volatile fatty acid as presented in Figure 3 (Tchobanoglus et al., 2003)

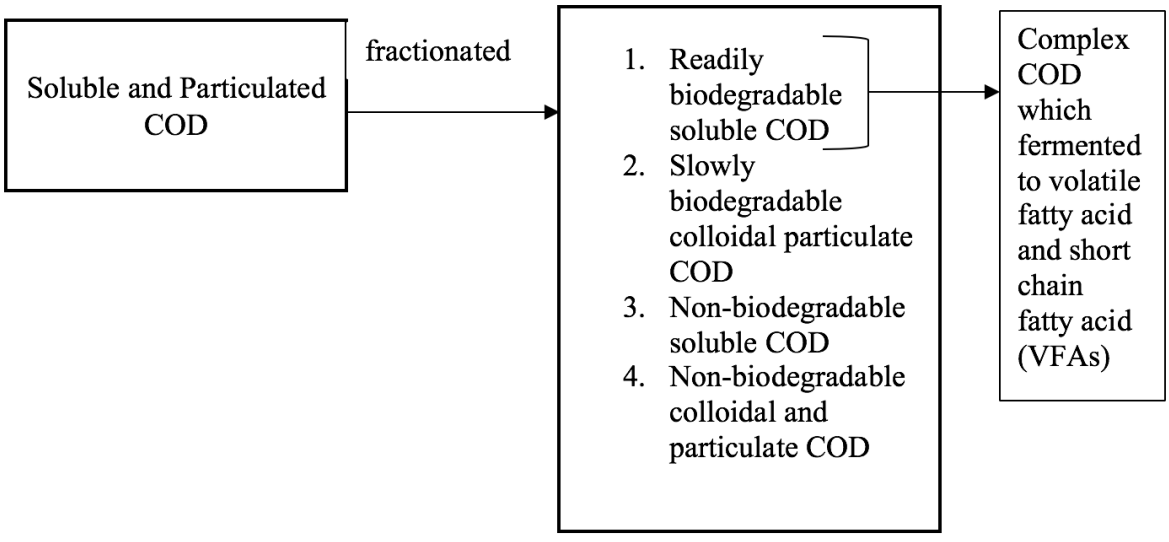

Figure 3 Fractionation of both soluble and particulate organic compounds in waste water

2.2 COD Mass Balance in Anaerobic Reactor

The overall COD balance as presented in Figure 4 is taken as a control tools to examine the operating condition of the anaerobic system. From the COD balance, it is said that there will be 10% sludge and 90% CH_4. But in general, 70% of COD flux is assumed to be converted into CH_4 gas which indicates that there is only rearrangement of COD and no destruction in COD (Henze, 2008). All the COD that added to reactor will end up into methane which is insoluble in water and get released into the gaseous form that can be collected and used as biogas. The best calculation of produced gas and measured effluent COD helps on estimating the amount of newly grown and entrapped biomass inside the reactor (Henze, 2008).

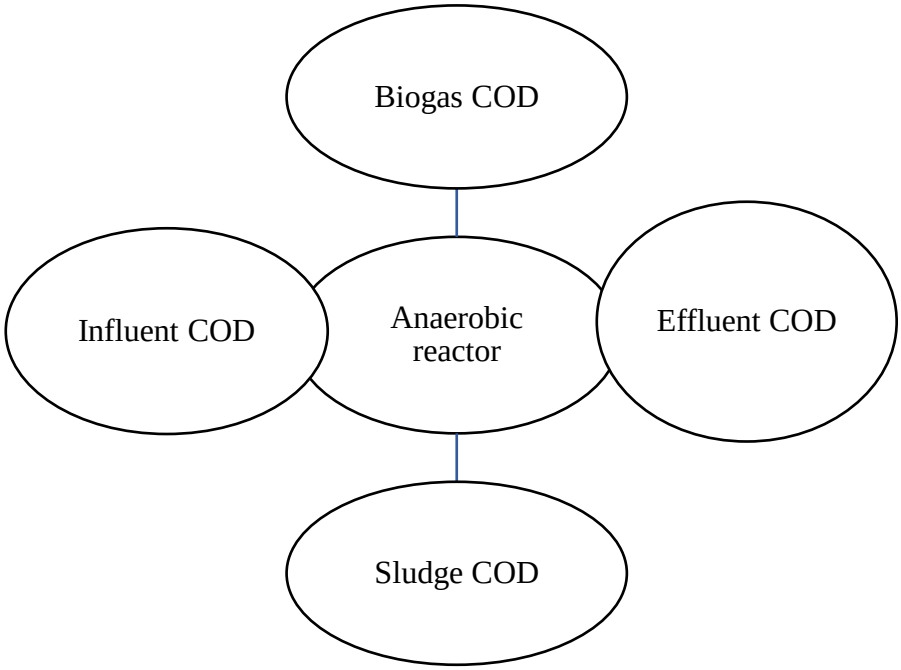

Figure 4 Mass balance of COD in an anaerobic system

So, finally COD mass balance can be calculated by measuring the COD of influent and effluent. The gap on COD balance due to some anions like SO_4^{2-} and NO^{3-} is consider either measuring all the concentration of electron acceptors or measuring all the reduced gases. The H_2S gas, and all inorganic compounds are supposed to be either end up into biogas or comes along with the effluents. Low methane production and high COD removal efficiency leads to huge gap in the COD balance which is due to Fats and Long chain fatty acid containing substrate that leads to the operational problem and failure in the anaerobic process (Henze, 2008).

2.3 Methane Production

The amount of methane produced per amount of substrate consumed at the specific time is defined as the methane yield (Henze, 2008). According to Universal Ideal gas law, volume of gas occupied by one mole of gas, at standard temperature (0^oc) and 1 atmospheric partial pressure (STP) is equal to 22.414 L CH4. This is calculated by using the Equation 2-1, defined by solubility of dissolved gas in Henry's Law, where P= pressure, V= volume occupied by gas, R= gas constant and T= temperature

$$PV = nRT \qquad (2\text{-}1)$$

Expected amount of methane production can be calculated based on influent characteristics like; flow rate, substrate concentration and biodegradability of the substrate (Tchobanoglus et al., 2003). The amount of COD of methane is the amount of oxygen utilized to oxidized methane into carbon dioxide and water as presented in the Equation 2-2.

$$\mathrm{CH4} + 2\mathrm{O2} = \mathrm{CO2} + H2O \qquad (2\text{-}2)$$

Theoretical Methane production at STP is 22.414 LCH_4 (22.4 m^3 CH_4). Methane requires 2 moles of COD (O_2), equivalent to 64 gO_2/mole CH4. i.e. conversion of CH_4 to COD under anaerobic condition is equal to 22.414 L/64 = 0.35 LCH_4 /gCOD. (1 kg COD can be converted into 0.35m^3 CH4). Similarly, theoretical COD production from the bacterial biomass ($C_5H_7NO_2$) is equivalent to 1.42 kg COD/Kg VSS.

In the experimental process, predicted methane production in the AD can be calculated by using the Equation 2-3, the mass balance of COD at steady state condition.

$$\mathrm{CODin} = \mathrm{CODout.\ Influent}_{\mathrm{COD}} = \mathrm{Effluent}_{\mathrm{COD}} + \mathrm{Biomass}_{\mathrm{COD}} + \mathrm{Methane}_{\mathrm{COD}}. \qquad (2\text{-}3)$$

In the carbonate system, the amount of CO_2 is dissolved in the liquid phase which directly influences the biogas composition as the production is highly depend on the pH change. The methane yield decreases at high organic loading rate and low retention time whereas the yield is maximum at low organic loading and high retention time (LCH4/gCOD) so, optimal loading rate and retention time is needed for AD (Sialve et al., 2009).

2.4 Digestion Steps of Anaerobic Process

Anaerobic digestion is takes place in four steps; Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis (Tchobanoglous et al., 2003; Jain et al., 2015; Ariunbaatar et al., 2014). The overview of the digestion process can be seen in Figure 5.

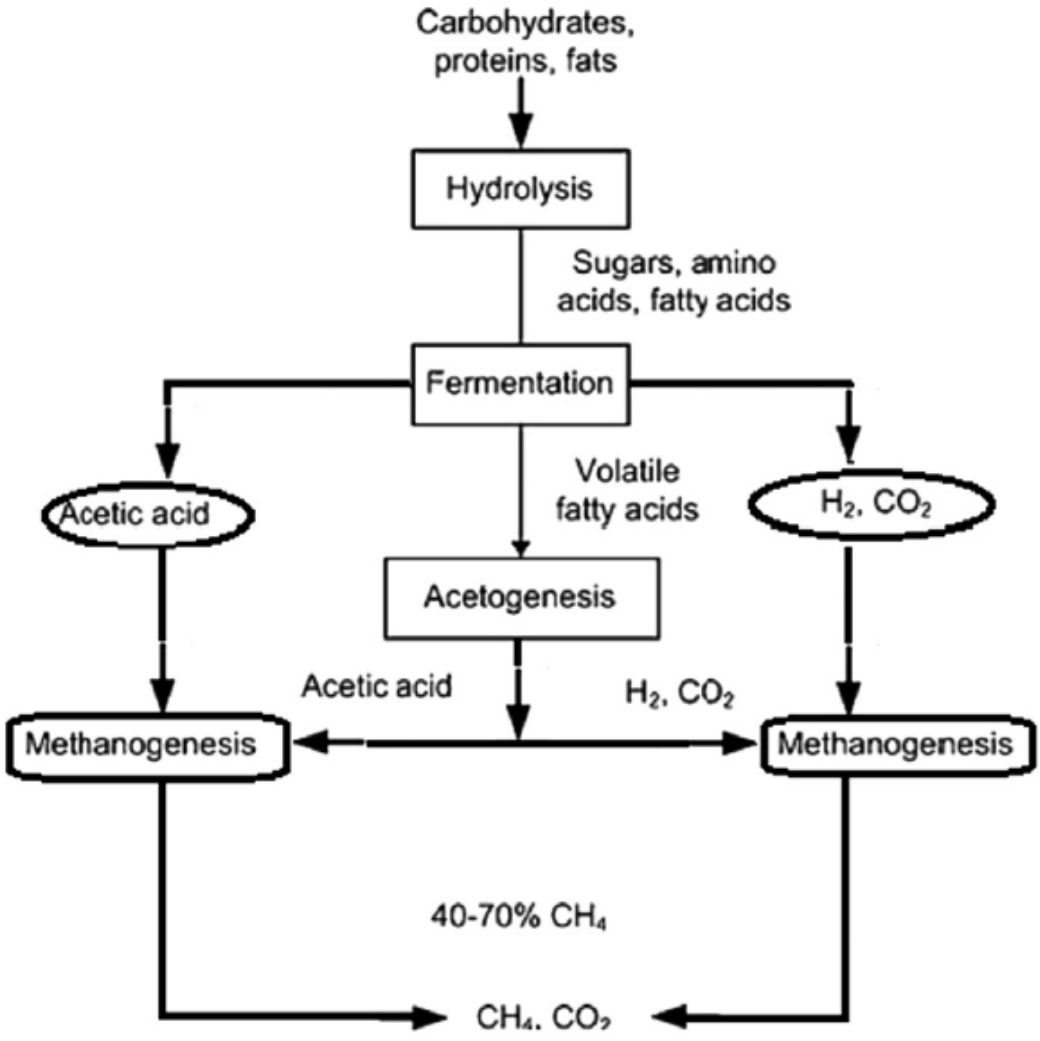

Figure 5 Steps of digestion in anaerobic process (adopted from Jain et al., 2015)

2.4.1 Hydrolysis

In this process of hydrolytic bacteria converts the complex particulate materials likes carbohydrate, protein, fats into soluble compounds like sugar, amino acids, fatty acid which will be further hydrolyzed to small monomers (Tchobanoglous et al., 2003). The degradation of complex particles into small molecules is through the action of exo- enzymes which can

pass through the microorganism cell barrier. During this process proteins are hydrolyzed to amino acids, polysaccharide to simple sugar and lipids to long chain fatty acids (LCFA) (Van Lier et al., 2008). These small monomeric and dimeric particles can be further used by acidogenesis bacteria. Hydrolysis process plays a vital role in controlling the rate of reaction and strengthen the conversion of substrate (Ariunbaatar et al., 2014). This process is more noticeable in semi solid and waste water with high suspended solid which is more sensitive to fluctuation in temperature, for example distillery slops and low temperature sewage (Van Lier et al., 2008). As an example for hydrolysis steps, triacylglycerol hydrolysis has been presented in Figure 6.

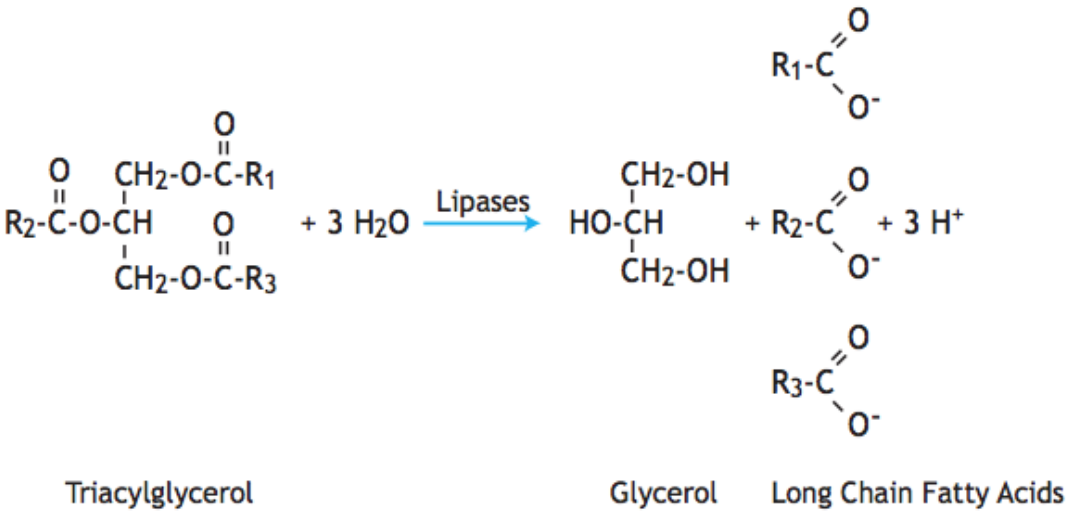

Figure 6 The Hydrolyses of lipids (adopted from Lier et al., 2008).

2.4.2 Acidogenesis

In this process acidogenic bacteria uses the soluble compounds produced from hydrolytic bacteria like amino acid, sugar, LCFA, and further ferments them into organic acid, butyric acids, propionic acids etc. (Tchobanoglous et al., 2004). The hydrolysis products are soluble and easily diffused to the bacterial cell through the cell membrane and oxidized anaerobically (Henze, 2008). Acetogenesis bacteria also produces VFA along with ammonia, CO_2, H_2S and other by-products based on source of organic waste (Appels et al., 2008). This process produces small organic compounds from the conversion of sugar and proteins mainly into VFA (i.e acetate) and carbonic acid and higher organic acids like propionate and butyrate as well as ethanol, lactic acids, and H_2. The acidogenesis reaction of sucrose as a substrate at temperature

25^0 C is presented in Table 1 where acetate, HCO_3^-, H_2 and H^+ are produces in each step of the reaction.

Table 1 Stoichiometric Reaction and Gibbs Free Energy from Sucrose as a Substrate (Van Lier et al., 2008)

Reactions	$\Delta G^{o'}$ (kJ/mol)
$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO_3^- + 8H^+ + 8H_2$	- 457.5
$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO_3^- + 6H^+ + 4H_2$	- 554.1
$C_{12}H_{22}O_{11} + 3H_2O \rightarrow 2CH_3COO^- + 2CH_3CH_2COO^- + 2HCO_3^- + 6H^+ + 2H_2$	- 610.5

The acidogenesis is the fastest reaction in the AD, resulting in the higher bacterial growth rates, conversion rate as well as higher yields which results in the accumulation of H_2, pH drop and souring inside the reactor which is well explained by the cycle diagram in Figure 7.

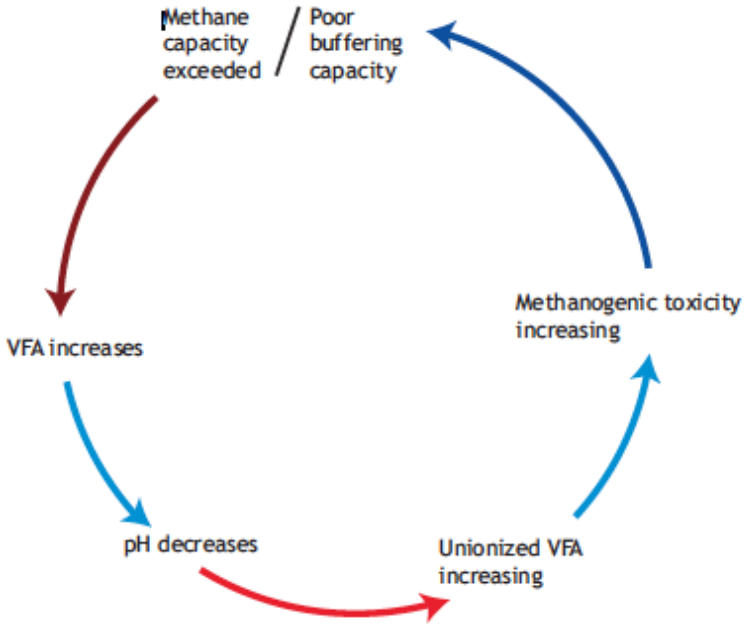

Figure 7 The result of pH drops and accumulation of VFA in the AD (adopted from Van Lier et al., 2008)

If the H_2 is removed by the H_2 scavenging organism, there will be acetate as an end product which later will be converted into methane. With the consumption of alkalinity by the produced acids, the pH will start to drop with the accumulation of unionized VFA leading to the inhibition on methane production. So, this is the main steps in AD to stabilize the system.

2.4.3 Acetogenesis

Acetogenic bacteria digested the volatile fatty acids, higher organic acids and alcohol produced by acidogenesis into hydrogen, carbon dioxide and acetic acids (Appels et al., 2008). Acetogens cannot survive in the high partial hydrogen pressure so symbiotic relationship exists in between acetogenesis and methanogenesis bacteria, where produced molecular hydrogen is utilized by methanogenesis bacteria to produce methane (Jain et al., 2015). The proper utilization of molecular hydrogen and production of methane gas takes place at the partial pressure of hydrogen between 10^{-3} to 10^{-6} as shown in Figure 8. The partial pressure of hydrogen above 8^{-10} will inhibit the methanogenesis activities and the production of acetate, H_2 and hydrogen ion takes place from the propionate and butyrate (Van Lier et al., 2008).

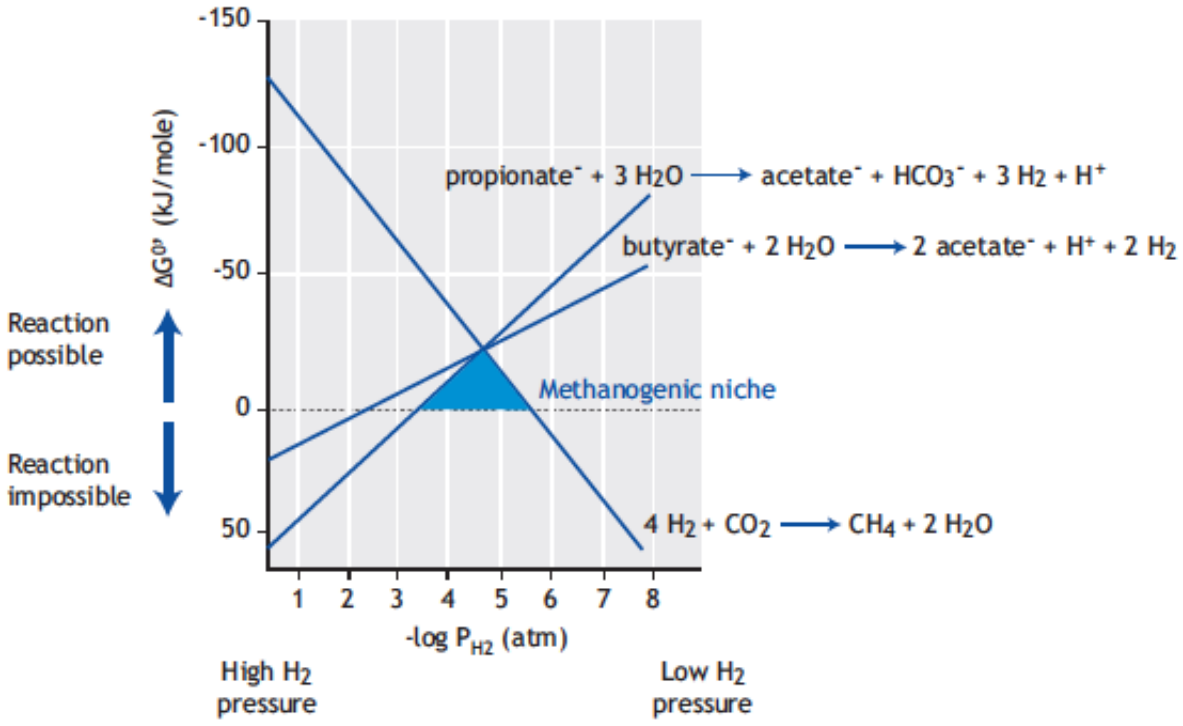

Figure 8 Change in Free Energy as A Function of Partial Pressure of Hydrogen (adopted from Van Lier et al., 2008)

2.4.4 Methanogenesis

Methanogenesis are the microbes which produces methane gas in the AD. There are two groups of methanogen organisms who consume acetates, hydrogen and carbon dioxide, produced from above processes, and convert them into methane and carbon dioxide. Organisms called aceticlastic methanogens (also called acetotrophic methanogenesis) help on splitting acetate

into methane and CO_2, while other hydrogen utilizing methanogens (called hydrogenotrophic methanogenesis) use hydrogen as electro donor and CO_2 as electron acceptor to produce methane (Tchobanoglous G., 2004; Van Lier et al., 2008). Jain et al. (2015) found that about 70% of the methane is produces from the degradation of acetic acid and about 30% from the redox reaction of hydrogen and carbon dioxide. Handling high rate of organic loading increases the specific activity of methanogenesis organisms resulting in the high methane yield. Methanogenesis process is much slower due to lower growth rate of aceticlastic methanogenesis which decoupled for several days in the production of methane. The inhibition and stability of anaerobic digestion is more depended on the utilization of intermediate product from hydrogenotrophic bacteria which has the fastest growth rate of 4 to 12 hours (Van Lier et al., 2008).

2.5 Stoichiometry Reaction in AD

The stoichiometry reaction and changes in free energy under the fermentation of different organic compound is presented in Table 2. If the fermentation process is maintained at neutral pH, room temperature 25^0C and pressure of 1 atm, the following reactions will take place where negative ΔG^0 indicates the possibility of reaction to happen and positive ΔG^0 indicated no reaction will takes place (Henze, 2008).

Table 2 Intermediate products and their reaction from AD (Henze, 2008)

Compounds	Reaction	ΔG^0 (kJ/mol)
Ethanol	$CH_3CH_2OH + H_2O = CH_3COO^- + H^+ + 2H_2$	+9.6
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O = CH_3COO^- + H^+ + 3H_2$	+48.1
Propionate	$CH_3CH_2COO^- + 3H_2O = CH^3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
Methanol	$4CH_3OH + 2CO_2 = 3CH_3COOH + 2H_2O$	-2.9
Hydrogen- CO2	$2HCO_3^- + 4H_2 + H^+ = CH_3COO^- + 4$ H20	-70.3
Acetotrophic methanogenesis	$CH_3COO^- + H_20 = CH_4 + HCO_3^-$	-31
Hydrogenotrophic methanogenesis	$CO_2 + 4H_2 = CH_4 + 2H_20$	-131

2.6 Glycol as Substrate for Anaerobic Degradation

Glycol is an organic compound belonging to the alcohol family. The most common glycol is ethylene glycol also called the 1,22- ethynediol with molecular formula $HOCH_2CH_2OH$. It is colorless, oily liquid, and toxic along with some of its derivatives. Ethylene, diethylene and triethylene glycols are easily biodegradable and can produce biogas (CH_4 and CO_2) at 106, 97 and 98% respectively (Battersby & Wilson, 1989). Propylene is another type of glycol also called 1,2- propanediol, which is similar to the ethylene glycol, but it is not toxic and used in food and cosmetics as preservative and moisture retaining agents. AD is more help for the degradation of higher molecular weight glycol compounds (Dwyer & Tiedje, 1983).

Microorganisms use glycol as a carbon sources which can be easily degraded in the absence of oxygen through acitogenesis and methanogenesis to produce biogas (Johnson & Taconi, 2007). The presence of hydroxyl group facilitates the biodegradability of ethylene in 1 to 2 weeks of incubation (Battersby &Wilson,1989). The fermentation process is highly based on the growth of specific microorganisms for specific substrate (Gaston & Stadtman, 1963; Amon et al., 2007). *Clostridium glycolicum* has unique ability to utilize glycol and to produce methane gas. They grow well only in ethylene and propylene glycol presence at temperature between 22 to 37^0C and pH 7.4 to 7.6. Carbon recovery is found 103% in ethylene and 102% in propylene (Gaston & Stadtman, 1963). It is very important to capture and utilize the methane gas produces from the AD of glycol compounds.

2.7 Growth Kinetics of Anaerobic Degradation

In the growth kinetics of AD, the kinetics of microbial process includes the kinetics of growth and substrate utilization which contributes to the biomass production in the bioreactor as Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS) (Nwabanne et al., 2009). The hydrolysis conversion rate only affects the total amount of solids converted, whereas the soluble substrate utilization rate for fermentation and methanogenesis is more sensitive for the establishment of stable anaerobic process (Tchobanoglus et al., 2003). Growth yield is lower than the values for the aerobic oxidation because the energy change for the anaerobic process is low (Tchobanoglus, 2003). Donoso-Bravo et al. (2011) proposed the first order kinetic to determine the hydrolysis rate constant, and Monod and Haldane kinetics for acidogenesis and methanogenesis respectively. First order kinetics gives the change in concentration of substrate

with time is calculated by using the Equation 2-4 (Schoenberg et al., 2001) and Monod concept for the growth kinetics is calculated using the Equation 2-5 (Nwabanne et al., 2009).

$$dc/dt = -kc \qquad (2\text{-}4)$$

where k is the degradation rate constant ($time^{-1}$) and c is the substrate concentration mg/L).

$$u = umax.\frac{S}{ks+S} \qquad (2\text{-}5)$$

Where u is the maximum specific growth rate (d^{-1}), S is the maximum substrate utilization (mg/L) and Ks is the saturation constant (mg/L)

In the anaerobic reaction typical synthesis yield for the fermentation and methanogenesis are given as; 0.10, 0.04 (gVSS/gCOD) respectively, whereas in endogenous decay for the fermentation and methanogenesis are given as; 0.04 and 0.02 (gVSS/gCOD) respectively. For the stability of anaerobic process, the concentration of VFA should be at minimum level which is the indication of existence of methanogenesis population and sufficient time for reducing VFA and H_2 concentration (Tchobanoglous, 2003).

Appropriate model is required to be used in the control theory of fermentation to maximize the production of biogas (Fedailaine et al., 2015). Anaerobic Digestion Model no.1 is the simple and more frequently used model developed by IWA (International Water Association) which includes both biochemical and physiochemical processes (Appels et al., 2008).

2.8 Pathway of Glycol Degradation

TEG contains two end hydroxyl groups and two vicinal ether groups so, it is always under influence of the hydroxyl groups and also to the ether groups. This type of PEG is expected to be susceptible to many types of reactions (Glastrup, 1996). Schink & Stieb, (1983) give the hypothetical pathway of Propylene glycol (PEG) where it is disproportionate to acetaldehyde and then to the corresponding acids and alcohol. There is no extracellular depolymerization to EG monomers. So, the propylene glycol is taken up inside the cell and undergoes into production of acid and alcohol as shown in Figure 9, 0.5 mol of ATP per mol of EG is conserved through acetate kinetic reaction. The growth yield and carbon recovery from the degradation of PEG has showed in Table 2.

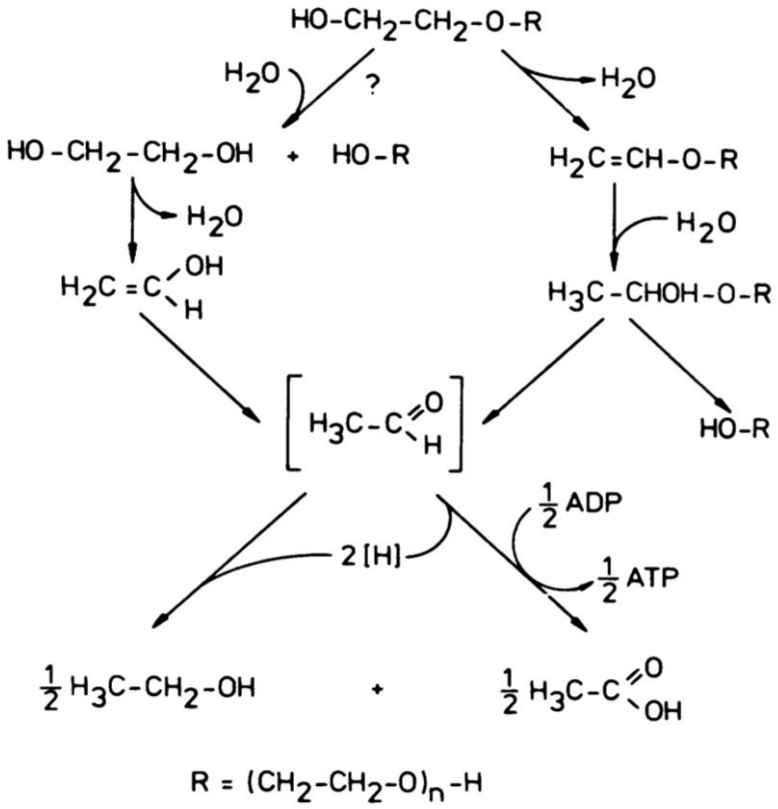

Figure 9 Hypothetical pathway of anaerobic degradation of PEG (Schink and Stieb, 1983)

Table 3 Growth yield and fermentation products of different products of glycol (Schink and Stieb, 1983)

Substrate	Amount in liter	Product of fermentation (μmol)		Growth yield	Carbon recovery
		Ethanol	Acetate	g/mol	(%)
PEG 20,000	1.0 g	218	206	3.02	101.6
PEG 6,000	1.0 g	228	190	1-25	94.1
PEG 200	1.0 g	208	200	1.94	103.2
Tri-EG	10 mmol	340	230	3.02	103.8
Di- EG	10 mmol	224	170	3.33	107.5

Dwyer & Tiedje, (1983) explained the pathway of fermentation of ethylene glycol (EG) which is shown in Figure 10.

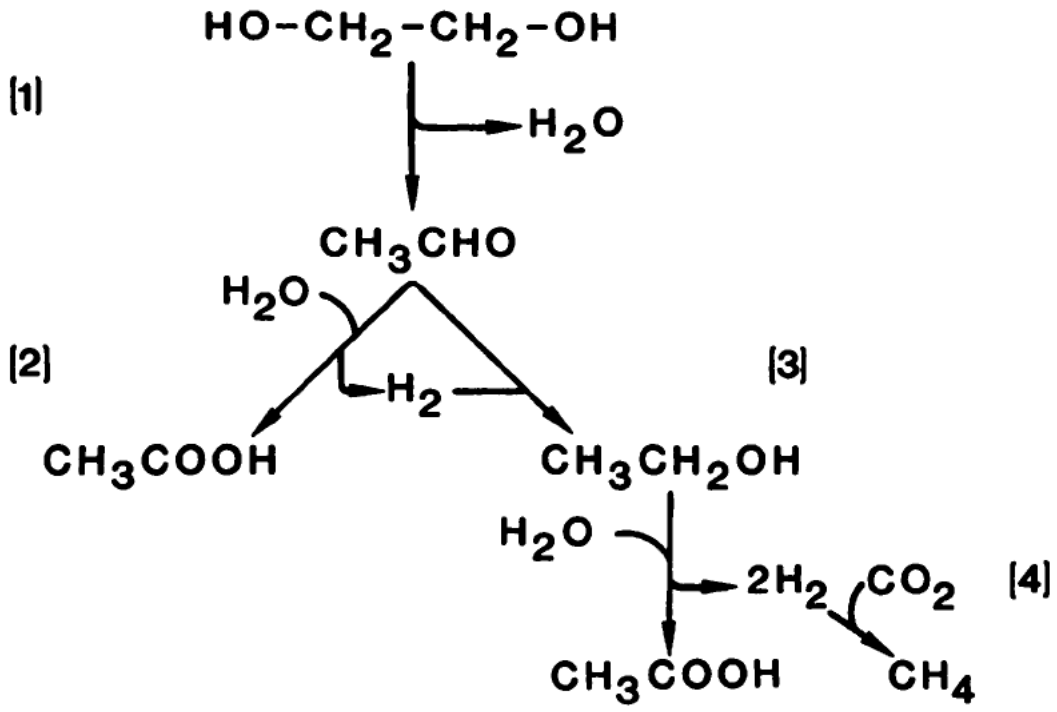

Figure 10 Proposed pathway of Ethylene Glycol (Dwyer & Tiedje, 1983)

The ethylene glycol produces ethanol by the hydrolysis process in the step 1. In step 2 acetaldehyde is formed. Step 1 and step 3 are energetically favorable. In step 4 oxidation of ethanol produces acetate and methane which will be more dependent on the low concentration of H_2. The presence of aceticlastic methanogens helps in the formation of methane with the consumption of hydrogen produced from the oxidation of ethanol.

Elreedy et al. (2016) proposed the degradation pathway of Monoethylene glycol (MEG) where the final degradation product is methane. The balanced equations are;

$$C_2H_6O_2 = C_2H_4O \text{ (Acetaldehyde)} + H_2O$$

$$C_2H_4O + H_2O = CH_3COO^- \text{ (Acetate)} + H^+ + H_2$$

$$C_2H_4O + H_2 = C_2H_6O \text{ (Ethanol)}$$

$$C_2H_6O + H_2O = HCO_3^- + CH_4$$

$$4H_2 + H^+ + HCO_3^- = CH_4 + 3H_2O$$

Under fermentation EG is converted into acetate and ethanol while the oxidation of propylene gives propionate and n-propanol and carbon recovery is 103% and 102% respectively (Gaston & Stadtman, 1963). The balanced equations for these fermentation processes are;

1. $2CH_2OH = CH_3COOH + CH_3CH_2OH + H_2O$
2. $2CH_2OH\text{-}CHOH\text{-}CH_3 = CH_3CH_2COOH + CH_3CH_2CH_2OH + H_2O$

Studies carried out by Schoenberg et al., (2001) found the low degradation rate constant of 3.5 d^{-1} for propylene glycol (PG) compared to EG of 5.2 d^{-1} at mesophilic condition (35^0C). The difference in degradation rate constants is due to complexity between anaerobic metabolic pathways of EG and PG. Low kinetic degradation rate of PE is due to the formation of propionate and n-proponal. Oxidation of propionate requires the additional metabolic steps and these steps are very sensitive to H_2 level. Degradation of propionate to acetate is favorable only at the low hydrogen.

2.9 Design Parameters for Anaerobic Digestion

The operational and environmental parameters of anaerobic digestion influence the population of microorganism (Yuan & Zhu, 2016). Digestion is very sensitive to different environmental parameters like pH, alkalinity and temperature. It is also affected by the different operational parameters like organic loadings, retention time, nutrients availability and metals.

2.9.1 Temperature

Temperature affects the physiochemical properties of substrate which influences the growth and metabolic activity as well as kinetics of different anaerobic microorganism in the fermentation process (Appels et al., 2008). Different microorganisms have their own temperature limits and digestion at higher temperature goes more rapidly than at lower temperature (Jain et al., 2015). The thermophilic bacteria grow at temperature from 50^0 C- 80^0 C, mesophilic bacteria - in between 20^0 C - 45^0 C and psychrophilic bacteria range is 4^0C - 20^0 C as shown in Figure 11. The mesophilic temperature range is the suitable for the growth of methanogenesis microorganism, so temperature of 35^0 C should be maintained for the production of methane (Jain et al., 2015). Excess temperature results in the increase of free ammonia which inhibits the growth of methanogenesis bacteria so maintaining the stable operating temperature is very important (Appels et al., 2008).

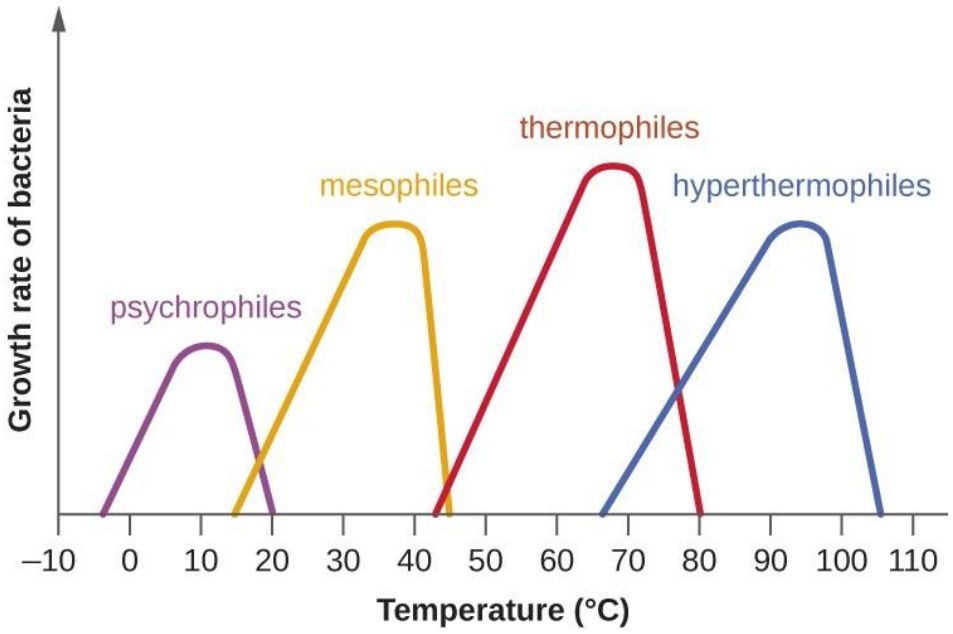

Figure 11 The growth rate of bacteria as a function of temperature (Lumen Microbiology,http://.)

2.9.2 pH

The anaerobic process is very sensitive to pH. It is the limiting factor for the growth of methane producing bacteria in the system. The pH value decreases at higher organic loading and low hydraulic retention time due to the production of volatile fatty acid. At high HRT the value of pH increases to about 7 (Zahedi et al., 2016). pH near to the neutral is preferred for enhancing the activity of methanogenic bacteria and below 6.8 causes the inhibition in the growth of these CH_4 producing bacteria. The microorganisms are very active for the degradation of organic matter in pH between 6.5 to 7.5 so the system should be buffered at this pH to prevent the failure in the system (Jain et al., 2015). The authors added the information about the important role of pH in the composition of biogas. According to their findings, CO_2 during the liquid phase in the reactor, is highly soluble in water and partially dissolved or converted to bicarbonate depending on the pH value. CH_4 end up in the gas phase as it is insoluble in water.

2.9.3 Alkalinity

There is a consumption of alkalinity during hydrolysis and fermentation stages (Yuan & Zhu, 2016). There is need to add alkalinity for the stability of the anaerobic process (Elreedy et al., 2016). The alkalinity in organic waste helps to resist changes in pH due to production of excess acid in the reactor. Due to production of CO_2 in the system the pH reduces so addition of

alkalinity as $CaCO_3$ is required in the range from 2000 to 4000 mg/L to maintain the pH at neutral level (Tchobanoglous et al., 2003).

2.9.4 Retention Time

Fermentation of organic matter is directly linked to solid retention time (SRT) and hydraulic retention time (HRT). SRT is the average time of solid spent in the digester and HRT is the average time of the liquid sludge in the digester. Growth of microbes is directly related to SRT based on which there will be decrease or increase in the rate of reaction. As shown in Figure 12, the longer the retention time, the higher is the biogas production. There will be decrease in methanogenesis population in short SRT with the increase in VFA (Appels et al., 2008).

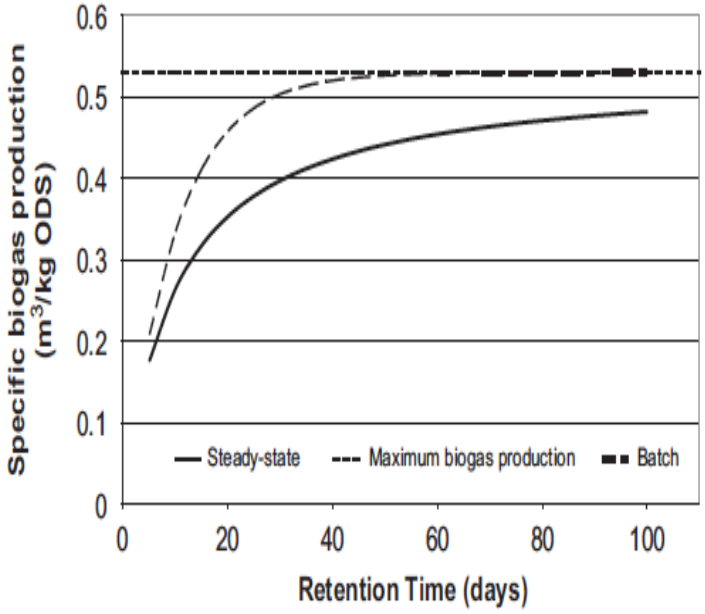

Figure 12 The Amount of Biogas Production Along Time (adapted from, Appels et al., 2008)

2.9.5 Organic Load Rate

The amount of organic materials that loaded into the reactor is called organic loading rate (OLR) which is calculated by using the Equation 2-6. Higher HRT leads to low OLR in the system which lead to higher biodegradability. At lower HRT there is the maximum production of H_2 and ethanol, whereas CH_4 production will be achieved at the peak HRT. There is increase in volatile fatty acid with the increasing OLR in the middle of the degradation process which is due to increasing in H_2 yield but further increase in OLR lead to decrease in H_2 yield (Elreedy

et al., 2016). The acidification yield is more influenced with OLR and not with HRT because yield increases with increase in the OLR in the beginning but decreases with the further increase in ORL as the acidogenesis bacteria affected and inhibited for the acid production (De La Rubia et al., 2009).

$$OLR = \frac{Flow * Concentration}{Volume\ of\ reactor} \qquad 2\text{-}6$$

2.9.6 Nutrients

Besides carbon, microorganisms also require nitrogen to form their cell proteins. But the presence of nitrogen in higher amount causes the serious problem in the production of biogas. So, the C/N ratio should be maintained at 20:1 to 30:1 (Jain et al., 2015). Besides the authors also found out the need of uniform feeding at the same time every day with the same quality and quantity to suppress the growth of acidic forming bacteria over methane forming bacteria.

2.10 Factors Inhibiting Anaerobic Fermentation

In anaerobic digestion different intermediate substances like volatile fatty acids, free ammonia and sulfate are produced. High concentrations of these substances cause the inhibition of the biogas production s and also causes the failure in the system (Yuan & Zhu, 2016). Anaerobic process is very sensitive to presence of toxic compounds in the industrial waste like organics, heavy metals and nanoparticles. Toxic components inhibit the metabolic activities of anaerobic bacteria by damaging the cell wall and result in the failure of the production of methane gas (Chen et al., 2014).

2.10.1 VFA/Alkalinity Ratio

The value of ratio between VFA and alkalinity in both acidogenesis and methanogenesis determines the stability of the system. Increased ratio causes the instability in the system thereby indicating increased H_2 yield and drop in the methanogenesis process. Further increase in the ratio results in the decrease of H_2 yield and increase in the production of CH_4 (Elreedy et al., 2016).

2.10.2 Sulphate Reducing Bacteria

In the anaerobic digestion sulphate is reduced to sulfide by sulphate reducing bacteria (SRB) (Chen et al., 2008). Colleran et al. (1995) has explained that reduction to sulfide is more favorable at pH< 6. The presence of sulphate components in the waste water has two major problems. The first one is competition between SRB and methanogenesis bacteria for the same substrate as alcohols, organic acids, fatty acids and hydrogen. Thermodynamic study shows that SRB has higher affinity for H_2 than methanogens, which can be observed also by amount Gibs energy for sulphate reduction bacteria was high than the methanogenesis reduction. It dominates over the growth of methanogenesis organism and inhibits the production of biogas

$4H_2 + SO_4^{2-} + H^+ = HS^- + 4H_2O$ ΔG^0= -151.9kJ (Sulphate reduction)

$4H_2 + HCO_3^- + H^+ = CH4 + 3H_2O$ ΔG^0= -135.6 kJ (Methanogenesis reduction)

And the second problem is that production of H_2S from sulfide is very toxic and reactive towards methanogenesis and also causes the corrosion problems in the system (Colleran et al., 1995). Inhibitory sulfide S^{2-} concentration in wastewater is 200 mg/l (Tchobanoglous et al., 2003).

$$SO_4^{2-} + C_2H_6O_2 + CO_2 + NH_4^+ + HCO_3^- + H^+ = C_5H_7NO_2 + HS^- + H_20$$

Hydrogen sulphide not only causes the 50% reduction on the biogas production, but also it is very corrosive to metal and can affect the reactor. Also, the combustion production from Sulphur oxidation causes the air pollution. The H_2S is highly soluble in water. The solubility of H_2S is higher than the methane at 35^0 C which 2650 mg/L. So, the concentration of oxidized compound in the form of Sulphur causes the inhibition of the anaerobic process. With the higher COD to sulphate ratio of substrate will produce high CH_4. During this process H2S is diluted and transferred to the gas phase. The sulphate in the aqua solution is remain as HS^- or S^{2-} form which can be determine by the Equation 2-7 (Tchobanoglous et al., 2003).

$$\text{H2S}, \% = \frac{[H2S]*100}{1+\frac{Ka1}{[H]}} \qquad (2\text{-}7)$$

2.10.3 Ammonia

Nitrogen present in the organic waste will be changed to ammonia which is the source of alkalinity for the anaerobic digestion. Optimal concentration of ammonia provides the

sufficient buffer capacity for the growth of methanogenic organism (Yuan & Zhu, 2016). Author also added that excess of ammonia may cause the failure in the system and it is toxic to methanogenic bacteria. So, proper concentration of ammonia is needed for the stability of the process. Extra cost may be needed for the further treatment of waste to meet the discharge requirement.

2.10.4 Presence of Ions and Heavy Metals

Chen et al. (2008) in their research found that the presence of ions like; Na^{+}, Ca^{2+}, Mg^{2+} helps in reducing the toxicity of ammonia. Toxicity of one ion reduces the toxicity caused by other ions. Addition of sodium ions provides good support in the methane production whereas the presence of chloride ions (Cl^{-}) inhibits the digestion. High concentration of Ca^{2+} leads to the scaling problem in the reactor and reduces the methanogenic activity (Chen et al., 2008). Heavy metals like copper, nickle, zinc, chromium, cadmium, lead play a very important role in affecting the biochemical reaction in anaerobic digestion depending on their concentration (Mudhoo & Kumar, 2013).

2.11 Anaerobic Growth and Biogas Production in the Different System

To examine the biomethane potential of the substrate, anaerobic microbes can be treated in both batch and continuous system. Both systems have its own principle and the % of methane production can be different based on all operating condition.

2.11.1 Principle of Batch System

The batch reactor is such reactor system where there is no inflow and outflow of the substrate from the system. The reactor is filled with inoculum and there is only transfer of substrate inside the reactor and filling with the inoculum. The growth of microorganism takes time to adapt to the new environment, so the number of microorganisms remains constant in the lag phase. As soon as the microorganisms adopt to the new environment the growth rate of microorganisms increases and multiplies rapidly. This phase is called acceleration phase. There is sufficient food for microorganisms for a certain hour but as long as the nutrients depleted and get some toxins inhibition there starts declining in the growth phase. The system reaches to stationary phase when the death rate is equal to the rate of cell synthesis as presented in Figure 13. The cells start using stored ATP (Adenosine Triphosphate) energy for respiration

and motion until the ATP is depleted, resulting in the endogenous phase. As ATP gets depleted the cell wall ruptures and releases carbon containing compounds as food for the remaining viable bacteria and finally decay process starts (Tchobanoglus et al., 2003).

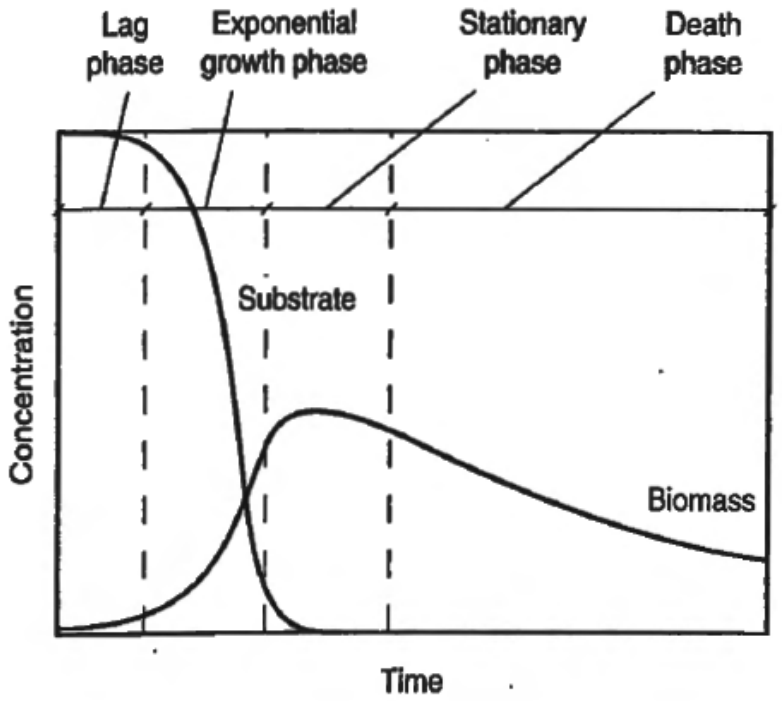

Figure 13 Microbial growth in the Batch system (adopted from Tchobanoglus et al., 2003)

The acceleration phase follows the first order reaction presented in the Equation 2-8; Kinetic model following the Monod, the material balances on substrate and biomass are presented in the Equation 2-9 and 2-10.

$$\frac{\mathrm{dX}}{\mathrm{dt}} = kX \qquad (2\text{-}8)$$

where, X= weight of dry cell/volume and k = Specific growth rate, time^{-1}.

$$-\frac{dS}{dt} = ko\,\frac{XS}{Y\,(Km+S)} \qquad (2\text{-}9)$$

$$\frac{dX}{dt} = ko\,\frac{XS}{(Km+S)} - kd\,X \;\ldots. \qquad (2\text{-}10)$$

Thus, the in the batch system both treatment and fermentation is in the same tank. The advantages for this system are ability of treating the wide range of influent volume, easy operation, effective quality control of effluent, highly flexible with low mechanical requirements and high biogas production (Mao et al., 2015).

2.11.2 Principle of Continuous System

In the continuous system input of substrate into the reactor and output of substrate takes place in a specific rate and this reaction into the system is analyzed by the mass balance (Vaccari, et al., 2006). CFSTR is used for this type of continues system as shown in Figure 14 where, the hydraulic retention time (HRT) and solid retention time (SRT) are considered to be same (Srinivas, 2008). This reactor is more suitable for more concentrated organic waste. There is no chance of separation of sludge from the wastewater due to complete mixing so there will not be the case of stratification (Srinivas, 2008). Due to rapid mixing and continuous stirring, acidification process gets more rapid resulting the accumulation of high VFA (Mao et al., 2015).

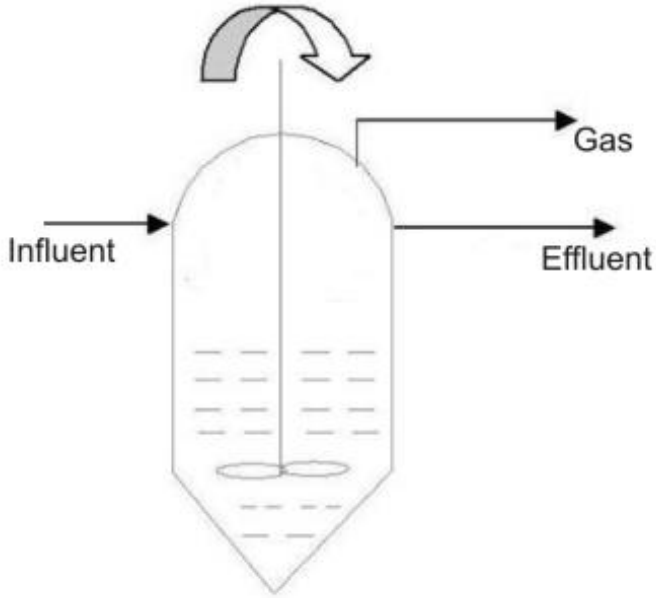

Figure 14 CFSTR used in the continuous system (adopted from Srinivas, 2008)

Inside of the well mixed CSTR the composition is uniform, so the effluent has the same composition as within the reactor. The mixing action should be sufficient to make this complete mixing of the incoming feed inside the reactor. The SRT is related to the growth of bacteria so,

to prevent the washout of microbes from the reactor, maximum SRT is best to select than the minimum SRT (Wold, 2007). The material balance in the steady state condition is given as:

Accumulation = input - output + formation by reaction.

The mass balance for the substrate and biomass are presented in the Equation 2-11 and 2-12 respectively.

$$V\frac{dS}{dt} = Qo\ So - Qo\ S + rs\ V \qquad (2\text{-}11)$$

$$V\frac{dX}{dt} = Qo\ Xo - Qo\ X + rx\ V \qquad (2\text{-}12)$$

Following the Monod kinetics, the rate of substrate conversion and biomass growth are presented in the Equation 2-13 and 2-14 respectively.

$$rs = ko\frac{XS}{Y\ (Km+S)} \qquad (2\text{-}13)$$

$$rx = ko\frac{XS}{Y\ (Km+S)} - kd \qquad (2\text{-}14)$$

Where, V= volume of reactor, S_0= Inlet substrate, S= outlet substrate, X_0= initial Biomass, X= outlet biomass, rs= specific substrate rate, rx = specific biomass rate, k_0= Specific rate constant, Y= yield of biomass

2.12 Biochemical Methane Potential Test

BMP is in widely used as an index in the anaerobic digestion of organic waste. This test is time consuming but give the significant information on the maximum potential of the substrate for the methanation. The biodegradability of the substrate can be investigated at different operational conditions. This is the best and inexpensive tools for investigating biodegradability

and bio- methanation of organic waste before implementing the biogas producing plant. In the batch system, the BMP is measured by a known quantity of waste.

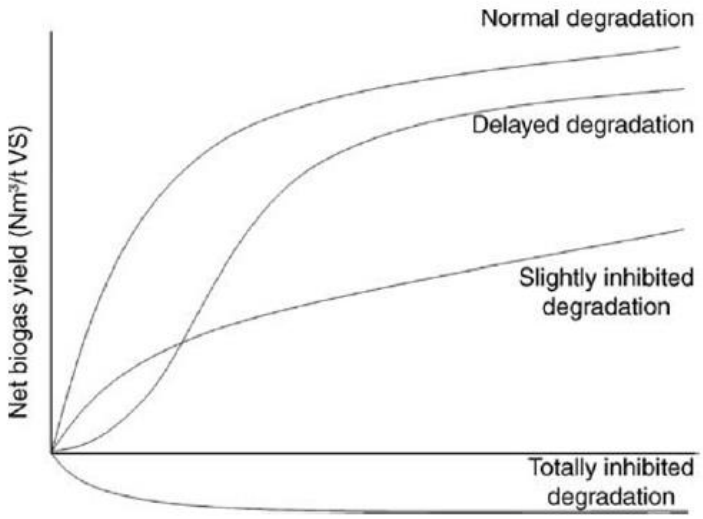

Figure 15 Explanation on degradation curves of BMP (Esposito, et al., 2012)

The plotted of data of cumulative biogas production explained the biodegradability of the substrate and its inhibition on the basis of curve's nature as presented in Figure 15. The closer the distance of the bio cumulative curve from the y- axis higher the biodegradability (Esposito, et al., 2012). The Biogas Methane Potential (BMP) is used to determine the possible methane yield and to get the information on the anaerobically degradability of the substrate that used as feedstock (Wellinger, 2013).

3. MATERIALS & METHODS

This chapter explains the laboratory experiment for BMP of glycol containing waste water from refinery gas industry, using anaerobic reactors; AMPTS II for the batch system and CSTR for continuous system. Laboratory work was started in February15, 2019 and ended in June 10, 2019.

3.1 Experiment Description

The experimental part has been carried out by implementing two types of industrial waste water containing glycol; 1) glycol waste with mixture of formation water and oil after the separation process. This type of waste is referred further in the text as Saline waste water and 2) waste water from the gas drying process after regeneration which mainly contain Tri Ethylene Glycol (TEG) and has not been in contact with formation water. This type of waste is referred further in the text as Not-Saline waste water. These two types of waste water have been provided by Norwegian Technology AS.

The initial plan was to test only one type of waste, but in the middle of experimental work the second type of substrate has been available for testing, what made the experimental work more interesting, providing opportunity not only test both types for different reactors, but also compare the biogas production potential. The performance of both Batch and CSTR reactors have been monitored and evaluated by pH, VFA, Alkalinity, COD and biogas production. The anaerobic sludge, provided by IVAR IKS, which contain various microbial populations, was used as inoculum for the experimental work.

The selected anaerobic digestion systems implemented for the experiment were the properties of the University of Stavanger (UiS), and the main laboratory work for this thesis had done in UiS. Additional test for hydrocarbon concentration, as secondary results, has been carried out by an external lab. The flow diagram of whole laboratory experiments has shown in Figure 16.

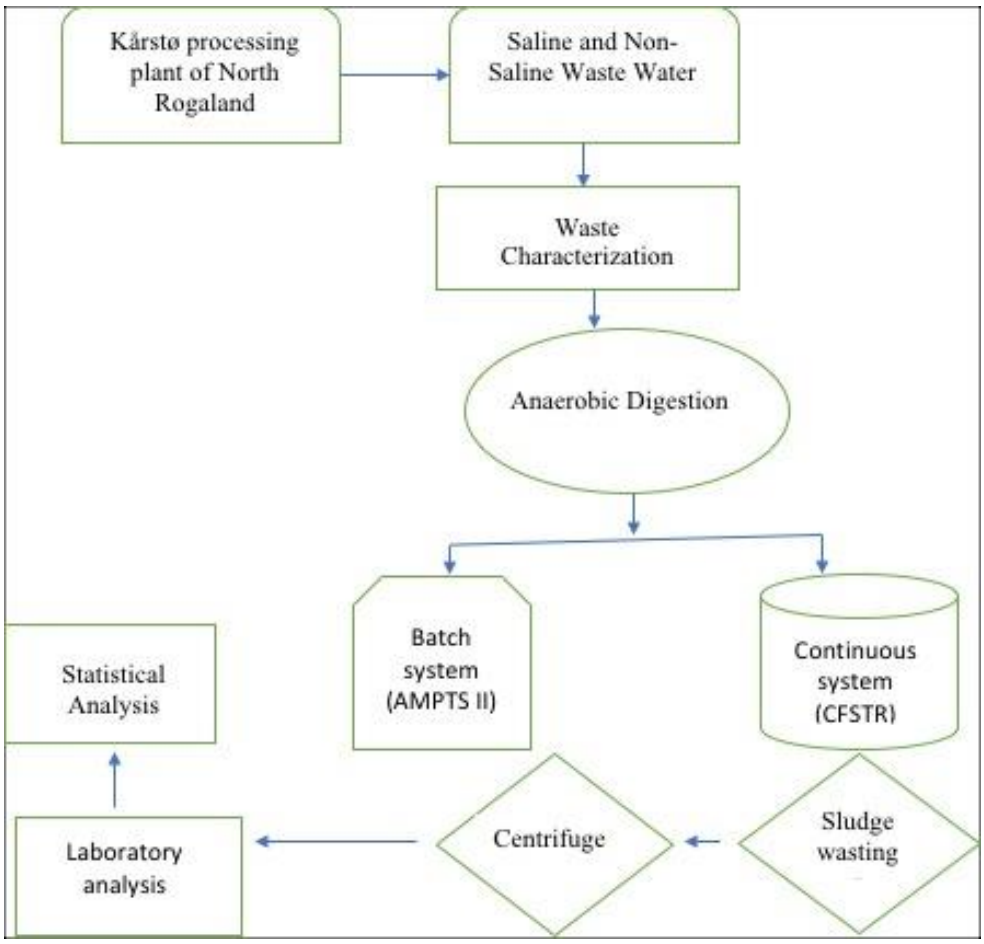

Figure 16 Flow chart of experimental process

3.2 Source of the Substrate

Two different types of substrates were investigated. As per the description of both substrates provided from Kårstø, it was collected from two different processes;

1) Saline type of substrate is the waste water after separation of light oil where the formation water was mixed. This source contains salts.

2) Not-saline type of substrate is the waste water from the regeneration after the gas drying process, which mainly contain Tri Ethylene Glycol (TEG) without salts.

In the initial the experimental work only involved the saline waste water, but later the new sample of not-saline waste water was provided for the same experiment with the same objectives as for saline waste water. However, due to late arrival of the not-saline waste water,

some tests like hydrocarbon concentration and Ion Chromatography, was not carried out for the not-saline sample.

Additionally, the visual observation of not-saline sample has shown absence of crude oil (uniform color without oil layer on the top), which was present for saline sample on the top of the barrel. That is why the additional hydrocarbon test has been decided not to carry out. However, it is worth to mention that both samples may contain some unknown components, which are not defined within this work, but which can potentially inhibit the biogas production. That is one of the points of the interest for the experimental part to see the biogas potential of these two types of raw industrial waste without any pre-treatment and define the key limiting factors.

3.3 Characteristics of the Inoculum

The inoculum from the anaerobic digestion plant of IVAR was used as biomass source for whole experimental. Data presented in Table 4 provided the details characteristics of inoculum tested by the lab technician of IVAR.

Table 4 Initial characteristics of inoculum used for both Anaerobic Systems

Characteristics	Used in saline source	Used in not-saline source
TS	6%	5%
VS	1-2%	1-2%
Alkalinity	6100 mg/l HCO_3	5566 mg/L HCO_3
VFA	423 mg HAC/l	316 mg HAC/l
pH	7.35	7.27

The total solid and total volatile solid were very less as compared to the substrate presented in Table 3. The calculated alkalinity of inoculum was much higher than the alkalinity of the substrate. It had the perfect pH value to start the digestion process. VFA of inoculum was lower than the saline waste water but was higher than the not-sample source.

3.4 Operation and Configuration of Batch Reactor

Biochemical Methane potential test (BMPT) was performed by using the instrument named Automatic Methane Potential Test System (AMPTS II) developed by Bioprocess Control, VWR. This instrument measures the amount of biogas produced from the anaerobic digestion of wastewater containing the biodegradable substrate.

The laboratory experiment was started with two batch tests. Both tests were operated for 22 days. Batch Reactor I (BR I) was run using saline waste water, and Batch Reactor II (BR II) was run with not-saline waste water, both at various COD loading. All the media in the vials were mixed by the slow rotating agitator. Nutrients and alkalinity were not added for batch test. The first unit of instrument includes incubation unit (unit A) where 15 vials containing 400 ml of anaerobic inoculum were incubated as bioreactor at a mesophilic temperature (35^0C). First two cells were used as blank using only inoculum, second two rows were used as positive control and remaining cells were used by duplication of each sample at various loading. The reactors were flushed by nitrogen gas to provide the anaerobic condition.

The second unit was CO_2 absorbing unit (Unit B), where 15 vials with alkaline solution of 3M NaOH (Product no. 106498) were kept. This unit contained two tubing parts, where one was connected to unit A and other to the biomethane gas volume measuring device, called unit C as shown in Figure 17.

Figure 17 Bioprocess control unit

The biogas produced in the unit A got passed through unit B where the traces of gas like CO_2 and H_2S were absorbed by the alkaline solution and allowed only to pass the methane gas to the unit C. Unit C measured the volume of methane gas passes, using wet gas flow measuring device with a multi-flow cell arrangement. The amount of methane gas production and flow rate per day and hour was recorded and display digitally on the computer. The details on experimental setup in both BR I and BRII were shown in Table 5.

Table 5 Experimental set up of Batch System

Cells	**Amount Added in Cells**		**Organic Loading**
1	400 ml inoculum = Blank		
2	400 ml inoculum= Blank		
3	400 ml inoculum= Blank		
4	3g starch+400ml inoculum= Positive Control		1.18 gCOD/VS*3= 3.54 gCOD
5	3g starch+400ml inoculum= Positive Control		1.18 gCOD/VS= 3.54 gCOD
	Saline waste water (BRI)	Not- saline waste water (BR II)	
6	12.34 ml of waste water + 400 ml inoculum	2.6 ml of waste water + 400 ml inoculum	1 g COD
7	12.34 ml of waste water + 400 ml inoculum	2.6 ml of waste water + 400 ml inoculum	1g COD
8	24.69 ml of waste water + 400 ml inoculum	5.2 ml of waste water + 400 ml inoculum	2g COD
9	24.69 ml of waste water + 400 ml inoculum	5.2 ml of waste water + 400 ml inoculum	2 g COD
10	37.03 ml of waste water + 400 ml inoculum	7.8 ml of waste water + 400 ml inoculum	3g COD
11	37.03 ml of waste water + 400 ml inoculum	7.8 ml of waste water + 400 ml inoculum	3g COD
12	67.72 ml of waste water + 400 ml inoculum	13 ml of waste water + 400 ml inoculum	5g COD
13	67.72 ml of waste water + 400 ml inoculum	13 ml of waste water + 400 ml inoculum	5g COD
14	98.76 ml of waste water + 400 ml inoculum	21 ml of waste water + 400 ml inoculum	8g COD
15	98.76 ml of waste water + 400 ml inoculum	21 ml of waste water + 400 ml inoculum	8g COD

3.5 Experimental Details on Continuous System

In the CFSTR system, the experiment was done in two different steps. In the first step, the experiment was done only in one reactor (RI) as a preliminary test using contaminated sources. In the second steps both contaminated and clean waste water sources were tested using two CFSTR. RI was feeded with contaminated waste water and R II was feeded with clean waste water as defined the nature of waste earlier. The both systems were operated in the dynamic conditions at different OLR, dilution rate, feeding nutrients every day and adding alkalinity directly into the feed whereas the flow rate, mixing speed, and retention time were kept constant. The reason for operating the system in dynamic mode was to observe all the probability of failure and inhibition in anaerobic digestion. The volume of the reactors was 1000 ml. Both reactors were made of glass. All the operating equipment were different as listed in Table 6 and 7. The CSTRs have been operated at the room temperature (20 -25^{0}C).

3.5.1 Configuration of CSTR

The reactors were designed and modified as per the requirement of experimental work. The specification of all equipment and materials used for both RI and RII were presented in Tables 6 and 7. Startup of the reactor was quite unstable in the beginning. The reactor was filled with 1000 ml inoculum. Inoculum was sieved with 5mm pore sized to separate fibers and to prevent disturbance in the stirring connected on the top the reactor. The gas counter filled with 1.8 % HCL was connected with the head of the reactor to observe the amount of biogas produced every day. Special gas tube was adjusted after failure in the system due to flow of HCl entering the reactor while wasting sludge. Under the stable operation the biogas produced in the system went out from the gas head to the gas counter due to pressure in the height of the biomass. The number displayed in the gas meter gave the volume of daily biogas production. Recorded biogas volume in ml was used for calculation of methane production per day.

Table 6 Specification of equipment used in R I

Equipment	Specification		Manufacture
Gas Counter	Model	MGC-1 V3.3 PMMA	Ritter
	Serial -no	0.53G.DJB/2016	
	Flowrate	1 mL/h - 1 ltr/h	
	Pressure	5- 100m Bar	
	Gas Accuracy in Measurement	3.34 ml ± 3	
Feed Pump	Type	DDA7.5-16 AR-PP/E/C-F-31U2U2FG	DDA Grundfos ® ALLDOS, France
	Voltage	100- 240 V	
	Frequency	50/60 Hz	
	Model	A9772193810001924P11411	
	Flow rate (Q)	Max- 7.5 ltr/h	
	Pressure	Max- 16 Bar	
GKH- GT Motor Control	Model	099B00002000010	Glos- Coll motor control, USA
	Serial No.	11322949	
	Voltage	240 V	
	AMPS	1.5	
	Frequency	50/60 Hz	
Mixing Rod	Cat No. Set No. Voltage	099B0000200004 11310694 130 V	ACG GLASS
	AMPS	0.3	
	Frequency	1/40 Hz	

Table 7 Specification of equipment used in R II

Equipment	Specification		Manufacture
Recirculation Pump	Pump drive 5201		Heidolph
	Serial -no	751128-1	
	Power	100-240V	
	Flow rate	5-120 l/min	
	Frequency	N/A	
		30 W	

Dosing pump	Peristaltic pump		ISMATEC®
	Power	100-240 V	REGLO ICC
	Frequency	50-60 Hz	
	Flow rate	5-120 l/min	
Gas Counter	type	MGC-1 V3.4PMMA	Ritter
	Serial -no	0.54H.A31/2017	
	Flow rate	1 ml/h – 1 ltr/h	
	Volume	3.16 ml	
	Pressure Loss	5 - 100 mbar	
	Packing liquid	HCL 1.8%	
	Accuracy in measurement	± 3	

3.5.2 Performance of CSTR

The feed, saline and not-saline waste water was pumped into the reactors at 60 ml/day which acts as food for growing biomass. The substrate was kept in the glass bottle and was continuously mixing with magnetic stirrer for homogeneity during feeding. The flow was control by dilution of the feed at different ratio. The sludge was wasted from effluent port every day to maintain the constant volume in the reactor. The system performance was monitored at different operating conditions. The adjustments for pH, alkalinity, and VFA accumulation, were done at different OLR, dilution rate. The total COD and TVS of the effluents were measured every day. Finally, the comparison on the performance of each reactor were explained in the result section. The outline of the proper installation of two CFSTR had drawn in Figures 18 and 19 for saline and not saline waste water treatment respectively. The photos have been listed in the Appendices A and B.

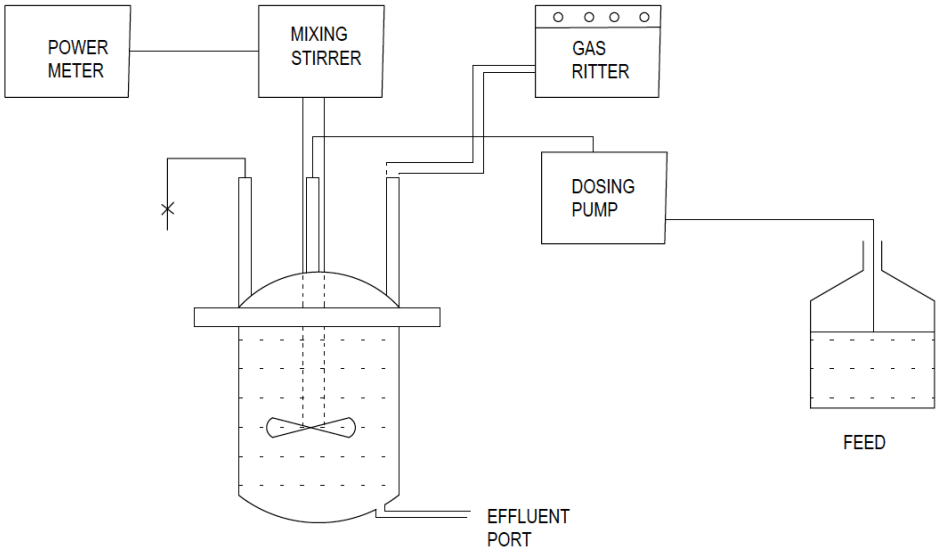

Figure 18 Experimental layout for the treatment of Saline waste water in RI

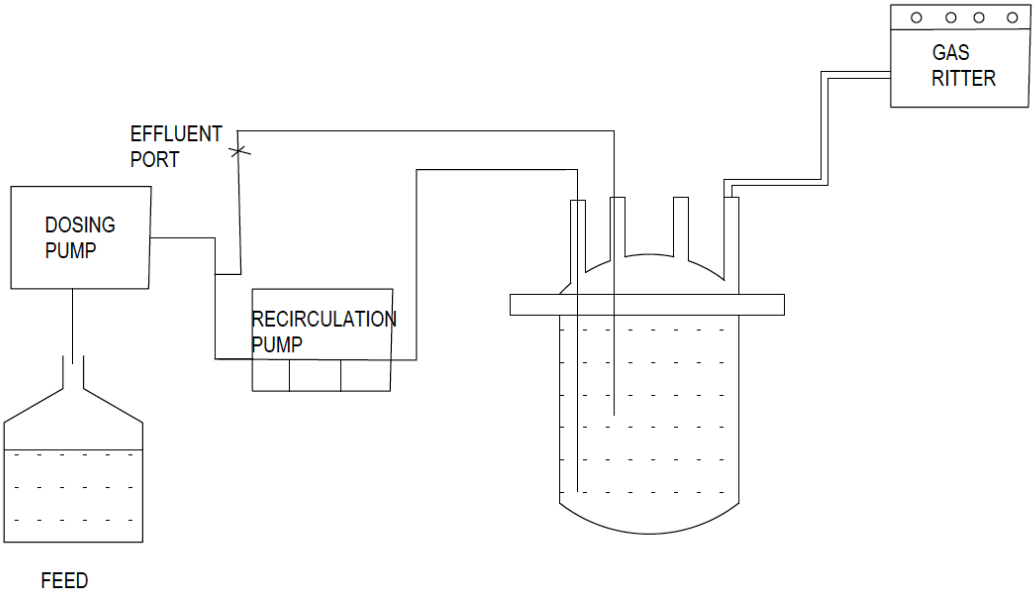

Figure 19 Experimental layout for treatment of not- saline waste water in RII

3.5.3 Analytical Methods

The different analysis on the effluent sample were carried out every day after wasting the sludge from the reactor. pH, conductivity, temperature and total COD were analyzed immediately. Samples were centrifuged for analyzing dissolved Alkalinity and VFA. TSS and VSS of the samples were calculated for analysis of biomass content from wasted sludge. The pH was maintained at optimum range between 6.5 to 7.5 for the proper production of methane

from methanogenesis. Extra alkalinity as NaHCO3 (8.4 g/L) was added in the feed to maintain the pH at optimum range (6.5 to 7.5). NaHCO3 was preferred than NaOH because it does not affect the physical and chemical environment of the system thereby no any effect to the microbial population.

The volume of biogas production was recorded every 24 hours. In the analytical process, distilled water was used for dilution and washing. The analysis of inorganic constituents of waste water is described below.

3.5.4 pH

The pH is expressed by the hydrogen ion concentration. The water with higher concentration of hydrogen ions is hard to treat with biological means (Tchobanoglus et al., 2003). The pH of sample was measured by the pH meter (VWR, Phenomenal, 1100L) immediately after the sample was collected by immersing the probe into the sample and reading was noted after the constant pH value was displayed into the meter. The pH meter was calibrated by using the buffer solution of pH 4 and 7 to minimize the error in the measurement.

3.5.5 Conductivity

The conductivity is a measure of concentration of ions in the water which increases with the increase of the ion's concentration. The unit are millisiemens per meter (mS/m). Salinity, Conductivity, and TDS of the sample have been directly determined by using the conductivity meter (VWR, Phenomenal, CO 3100L).

3.5.6 Alkalinity and Volatile Fatty Acid

The sample from the CFSTR was taken every day and centrifuge in Biofuel 17S/RS at 1000 rpm to decant water for further analysis. This process is very effective for measurement of VFA as it helps in biochemical assays by the separation of proteins, enzymes and other bio cell present in the effluent. After centrifuging the sample, it diluted to 1:10 ratio and HCl of 0.1 M is used for titration. The auto titration equipment (TitroLine@50000) consists of different units like probes, acid dosing units, stirrer, titration units and acid containing bottle. The diluted sample was stirred during titration at low rotation speed to avoid the CO_2 interference. The titration was noted in five different pH; 6.7, 5.9.5.3 and 4.3 and volume of acid used for titration at different pH was noted. The sample with pH lower or higher than 6.7 will be adjusted by

adding NaOH or HCl respectively. The obtained value of different pH was inserted in the software called Tetra 5 which gives the data for total alkalinity as CaCO3 and VFA as HAC content in mg/L. This parameter is very important for chemical and biological treatment.

3.5.7 Solids

TS, TVS, TSS and VSS were the major solid characteristics to be measured in this glycol containing waste. The amount of solid represented the carboceous compounds which is volatile and easily oxidizable. The standard methods of testing (D1252-95) was used to process and to determine the solid characteristics (Clesceri et al., 1998)

3.5.8 Gases

N_2, O_2, carbon dioxide (CO_2), hydrogen sulfide (H_2S), ammonia (NH_3) and methane (CH_4) were the common gases found by the decomposition of organic matter in the waste water, which are consider toxic, as well as had high energy value like CH_4 to produce biogas. Volume occupied by gas in ml was noted in the gas Ritter. Assuming 70% of CH4, 25 % of CO2 and 5% of other gases, the amount of gas is calculated by the ideal gas law; PV= nRT, where expected methane production after complete digestion at different temperature was listed in Table 8, which showed that higher the temperature higher will be the methane production.

Table 8 Methane production varying with temperature

Temperature	Theoretical Methane Production ($mlCH_4/gCOD$)
0^0 C	350
20^0 C	370
22^0 C	375
25^0 C	380
35^0 C	400

3.6 Measurement of Organic Constituents of Waste Water

Both total and dissolved COD was determined by using the COD test kits, ranges from 100 – 1500 mg/L). The sample was diluted by using distilled water to different ratio of 10, 20, 50, 100, 150, 200, 300, 400, 500, and 1000 since the COD concentration of the sample was so high. To determine the dissolved COD, the sample was filtered and followed same dilution ratio as above. After that the 2 ml of sample was taken and inserted into the test kits and digested into the thermo reactor for 2 hours at 148 ^{0}C. The test kits contained an acidic solution

of potassium dichromate for chemical oxidation of carboceous compound. It represents all organic and inorganic carbons that can be oxides by means of acid. The sample was swiped after 10 minute of digestion and allowed to cool for 30 mins at the room temperature in a metallic rack. The barcode in the test kits will measured the COD concentration in mg/L in the spectrophotometer (Spectroquant® MERCK TR 620).

3.7 Nutrients Analysis

Nutrients for the biomass growth in the reactor are supposed to be sufficient as inoculum from the digested reactor of IVAR requires it, and the substrate provides the good sources of nutrients as carbon, but not phosphate. As an extra source of nutrient, 30 mg/L, yeast solution was prepared. Both reactors were fed by 0.1 ml of yeast solution (MERCK, Product no.1.03753.0500) every day at the same time which is equivalent to 3 mg yeast/d. In COD unit, 4.5 mg COD/d, calculated from the specific COD of yeast which is 1.5 g_{COD}/g_{yeast}.

Total and Orthophosphate (Spectroquant®, 1.14729.0001, MERCK) and Nitrogen (Spectroquant®, 1.14763.0001, MERCK) test kits were used in the middle of the experiments to cross check the availability of nutrient inside the reactors. This analysis is more useful to control the failure in the performance of the reactor due to deficiency of nutrients for the growing microbes in AD process.

3.8 Inhibition Test

Sulfide (S2-) (Spectroquant®,1.14779.0001, MERCK) test kit was also used in the middle of the experiment to examine the inhibition by sulfide in the system during digestion. The details procedures provided by the product was followed to analyze in the effluent sample and was determined photometrically (Spectroquant Pharo 300).

3.9 Ion Chromatography Test

The Ion Chromatography test (IC) was performed to analyze the cations and anions contained in the "Saline" substrate. Synthetic sea water (SSW) was prepared using different the composition of salts. Different salts like; NaCl (0.400 mol/L), Na_2SO_4 (0.024 mol/L), $NaHCO_3$ (0.002 mol/L), KCl (0.010 mol/L), $MgCl_2.6H_2O$ (0.045 mol/L), $CaCl_2.2H_2O$ (0.013 mol/L) were taken and mixed with one liter deionized water for the preparation of SSW. The

wastewater sample was filtered with IC Acrodisc with 0.2um super membrane to filter the oil contained. The sample containing oil cannot be inserted in the IC instrument. The wastewater sample and the SSW were diluted 500 times. These diluted samples were used in IC instrument for analyzing the cations and anions existing over there. For the analysis of cations, Na^+, K^+, Mg^{2+} and Ca^{2+} were tested, whereas for the anions Cl^- and SO_4^{2-} were tested.

3.10 Hydrocarbon

Hydrocarbon test was performed as additional test to quantify the hydrocarbon concentration in saline waste water. The test has been performed by the external lab, ALS laboratory Group Norway AS by using the standard method EN ISO 9377-2.

3.11 Statistical Analysis

The data obtained from both batch and continuous system were inserted in Excel and used for further analysis for standard deviation (SD), standard error (SE) and relative error (RE). The mass balance analysis was done only in the optimized experiment of the continuous system. The potential methane production, degradation time of substrates was calculated using the cumulative methane production and Methane flow rate. The SD, SE and RE was calculated to show the accuracy in the measurement value by using Equations 2-14, 2-15 and 2-16. But this calculation of SD and SE were not carried out in single observation experimental data.

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}} \qquad (2\text{-}14)$$

$$Standard\ Error = \frac{Standard\ Deviation}{\sqrt{n}} \qquad (2\text{-}15)$$

$$Relative\ Error\ \% = \frac{Measured\ Value - Expected\ Value}{Measured\ Value} \qquad (2\text{-}16)$$

4 RESULTS

This section presents the overall results obtained from the experimental work on the industrial wastewater. Both Batch and CSTR are cost-effective biological treatment techniques with the production of methane as a bio-product. In section 4.1, the characteristics of the wastewater from both sources had explained. In section 4.2, the BMP for both waste water source and time required for degradability had explained. Finally, data on various operating parameters, probability of failure in the CFSTR system were described. All the experimental data will help to find out the way for further research on the selected samples.

4.1 Characteristic of Glycol Waste Water

Wastewater was characterized by its physical and chemical characteristics. The TVS of saline source was much higher than not-saline source, but not-saline source had more COD content along with the particulate and dissolved COD. The measured parameters are presented in the Table 8, 9, 10.

Table 9 Characteristics of Saline and Not-saline Waste Water

Parameters	**Units**	**Values**	
		Saline Waste Water	**Not-Saline Waste Water**
Alkalinity	as CaCO3 mg/L	3270.6	3563.3
pH	pH unit	7.4	7.5
Salinity	uS/cm	6.8	0.9
Volatile fatty acid (VFC)	as HAC mg/l mg/L	1770.9	197.1
Total Solid (TS)	mg/L	10510	1430
Total Fixed Solid	mg/L	6850	1230
TDS	mg/L	10500	1380
TSS	mg/L	10	50
Total volatile solid (TVS)	mg/L	3660	200
Total COD	mg/L	81000	383000
Dissolve COD	mg/L	48000	214000
Particulate COD	mg/L	33000	169000

The experimental data on hydrocarbons content of the saline source from the external lab is shown in Table 10. The higher fraction of hydrocarbon elements was found in the surface of the bulk sample which was present as an oily layer of hydrocarbon on the surface of the wastewater, as compared to bottom sample. Sample from the bottom was taken for experimental part. Due to low hydrocarbon content in the bottom sample, serious inhibition did not happen during the AD process.

Table 10 Experimental data on hydrocarbon content in the saline source

Elements		**Bottom sample**	**Surface Sample**
		ug/l	**ug/l**
Hydrocarbon fraction	>C10-C12	1110	2230
	>C12-C16	3440	6120
	>C16-C35	9910	132000
	>C35-C40	1040	33600
	>C10-C40	15500	174000
Average	>C12-C35	13400	138000
Homogenization		yes	yes

Furthermore, the experiments were carried out to examine the number of selected anions and cations in order to analysis the inhibition factors on the digestion system. The results obtained from the Ion chromatography presented in Table 11 and compared to the possible inhibition with defined range by (Tchobanoglus et al., 2003).

Table 11 Concentration of Ions Present in Saline Source

Ions	**Name of ions**	**Concentration of ions in the sample (mg/L)**	**Inhibition Range (mg/L)**	**Reference**
Anions	Sulphate ions	458.51		Tchobanoglus et al., 2003
	Sulfide S^{2-}	-	200	
	Chloride ions	1943.32		
Cations	Sodium ions	89.37	3500-5500	
	Potassium ions	93.45	2500-4500	
	Magnesium ions	17.23	1000- 1500	
	Calcium ions	166.93	2500-4500	

4.2 Experiment on Batch System

Batch tests were conducted from mid of January 2019 to mid of March. This test was used to determine the Biochemical Methane Potential (BMP) and degradation degree of the selected substrate which was explained with Figures and Tables in the following section. BMP was used to determine the possible methane yield from the selected samples.

4.2.1 BMP test of Saline Waste Water

The methane production started to increase after 1 day of lag phase. The bulk of COD was converted to methane within three days. Maximum methane production from all COD series was observed within 3 days. This indicates the saline waste water got digested within short day. After 5 days there was reduction in methane production from all series of COD which indicated by the flat curve as shown in Figure 20. The more data was not observed, as the batch test was run only for 22 days. All the experimental data obtained from batch reactor has been listed in Appendix Tables C.1 and C.2.

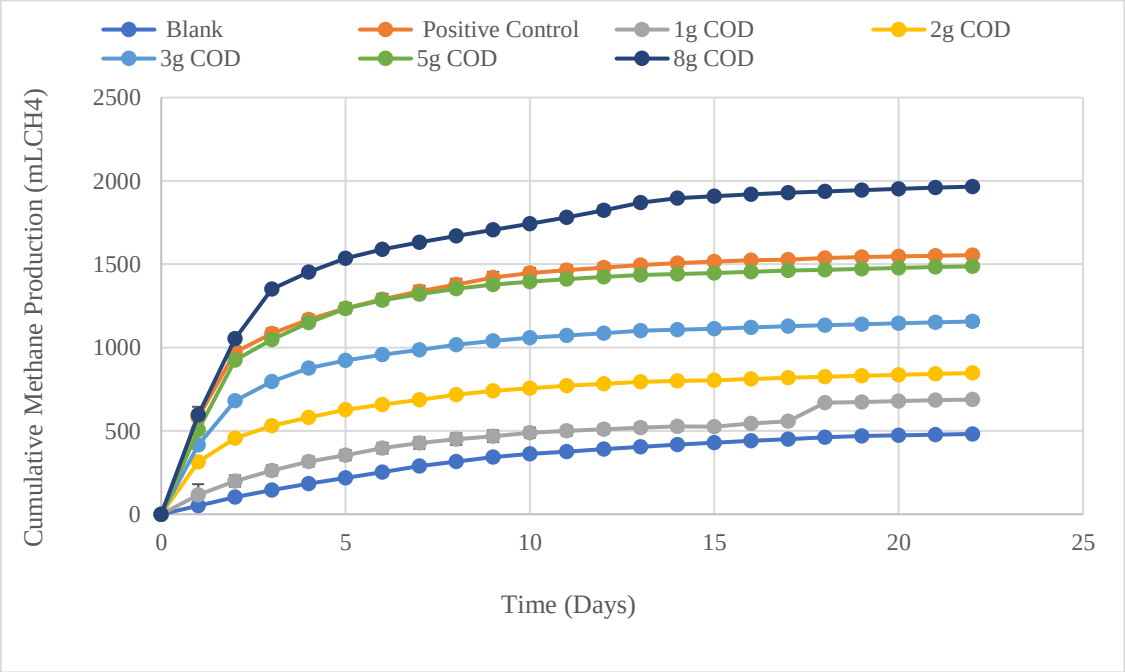

Figure 20 Cumulative Methane Production of Saline Source from BMP Test

The flow rate of methane production with respect to time for saline source was expressed in mL/day in Figure 21. The flow rate of methane reached to peaked after one day and showed

the complete degradation of COD within two to three days. All the series of COD showed the same degradation time. But the flow rate of methane was high for high COD loading series.

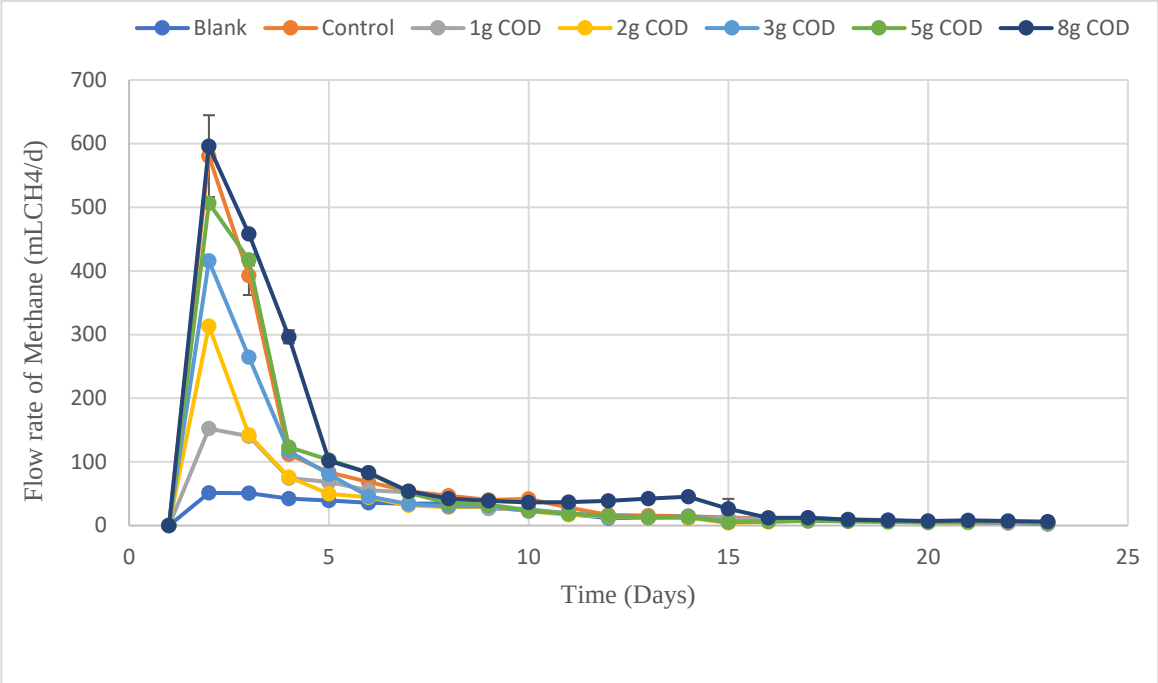

Figure 21 Methane Flow Rate at different COD Loading of Saline Source

8 g COD has high flow rate followed by 5g, 3g, 2g and 1g COD. Positive control with 3.5g COD showed the similar flow trend as 8g substrate but the deviation was high so dependency on the accuracy of the data is quite low as compared to all other substrate loading. The flow rate of methane in all COD series decreased after 5 days. The flow rate was equal to the blank flow rate after day 8. It can be concluded that the degradation time of such saline substrate is between 3 to 5 days.

The experimental data obtained from testing the digested sludge from the batch reactor was summarized in Table 12. The pH was observed maintaining at the neutral range 7.5 - 7.6, alkalinity concentration was between 5000 to 7000 mg/l as $CaCO_3$, and VFA was zero in four reactors and one reactor with 1 g COD loading has some VFA contained.

Table 12 Experimental Results of the Digested Sludge in Saline Source

Experimental Products	Units	COD Loading				
		1g	2g	3g	5g	8g
VFA	mg COD/L	215	NA	0	0	0
Alkalinity	mg CaCO3/L	5062		7053	5478	4842
COD	mg COD/L	59		45	50	50
pH	pH unit	7.6		7.6	7.5	7.6

The results showed no inhibition in the digestion as the pH and alkalinity were in good range for mesophilic activity.

4.2.2 Methane Production and Yield of Saline Waste Water

The total methane production was calculated with subtracting the volume of methane production from the blank. Theoretically, the specific methane yield (SMY) of 1 g COD is 400 ml CH_4 as presented in Table 8. The SMY was calculated for all COD loading and was presented in Table 13. In an average only 0.50 ± 8 g COD (as CH_4) per g COD (as ethylene glycol) was obtained by anaerobic digestion in batch test. Experimentally produced methane was lower than the expected methane production at 35^0C. It was observed that the saline waste water source has 47% of biomethane potential.

Table 13 Methane production and Yield of the Saline source

Substrate Loading		Without Blank		Expected Methane production
mL	g COD	Methane production	Specific Methane Yield	
		mLCH_4	gCOD/ gCOD	mL CH_4
12.34	1	206 ± 2	0.52	400
24.69	2	365	0.45	800
37.03	3	675 ± 16	0.56	1200
76.72	5	1005 ± 8	0.50	2000
98.76	8	1484±9	0.46	3200

4.2.3 BMP Test of Not-Saline Waste Water

The standard error and accuracy were not calculated in the batch test of non- saline waste water because there was failure in some reactors, while some reactors were running without duplicating the samples. There was crowd on using the same AMPTS II by other researchers simultaneously in lab, so we share the reactors cells and ran the samples without duplication. All the experimental data of lab work has been listed in Appendix C Table C.3 and C.4.

Cumulative methane production at different COD loading of this not-saline waste water as shown in Figure 22 showed that the bulk of COD converted to methane was slow. Higher methane was produced by higher loading i.e. 8 g COD. The conversion of COD to methane was completed within 5 days. All the series of COD turned to flat after 5 days which is the indication of low methane production. The COD conversion from positive control was faster than that of other COD loading which also showed the low degradability and low conversion of COD to methane production from not-saline waste water. The reactor with 5 g COD had stopped working after 3 days so, there was no result to explain.

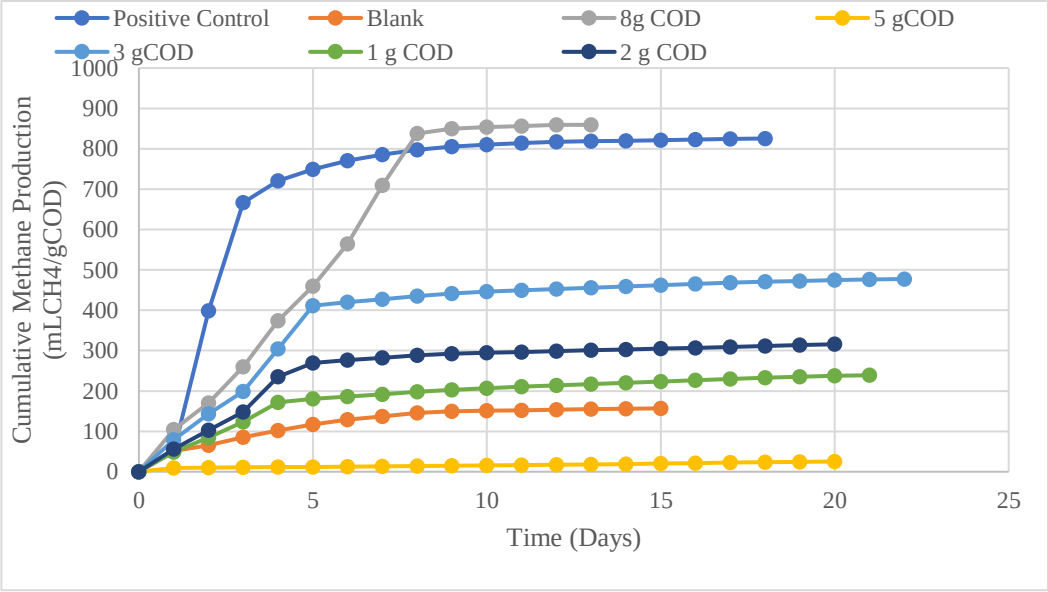

Figure 22 Cumulative Methane Production of Non-Saline Waste Water

The methane flow rate data in Figure 23 showed that the degradation was not taken place smoothly. The time required for degradation was slow so, it did not show the high methane flow rate. All COD series showed the similar trend of flow rate. There was decreased in

methane flow rate from the day 8 after all the flow rate of different COD series observed to be equal with blank. The methane flow rate of 5g COD series was failed due to failure in the reactor cell.

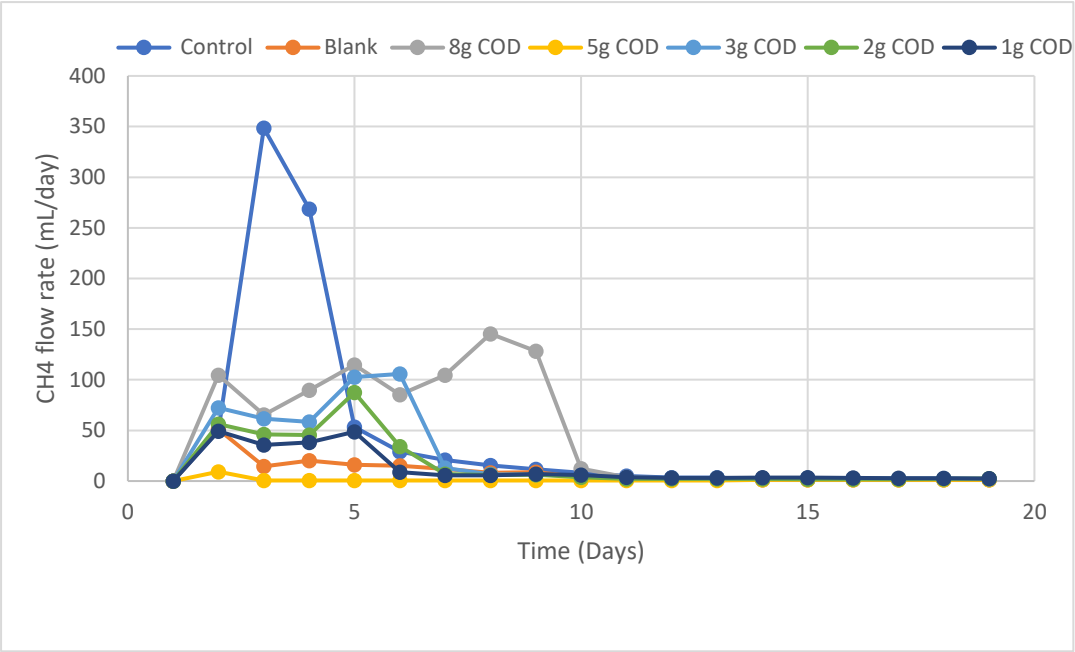

Figure 23 Flow rate of methane production at different COD loading

The experimental data from the digested sludge was presented in Table 14. The results showed VFA accumulation in all reactors cell with high pH above 7.8 and alkalinity below 2000 mg/l.

Table 14 Experimental results of digested sludge of not saline source

Experimental products	**Units**	**COD Loading**				
		1g	**2g**	**3g**	**5g**	**8g**
VFA	mg COD/L	59	142	323	142	216
Alkalinity	mg CaCO3/L	1898	1884	1672	1885	1734
VFA/Alkalinity	-	0.03	0.07	0.19	0.07	0.12
pH	7.3	7.8	7.9	7.9	7.9	8.2

4.2.4 Methane Production and Yield of Not-Saline Waste Water

The calculated results of methane yield of different COD were presented in Table 15. The SMY was 0.22 g COD (as CH4)/ g COD (as Ethylene glycol). All COD loading showed the same SMY. Methane production for 5g COD was not observed due to failure in the electric wire. Only 20 % of methane production was obtained from this source.

Table 15 Methane production and yield of the not-saline source

Subtract Loading		**Methane Production**	**Specific Methane Yield (SMY)**	**Expected Methane Production**
mL	**gCOD**	**ml CH4**	**gCOD/gCOD**	**mLCH4**
2.6	1	81	0.20	400
5.22	2	160	0.20	910
7.83	3	318	0.26	1365
13.05	5	Failed		
20.8	8	704	0.22	3640

4.3 Experiment in CSTR System

The CSTR testing was performed at the room temperature (20-25^{0}C). The Experimental scale analysis was performed after some preliminary test on the raw industrial waste. The CFSTR named as RI was run with saline sample to examine operation and limitation in the digestion process. Two experiment (I and II) was conducted from February to mid of March 2019. This startup experimental results from I and II were presented as preliminary experiment in sub-Section as 4.2.1 and data were presented in Appendix D.1 and D.2.

In Section 4.2.2, two reactors were used; one for saline source (RI) and other for not-saline source (RII). The digestion process was operated at different OLR and at different dilution rate. In this research different intermediate products of the anaerobic digestion were not studied experimentally. The HRT was 16 days and flow rate were 60 ml/day throughout the whole experiment.

4.3.1 Preliminary Experimental

The preliminary experiment was done only in saline waste water source because there was no plan to experiment on not saline source. But after some interesting conclusion from preliminary experiment with some findings on the operation condition, the laboratory scale experiment was performed with new CFSTR in not-saline waste water too. The laboratory scale experiment with added operating parameters were presented on Section 4.3.

Two experiments (I and II) were carried out with saline waste water at different operational conditions. In the Experiment I, the reactor was feeded at flow rate of 60 ml/day and OLR of 4.86 g COD/ld. The experiment had started without adding extra alkalinity and nutrients with retention time selected of 16 days. In this preliminary test only pH, alkalinity and reactor response were tested under anaerobic condition. Different results were obtained and presented graphically.

Results obtained from the **Experiment I** in Figure 24 and 25 showed that there was drop on alkalinity after 3 days, from 1753 mg/l to 185.4 mg/l and pH from neutral range reached to 5.7. At this situation the inhibition was supposed to high VFA (4993 mg/l) and also the production in biogas drop from 273 ml/day to 39 ml/day. So, the feeding was stopped for two days. No changes on pH and alkalinity concentration was observed and the experiment was restarted by replacing new inoculum by 50%. This results on increasing pH and alkalinity was observed to some extend with increase in biogas production. Later after 2 days, pH and alkalinity started to decrease with increasing VFA concentration, and no biogas production was observed. This added inoculum did not support for long. Reduction on biogas production with low pH and higher VFA accumulation was continuously observed. At the end of 22 days the system stops to produced gas when VFA concentration reached to 5883 mg/L. Decreasing trend of VFA can be seen in the Figure 24, but the experiment was stopped for new observation.

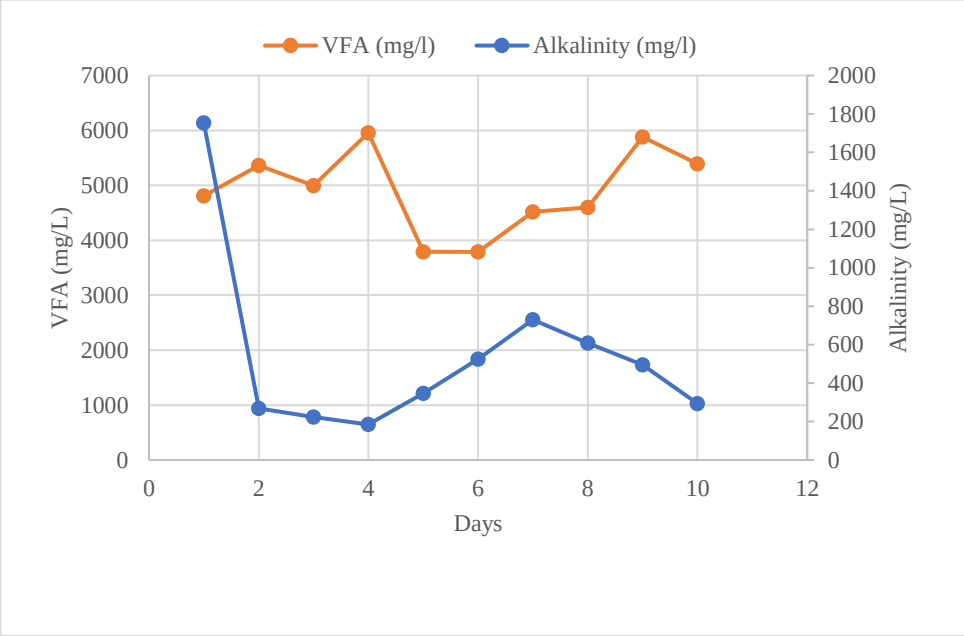

Figure 24 Changes in alkalinity with VFA accumulation

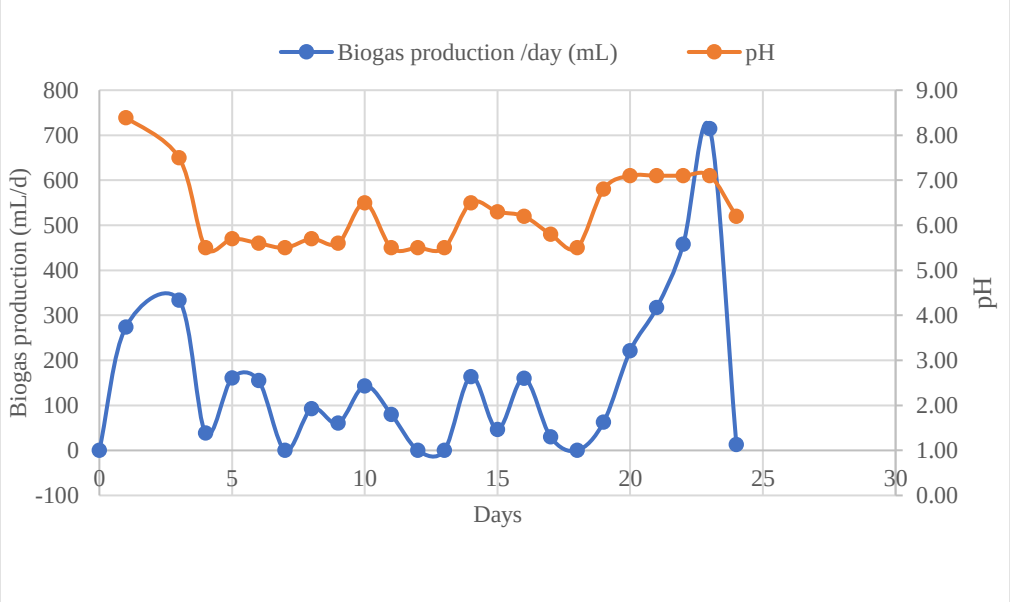

Figure 25 Effect of pH on biogas production

From Figure 25, it is visible that the biogas production was affected by change in pH. The biogas production was high in the natural pH range and get reduces when the pH reached to 6.

Experiment II was started with dilution of the feed with tap water at 1:1 ratio to reduce the OLR by 50%. The feed was loaded with OLR 2.43 gCOD/l.d with 16 days retention time and flow rate of 60 mL/day. The system was observed for 6 days and analyzed the changed on the

alkalinity, VFA and pH. The following results were obtained as presented in Figure 26, 27 and 28.

Increase in biogas production was observed from 147 mL/day to 332 L/day where pH was in stable condition from 7.5 to 7.1 But reduction in alkalinity and accumulation of VFA was continuously observed. So, at the pH 6.4, extra alkalinity of 0.1 M (8.4 g/L) sodium bicarbonate ($NaHCO_3$) was added as a source of alkalinity. The added alkalinity helped to increase pH to the neutral range. This helps in stopping the reactor from shutting down and continuously maintain pH and biogas production. The slow reduction on VFA concentration and increased in pH was not observed because the VFA accumulation was already overloaded to high concentration to 6907 mg HAC/l as shown in Figure 26. Lowering in alkalinity and VFA was observed before stopping the observation and the further experiment.

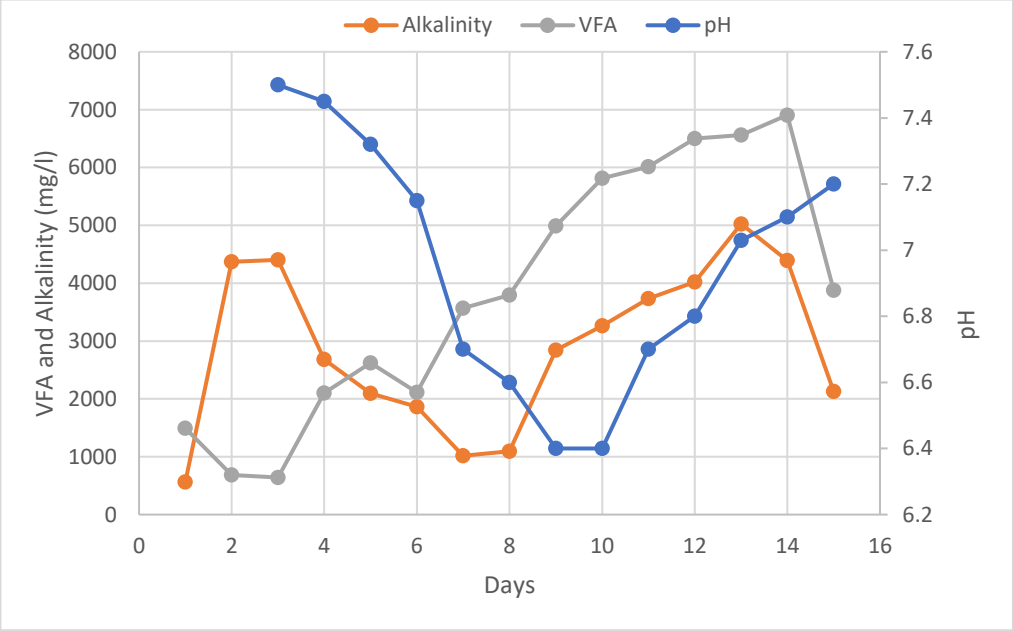

Figure 26 Observation on VFA and alkalinity by changing pH

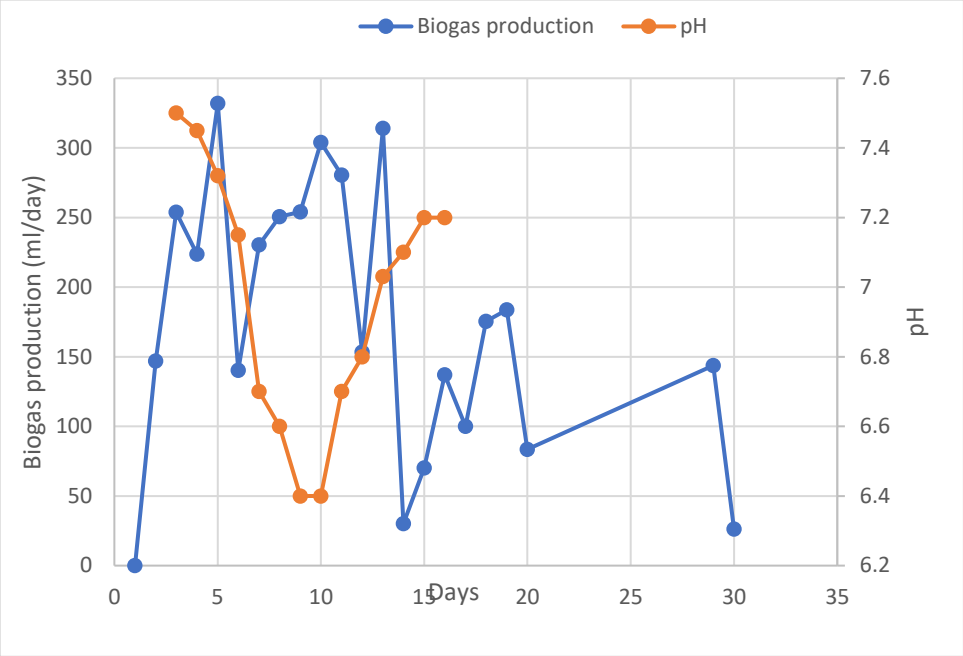

Figure 27 Effect of pH on biogas production

The effect of pH on biogas production was observed as shown in Figure 27. Only Biogas production data was taken after maintaining the pH by adding extra alkalinity in the feed.

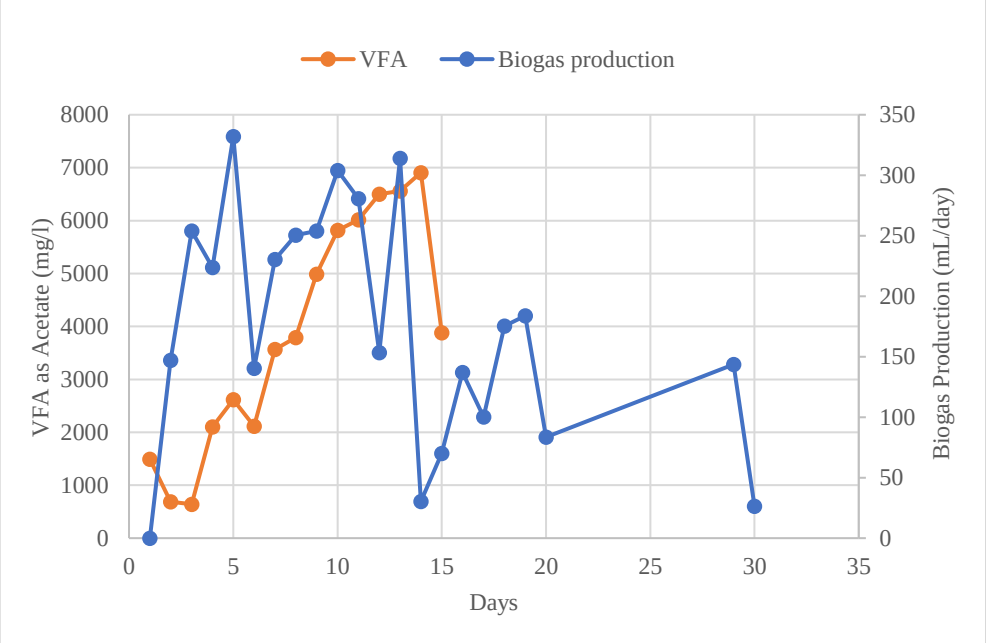

Figure 28 Effect of VFA in biogas production

Feed was stop for some days. There was observed with some reduction of VFA to 3880 mg HAC/l and decreasing in alkalinity as shown in Figure 28. But there was failure in the system

failure and no biogas production was observed. The whole experiment was stopped and decided to feed the reactor with low loading from the beginning and also adding the alkalinity in the feed from the very beginning.

4.3.2 Effect of Dilution on COD

In this preliminary Experiment II, the effluent soluble COD was also analyzed which was presented in Figure 29. Higher COD in the effluent sample was observed after reducing COD loading at OLR 2.43 gCOD/d.l by 1:1 dilution rate. The effluent COD concentration was less in the beginning, but the dilution resulted into wash out of particulate COD and increased effluent COD concentration continuously. There was also observed reduced in concentration after some days. The COD mass balanced was not done in this experiment because of instability in the system and experiment was just done for testing the operating condition to run other experiment later with optimized conditions.

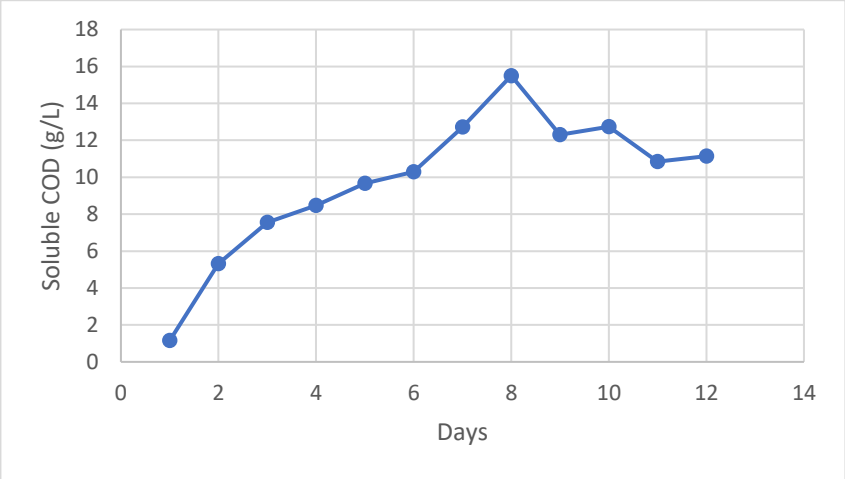

Figure 29 Effluent soluble COD concentration data after dilution

4.4 Optimized CFSTR Experiment

The experiment III was started in two properly designed CFSTR. RI reactor was fed with saline waste water having COD concentration of 81 g/L and RII was fed with not-saline source with COD concentration of 383 g/L. In this experiment the loading of substrate was started with lower OLR and alkalinity was added directly into the feed from the beginning of the experiment. To lower the OLR at different concentration, dilution of feed was carried out with

tap water. The flow rate of feed to the reactor was constant with 60 ml/day and HRT of 16 days. All the inhibition parameters were properly handled and operated in the good condition.

Experiment was started in May 11, 2019 and ended in June 15, 2019. Operational parameters and condition applied for testing both the Saline and non- Saline waste water in the laboratory scale were presented in Table 16 and 17 respectively. Data obtained from the experimental analysis were presented in Appendix Tables E.1, E.2. F.1, and F.2.

Table 16 Operational parameters for R1

Dilution Ratio	Daily organic loading rate (OLR)	Added Alkalinity ($NaHCO_3$)	Initial condition
	gCOD/day	g/l	OLR= 4.86 g/l.d
1:8	0.61(May 11- 20)	8.4	Flow rate= 60 ml/day
1:4	1.21 (May 21-24)		HRT= 16 Days
1:1	2.43 (May 25- June 15)		COD = 81 g/l

4.4.1 Optimized Experimental Analysis for Saline Waste Water

Methane production was measured in g COD/day with respect to COD loading at OLR g COD/d.l. There was increase and decrease in production at OLR, 0.6 and 1.21 g COD/l. d. OLR was increased when there was low methane production as shown in Figure 30. As long as the OLR increased to 2.43 g COD/l. d, the maximum production was observed which was 0.39 LCH4/d, but it did not continue and started to reduce. The digestion process gets failure after continuously feeding at OLR, 2.43 g COD/l. d which results into no biogas production at the end.

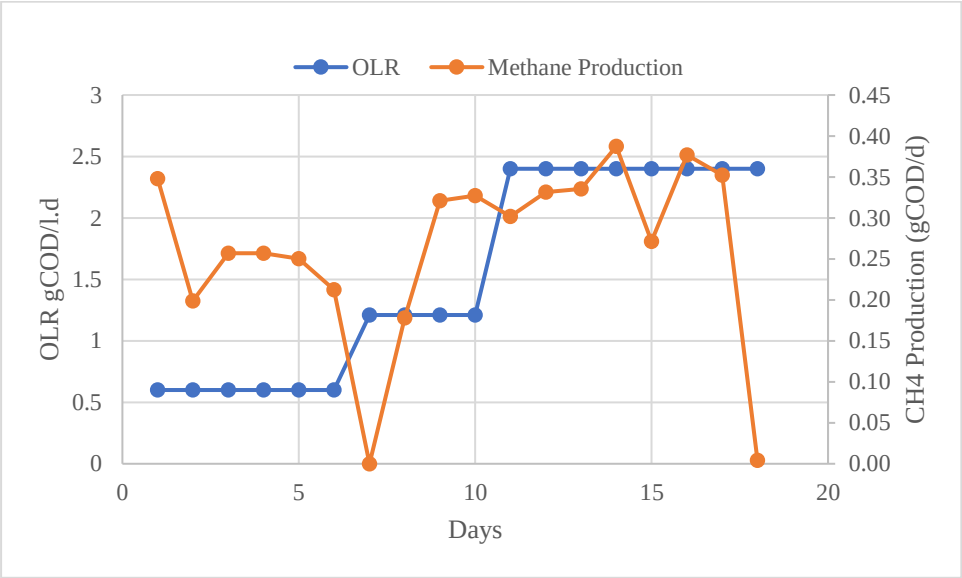

Figure 30 Change in methane production at different COD loading

SMY as g COD/g COD was calculated with an assumption of 62 % of theoretical methane production during digestion of EG. There was negative SMY at lower OLR of 0.6 g COD/l. d as showed in Figure 31. Increase in yield was observed at OLR 2.1 g COD/l. d. The yield remained constant with increase in OLR from 1.21 to 2.43 g COD/l. d.

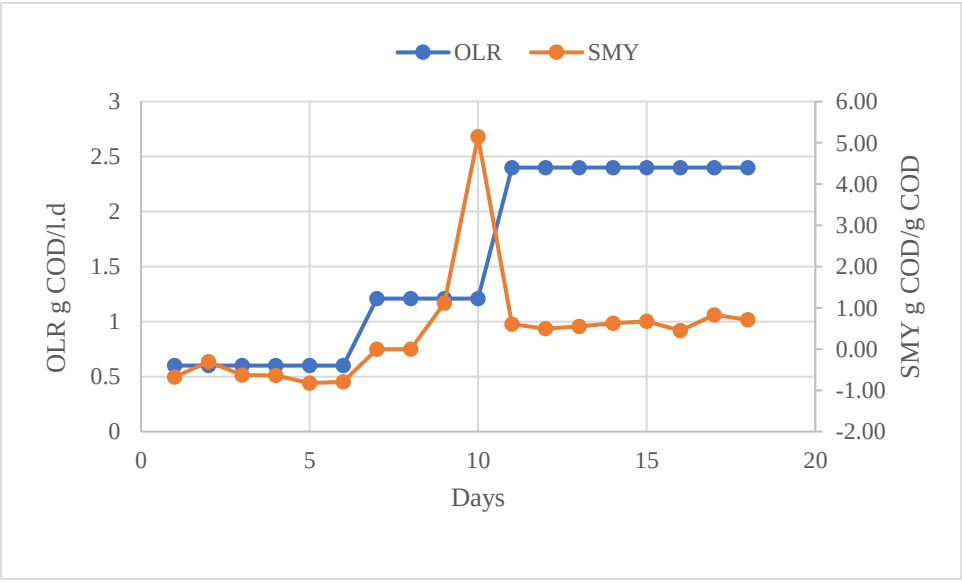

Figure 31 Effect on specific methane yield at different COD loading

The flux of COD_{out} was higher than the flux of COD_{in} at low OLR, as per the results presented in Appendix Table G.1. So, the COD removal efficiency was negative as shown in Figure 32 at OLR 0.6 g COD/l. d. As long as the OLR increased to 1.21 and 2.43 g COD/l. d, the % of COD removal also increased with positive value and remain constant at 50-60%.

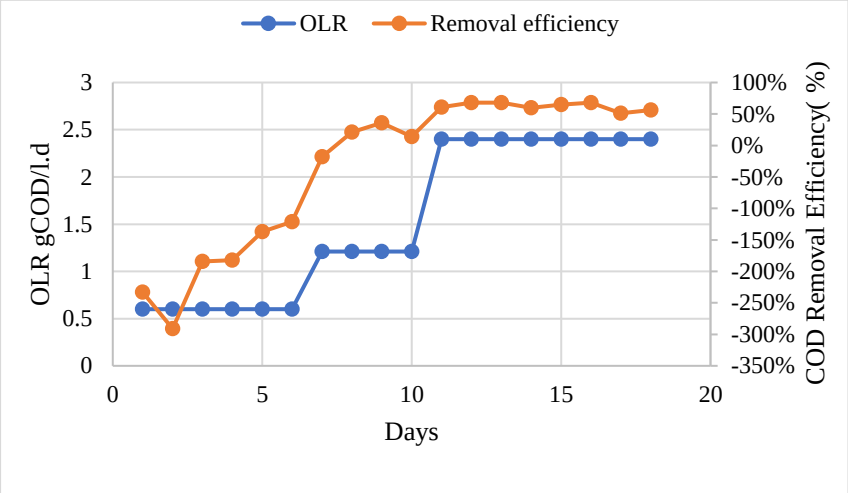

Figure 32 COD removal efficiency at different loading

Different environmental factors like, alkalinity, VFA and pH were observed to analyze the change in biogas production. The results obtained on the influence of environmental parameters were defined graphically in Figure 33 and 34.

The daily biogas production, as presented in Figure 33, showed that the optimal biogas production was observed at pH between 7.1 to 7.5. As soon as the pH increased above 7.5 and below 6.8, there was reduction in biogas production. The production was totally inhibited at pH below 6.8. The maximum biogas production was recorded at 7.5 which was 625 ml/day.

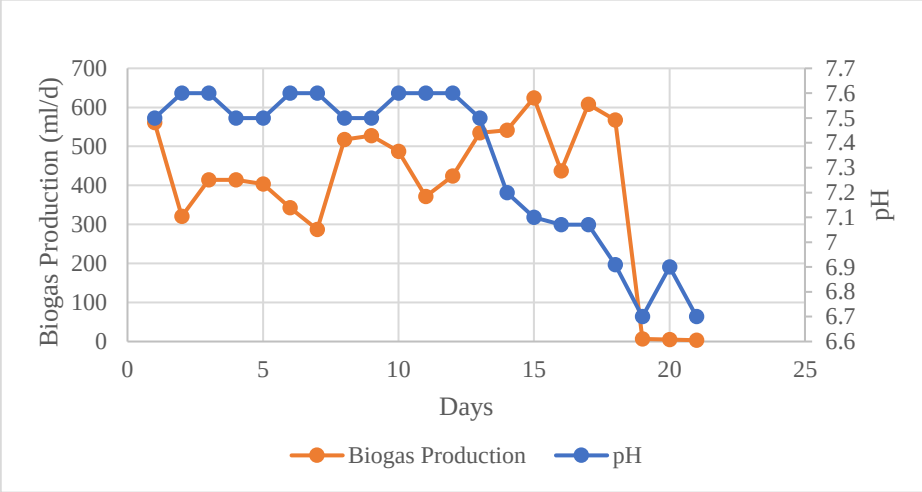

Figure 33 Influence of pH on daily biogas production

The change in concentration of alkalinity with respect to pH was clearly observed in Figure 34. Concentration of Alkalinity was high in the beginning of the AD process which was 6662 mg/l at pH was at 7.5. As long as the alkalinity was consumed, there was reduction in pH. The reduction in pH was also observed when there was increased in alkalinity concentration at pH 7.5. The decreased in alkalinity was continuously after the pH dropped from 7. The reason of dropping pH at this point after continuously maintaining alkalinity by adding NaHCO3 was not due to the process but its due to system failure due to flow of HCL from the ritter inside the reactor during wasting of sludge.

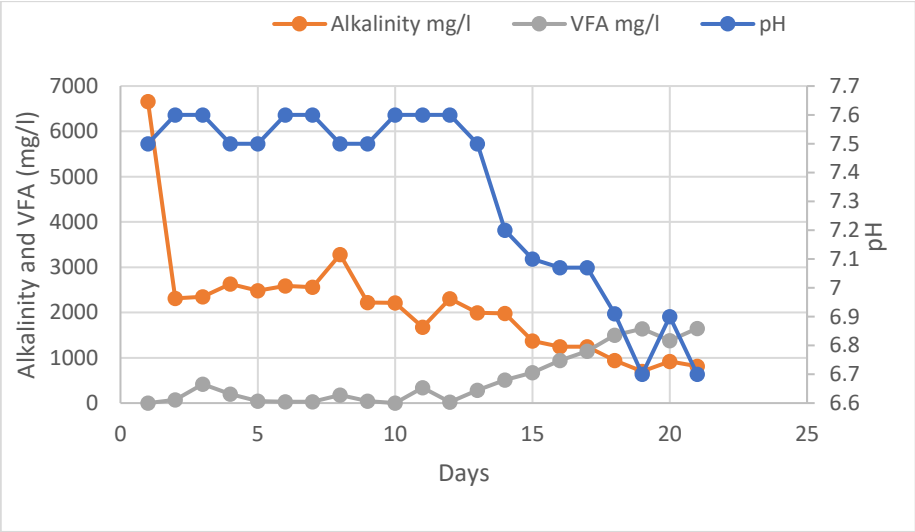

Figure 34 Change in concentration of alkalinity and VFA with respect to pH

The concentration of VFA as showed in Figure 34 was not accumulated as long as the pH was in maintained between 7.1 to 7.5. The concentration of VFA during the biogas production was

low at pH 7.5 to 7.6. As long as pH reached 7.2, the accumulation of VFA in the system started and at the same time the concentration of alkalinity also decreases. The high concentration of 1640 mg HAC/l was observed at pH 6.7 where the concentration of alkalinity was 813 mg/l. This was the point where system get inhibited and digestion process stopped.

4.4.2 Optimized Experimental Analysis of Not- Saline Waste Water

The experimental analysis on the not- saline waste water was done at different operating parameters as presented in Table 17. No preliminary test had been done for this sample. After finalizing the inhibition condition that could take place in the CFSTR system from RI, the new feed was loaded at same flow rate which was 60 ml/day and selected HRT was 16 days in RII. There was poor installation in this reactor with head tube for balancing pressure, which causes the back flow of HCL liquid into the reactor during wasting of sludge. Due to the gas leakages in the middle of the experiment no biogas reading was observed for some days.

Table 17 Operational parameters for not- saline source

Dilution Ratio	Daily organic loading rate (OLR)	Added Alkalinity ($NaHCO_3$)	Initial condition
	g COD/ day	g/l	OLR= 22.98 g/l. d
1:35	0.61(May 11- 20)	8.4	Flow rate= 60 ml/day
1:15	1.53(May 11- 20)		HRT= 16 Days
1:4	5.74 (May 25- June 6)		COD = 383 g/l

The effect of COD loading on methane yield at different OLR was observed and presented in the Figure 35. At low loading (0.6 g COD/l. d) negative yield was observed. By increasing the OLR to 1.53 g COD/l. d, the value of yield was increased and showed the positive value. The yield was slightly constant 0.22 at OLR 5.41.53 g COD/l. d.

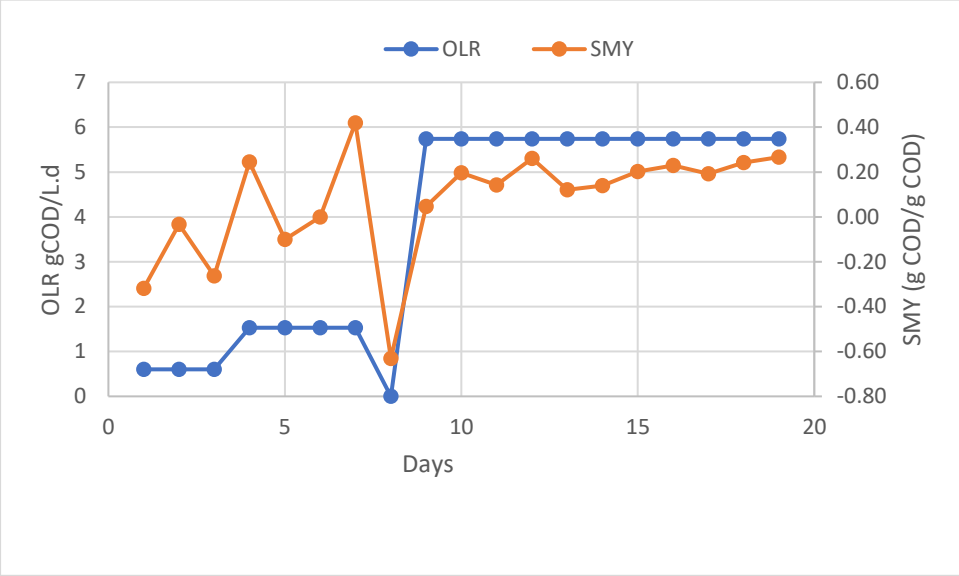

Figure 35 Effect of different COD loading on methane yield

The higher methane production was achieved at higher COD loading at OLR 5.74 g COD/l. d as shown in Figure 36, there was increasing and decreasing trend during high and low COD loading.

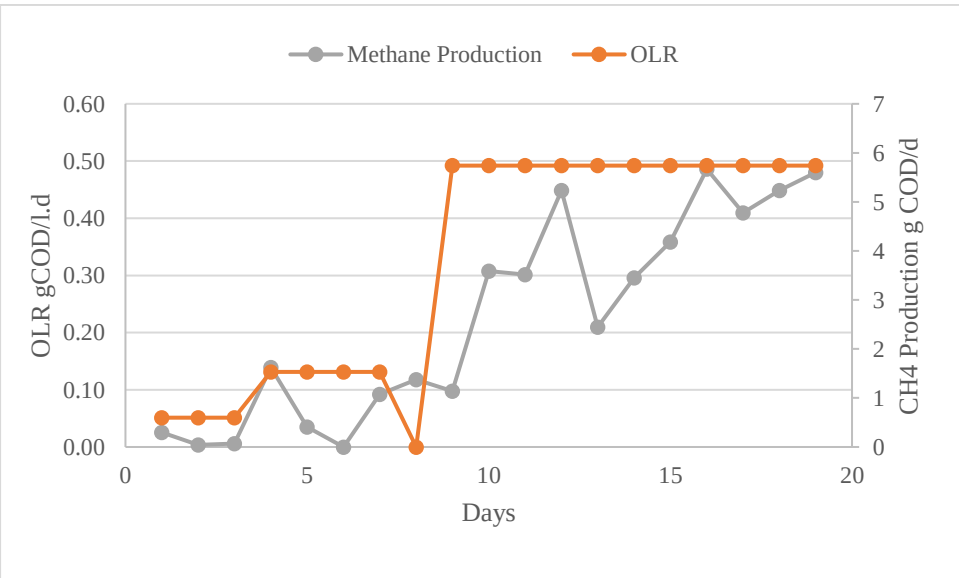

Figure 36 Methane production at different COD loading

The flux of COD_{out} was higher than the flux of COD_{in} at low OLR, as per the results presented in Appendix Table G.2. So, the percentage of COD removal was negative as shown in the

Figure 37 at OLR 0.6 g COD/l. d. As long as the OLR increased to 1.53 and 5.74 g COD/l. d, the COD removal efficiency also increased with positive value, but stability was not observed.

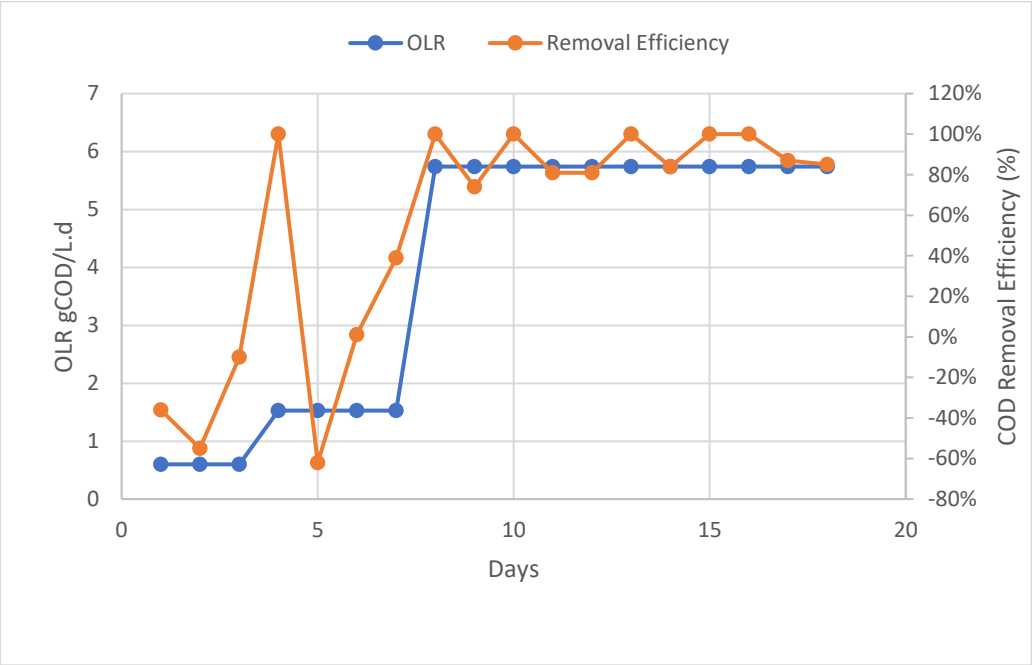

Figure 37 COD removal efficiency at different loading

The effect of pH on changing the concentration of VFA and alkalinity in mg/l was presented in Figure 38. The concentration of VFA was low throughout the experiment. The highest VFA concentration observed was 461 mg/l. In this digestion of not saline waste water pH was always higher than the neutral value which was 7.2 to 7.9. Alkalinity concentration showed the increasing and decreasing trend throughout the experiment where highest value observed was 2618 mg/l at pH 7.6. The change in concentration of alkalinity followed the similar trend with change in pH value.

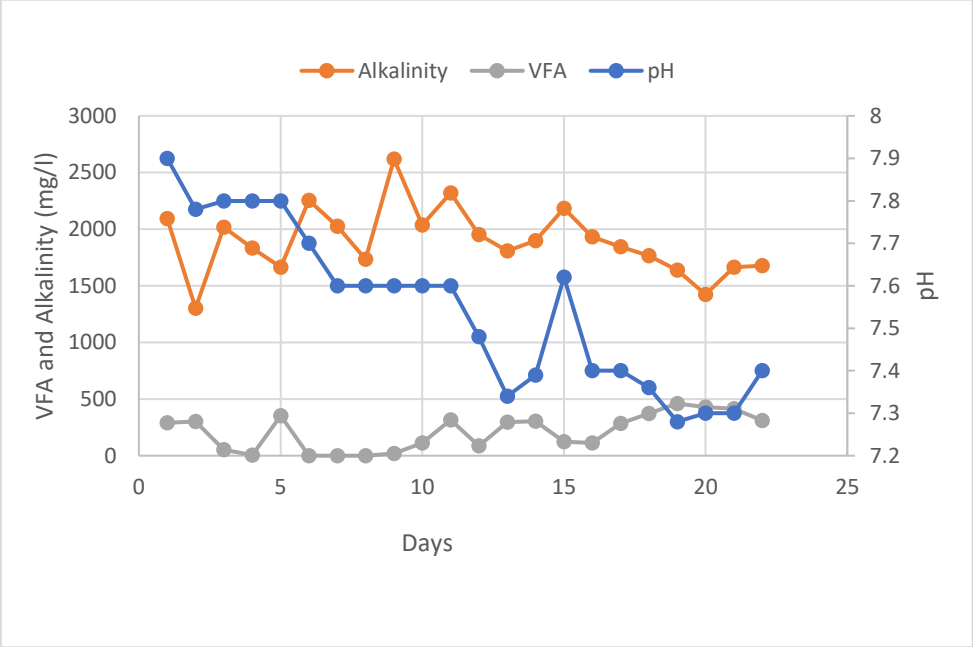

Figure 38 Change in VFA and alkalinity concentration with respect to pH

Combined effect of pH, VFA and biogas production was presented in Figure 39. The production of biogas was observed to be influenced by the concentration of VFA inside the reactor. In the beginning the concentration of VFA was around 300 mg/l. As long as the VFA was converted into biogas, the production of biogas increased from 41 to 225 ml/day. The reduction in biogas production went low with low concentration of VFA. After 10 days of digestion the concentration of VFA decreased but production on biogas increased continuously. This trend showed that VFA is converted into the biogas as the same rate with respect to production. The negative effect of pH on the concentration of VFA and biogas production was high at pH 7.4 to 7.9. As long as the pH decreased to 7.2 the production of biogas increased to 783 ml/day.

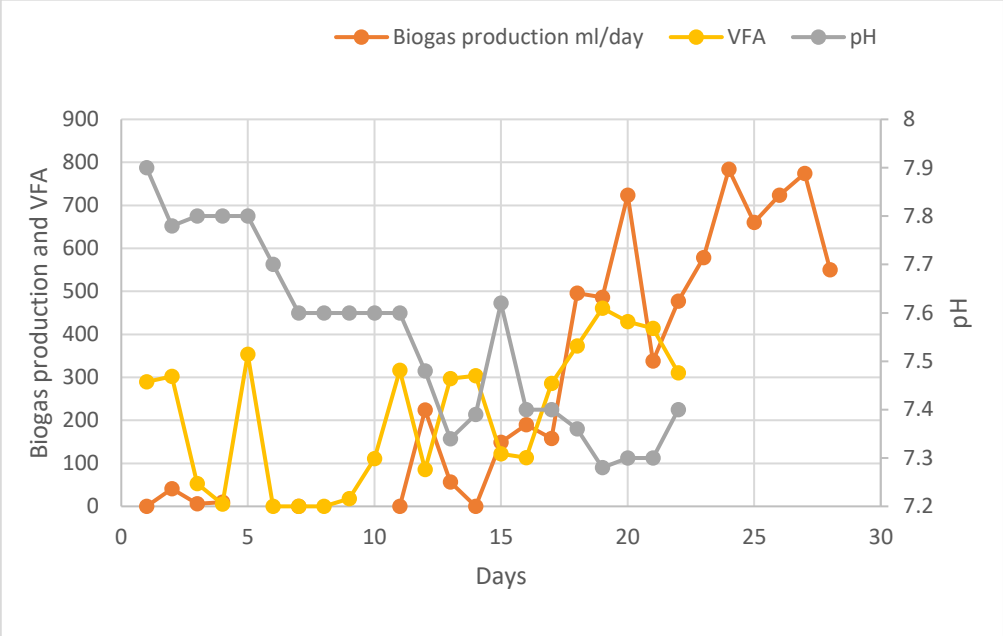

Figure 39 Effect of pH in the production of Biogas and VFA

4.5 COD Mass Balance

COD mass balance has been calculated using flux of inlet COD, flux of effluent COD, flux of methane production by applying the Equation 2-1. The details COD mass balance calculation of both reactors RI and RII has been tabulated in Appendix G.1and G.2 respectively.

4.5.1 COD Analysis in Saline Waste Water

The results on COD mass balance presented on Figure 40, it was found that in the starting, the COD mass balance percentage was higher than 100 %. The COD loading was low with OLR 0.6 g COD/l. d with 15 times dilution rate. The OLR increased to 1.5 g COD/l. d with lowering the dilution rate to 4. This result into lowering the COD system mass balance to 158%. Further increase in OLR to 2.4 g COD/l. d with 1:1 dilution, there achieved the stable mass balance percent between 75-80%. The stable mass balance percentage indicates the system reached to the steady state condition with SMY of 0.45 g COD/g COD. All the observed and calculated data has been listed in Appendix Table G.1.

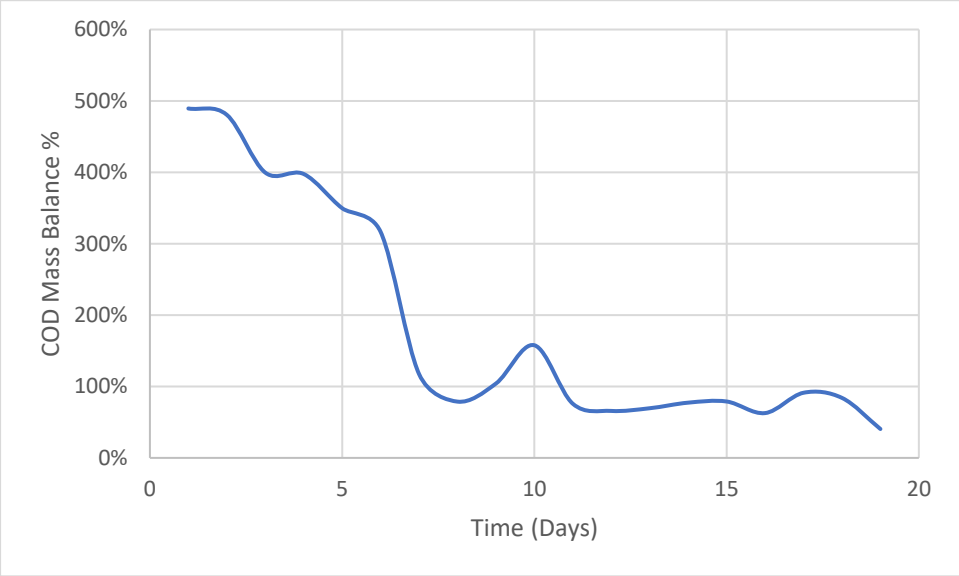

Figure 40 COD mass balance in CFSTR (RI)

From this experiment the optimum COD loading was observed between1.21and 2.43 g COD/l. d for saline waste for reactor volume of 1000 ml. The system was approaching to the steady state condition, but the observation was stopped due to time constraints for this experimental work.

4.5.2 COD analysis in Not- Saline Source

The results on COD mass balance analysis showed the higher COD percent when the COD was started with 0.6 g COD/l.d at dilution rate of 35. The system approach to the stable mass balance condition when COD increases with decreasing dilution rate. The second COD loading was started with OLR 1.53 g COD/l.d at dilution rate of 15 which also results in higher percent in COD mass balance. The stable COD mass balance was observed at higher COD loading with OLR 5.74 g COD/l.d, The SMY observed at stable condition was 16%. Due to time constraint, further experiment was not carried out after 15 June 2019. COD mass balance was not clearly observed in this not-saline waste water. The system was imbalanced with very little methane production and yield. All the data has been listed in Appendix Table G.2.

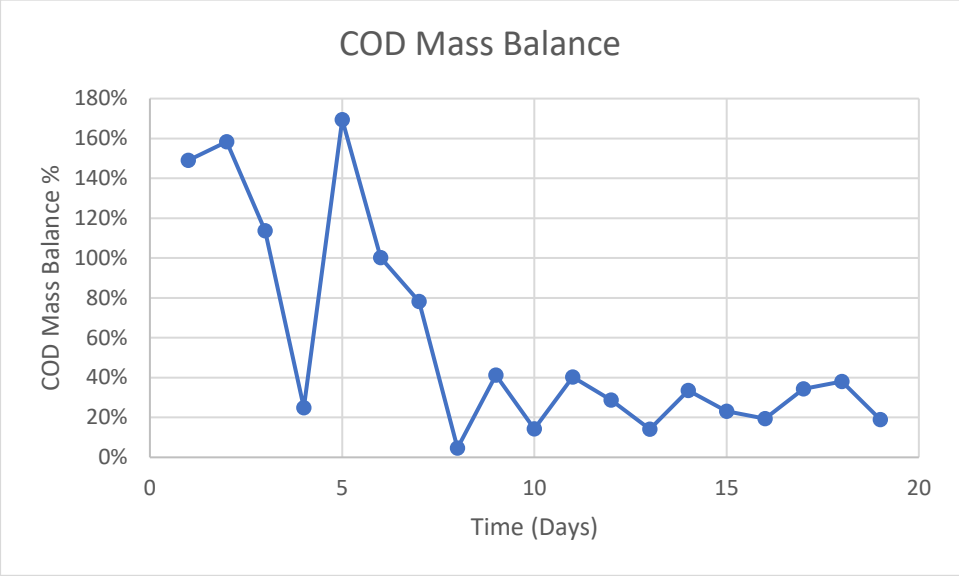

Figure 41 COD mass balance in CFSTR (RII)

4.6 Inhibition Analysis

Inhibition of the digestion process due to sulfide, lack of nutrients and ammonia have not been observed in both waste water types. The concentration of S^{2-} were examined randomly especially after observing the reduction in biogas production. The observed concentration was approximately within the range of 28-32 mg/l for saline waste water and 5.2-7.8 mg/l for the non-saline waste water. The fresh inoculum was supposed to be the good source of nutrients for the available microbes. The experimental results on the concentration for both total phosphorous and nitrogen were higher than the limits range of kit. So, no deficient of nutrients in the digestion process was concluded. The potential inhibition on testing of this types of waste could be presence of some unknown components not suitable for the anaerobic digestion, the molecular weight of different compounds of glycol, half-life of degradation of different organic compounds, as well as deficient of some ions necessary for efficient degradation (such as sodium, chloride, magnesium).

4.7 Error Analysis

The error analysis was carried out in the samples that have been tested with duplication. The analysis of error on the COD measurement of the samples which contained only dissolved COD was estimated as 80 % confidence level which will give the relative error of 7.3 %. This error was due to different dilution rate of 1:1000, 1:500, 1:400, 1:200, 1:100. The relative error

had increased from 2 to 7 % on different ratio from 100 to 1000. The dilution was carried to fit the COD measurement range, which was 100- 1500 mg COD/L.

The sample was digested in the inoculum so the COD analysis of the sample from the effluent was estimated to have higher error and this relative error was estimated as 10.1 % with 90 % confidence level. Since the sample needs dilution to cover the range of measurement and also due to presence of particulates COD from sludge, relative error was higher.

The calibration of all instruments was done before the experiments, so relative error for the measurement of alkalinity, VFA, pH, dosing pump, recirculation pump, mixing stirrer were assumed to be less than 2 % with confidence level of 95 %.

4.8 Comparison on SMY from Batch and Continuous System

The comparison on Anaerobic Digestion of saline and not-saline waste water was done based SMY. It was observed that the SMY of saline waste water was 0.47 g COD/g COD and 0.45 g COD/g COD in batch and CFSTR respectively. Similarly, the SMY of not-saline waste water in batch and CFSTR were 0.2 and 0.16 g COD/g COD respectively. The difference in SMY was due to high retention time for the batch system which was 22 days and for CFSTR was 16 days. the saline source has 47 % of methane potential and not- saline waste water has only 20 % of methane potential.

5 Discussion

This section provides discussion and interpretation on obtained results from whole experimental work. The main discussion is dedicated to BMP of selected waste water types in batch system, limiting operating parameters and COD mass balance in continuous system, as well as major environmental factors like pH, Alkalinity, VFA, OLR, which indicate the system stability and digestion process.

The fermentation of glycol produces the ethanol as the intermediated product as explained by different researchers mention in Chapter II. The proper utilization and conversion of intermediate products, which is acetate and hydrogen in case of glycol containing waste, indicates the overall performance of the reactor as explained by Tatton et al., (1989), but the concentration of different intermediate products of the fermentation process were not analyzed in this experiment.

The characteristics of saline and not-saline waste water presented in Table 9 found that the saline waste water is more suitable for carrying the anaerobic digestion than the not-saline waste water with an assumption for containing high TVS which is approximately 10 times higher than the not- saline source. The analysis of hydrocarbon in saline waste water in Table 10 also assumed to contain other long chain hydrocarbon groups which might have steric hindrance characteristics, lack of degradation, lack of cell uptake mechanism which might limit 100 % biodegradation under anaerobic condition. Due to lack of functionality of hydrocarbon compounds, anaerobic bacteria have to introduce functional groups with H_2O, HCO_3^- and organic acid. Bactria also need to establish synergetic relationship at both methanogenesis and hydrogenases steps to make the reaction thermodynamically feasible (Field, 2002). The presence of different ions in saline waste water given in Table 11 was much lower than the inhibition ranges, so, higher and lower ranges could have affected the growth of microbial community present inside the reactors.

5.1 Biomethane Potential of Saline Waste Water

On the basis of explanation in Figure 13, on growth pattern of biomass in the batch system and biodegradability rate explanation by Esposito et al. (2012), the flow of methane production from the series of different COD in saline water was observed following the general pattern of

biomass growth per substrate consumed referring to Figure 20. The lag phase was less than 1 days for acclimation to microbes in new environment before they start to grow with the consumption of substrate. As soon as the biomass acclimatized, they start to grow rapidly with high methane production from high COD loading. The exponential growth of biomass stops after 3 days and turn to intermediate phase. Referring to Figure 21, the flow rate of methane production was high in 2 days which indicates the rapid biodegradation with sufficient substrate and nutrients in the system. So, the degradability of saline waste water was concluded to be 3 days. The biomass growth phase reached to intermediate phase after 5 days might be due to limitation of substrate and nutrients availability. To get the final growth phase, the digestion needs to be run for 40 days as suggested by Esposito et al. (2012).

The BMP of saline waste water was approximately 47 % with average methane yield of 0.50 ± 8 g COD/g COD. The remaining 50 % of COD loss can be assumed to be used some for assimilation of bacterial cell, respiration and maintenance. There might also contain some undegradable COD and some COD might have remained attached to the sludge which did not come to the solution during centrifuging of sample. The values of neutral pH, alkalinity range of 5000 mg/L and negligible VFA concentration in the digested sludge is good enough for concluding it as good source for methane production.

5.2 Biomethane Potential of Not- Saline Waste Water

Based on batch results from not-saline waste water as presented in Figure 22 and 23, the methane production and biodegradability rate seem to be inhibited by unknown organic compounds. Referring to Figure 22, the COD series do not follow the regular biomass growth pattern. This type of waste water had much higher COD concentration than the saline sample, but BMP was only 20 % with average SMY as 0.22 g COD/g COD. Methane flow rate was below the positive control which also indicate the slow degradation and slow conversion of COD into methane. Low methane production might be due to inhibitory effect of EG during utilization by blocking of the cellular uptake system, which inhibited the production of acetate. Microbes required acetate for growth, if other products get accumulated rather than acetate it also inhibits the digestion process.

The not-saline waste was said to have TEG and it was collected after gas drying. So, it might contain other chemicals like biocides. The not saline sample was also suspected to contain the nonionic surfactant with the observation of soapy foams during dilution and shaking of the sample. This might be also the supportive statement for the partial degradation in anaerobic condition. This type of nonionic surfactant source is independent to growth factor and did not reduce sulfate or nitrate. The growth yield of this type of substrate could be higher in the addition of 10 g of NaCl or 1.5g of $MgCl_2.H_20$, except this, such waste does not require any other growth factor and minerals (Schink and Stieb, 1983). As a result, referring to Table 14. Low COD loading gives high specific methane yield of 0.59 g COD/g COD and vice versa. So, inhibition might have occurred at higher COD loading. But the value is not that much accurate since no parallel tests were performed for not-saline waste and also have some failure in reactor cell containing 5 g COD.

The results of high pH, lower alkalinity and accumulation of VFA of the digested samples also indicate the inhibition in the system during digestion. Methane production decreased at high COD loading with increased in pH, what is the indication of failure in the digestion process. This might be due to the presence of dissolved concentration of H_2 which gives the accumulation of VFA not in the form of acetate but in the form of butyric or propionate acid (Boe et al., 2010).

5.3 Anaerobic Digestion Process in the Continuous System

The failure in the biogas production in the two-preliminary experiment (I & II) was due to improper installation of the reactors, leakage of biogas from the system, high OLR and not adding of extra alkalinity.

In the Continuous system, as soon as the microorganisms get the glycol containing waste water as a source of carbon, the presence of hydroxyl group facilitated the biodegradability through acitogenesis and methanogenesis process to produce biogas (Johnson & Taconi, 2007; Battersby & Wilson, 1988). During degradation of this saline waste water, acidogenesis could be the fastest process resulting into high accumulation of VFA, dropping alkalinity concentration and pH value within 3 days as observed in Figure 24 which is the indicates of

instability and failure of the system. The inhibition and stability of anaerobic digestion is more depended on the utilization of intermediate product (Van Lier et al., 2008).

For Experiment II with lowering the organic loading to 2.43 g COD/l. d and adding of alkalinity into the feed, this operational condition helps the system to lower the VFA accumulation. Added alkalinity increases the buffering capacity in the system. This help to maintain pH in the neutral range which favor the growth of acetogenesis and methanogenesis bacteria to produce more biogas. Adding alkalinity only after observing the reduction on pH to 6.4 was the major cause of VFA accumulation which inhibits the symbiotic environment of both acetogenesis and methanogenesis to convert the intermediate product into the methane. The higher COD effluent concentration observed after dilution which lower the OLR from 4.86 to 2,43 g COD/l.d. This might be due to washout of biomass from inoculum and low retention time for growth and consumption of substrate.

5.4 Optimized Experimental Condition

This experiment was started with low COD loading to reduce the accumulation of VFA inside the reactor, dilution was carried out to lower the loading, and addition of alkalinity was added to maintain the neural pH in the system and to help in buffering the system. The VFA concentration remain below 400 mg/l and inhibition due to accumulation of VFA was control after optimizing the COD loading and balancing the alkalinity in the system. The high dilution rate with low HRT is the case of washing out of bacteria with lower methane yield. These findings of the experiment also support the findings of Sialve, et al., (2009).

5.4.1 COD Mass Balance in RI

In the RI, the organic loading was started with OLR 0.6 g COD/l. d. The dilution rate at this point was 1:15. Negative value in yield and removal efficiency presented in Appendix Table F1 was due to death of biomass with low COD loading. The feed was not sufficient for the growth of biomass and they start to digest themselves. The biogas production was low and give the negative yield at this OLR. There was low COD balance which result into high COD value in the effluent sample. The SMY calculated in the Appendix Table F.1, increased to 0.83 g COD/g COD with increasing OLR to 2,43 g COD/l. d and system COD balance also increases.

At higher OLR, the biomass present in the inoculum get more substrate to grow and starts producing biogas. With decreasing the dilution rate results into increasing COD loading, which give the positive yield value. This is the indication of consumption of COD and conversion of COD to methane production. This result of positive COD removal efficiency suggests the growth of biomass and lower the death rate. The COD balance reached to nearly 100 % indicates the sufficient substrate concentration to prevent death and decay. The constant removal efficiency and yield also indicate the stability in the system.

In an average methane production from this saline waste water was 45 %. The lost in 55 % of methane production might be due to high biodegradability of substrate, rapid growth of biomass in the system. This could be also due to unseen methane leakage, high solubility of methane in the liquid phase or inaccuracy in the measurement in the gas ritter. The COD lost also might be capture of COD inside the reactor in the sludge. There might also have error during centrifuging sludge and COD measurement.

5.4.2 COD Mass Balance in RII

The low COD loading with 35 times dilution rate result into lower removal efficiency as presented in Appendix Table F.2. This is the indication of lower adaptation or lower contact time for the microbes to consumed substrate for acclimatization. With increasing OLR from 0.6 to 5.7 g COD/l. d, bacteria get more substrate, the consumption and assimilation rate were high resulting in the large biomass production with multiplication of bacterial cell. But lower methane yield was observed throughout the experiment. The yield was negative in the beginning might be due to improper installation of reactor. There was leakage of biogas from the ritter.

COD mass balance was not observed which might be due to quick changed in OLR before it reached steady state condition or there might be some unbiodegradable COD that inhibits the digestion process. This sample might not suitable for biogas production without some pre-treatment. The instability in the reactor might be also due to slow degradation as observed in the batch test. Only 19% COD mass balance was achieved with specific methane yield of 0.20 g COD/g COD at high COD loading is the indicates that this waste water cannot be used for methane production without pretreatment. The COD loss can be determined by collecting the

gas from the reactor for the gas specification which was not done during this experimental work.

5.4 Installation and Performance under Anaerobic Condition

For such types of industrial waste, VFA to alkalinity ratio, retention time, substrate to inoculum ratio, dilution rate, organic loading rate have been decided as major operating parameters for the anaerobic digestion. The performance of the reactor depends on the proper installation. The consumption of alkalinity by the produced acids in the system should be balanced by the supply of extra alkalinity which helps to maintain the pH and stops the accumulation of unionized VFA leading to the inhibition and stop of methane production.

Furthermore, the hydrogen produced by the digestion of VFA should be maintained, which could establish the symbiotic relationship between acetogenesis and methanogenesis bacteria to produce methane (Jain et al., 2015). Methanogenesis process is much sensitive and slower process due to the lower growth rate of aceticlastic methanogenesis (Henze, 2008). So, instability in the process is all depended on the operating parameters. The trend of increase and decrease in yield with increasing OLR, as explained by De La Rubia et al. (2009), where the acidogenesis bacteria affected and inhibited for the acid production, could only studied by measuring the daily VFA concentration. The drop in pH after dilution and low OLR might be due to high dilution which imbalances the rate of production and disturbs the activity of acetogenesis microorganism for the removal of acetic acid (Tatton et al., 1989).

Inhibition due to higher loading can be minimized by selecting longer SRT, where COD removal and methane production gets higher at SRT more than 36 days (Wold, 2017). Inhibition by high COD contains industrial waste also might be due to dissolved COD which is either unbiodegradable or slowly biodegradable soluble COD. There might also contains other traces gases like H_2S in higher amount. But there no sulfide (S^{2-}) inhibition was observed in the system, so it was taken into account that H_2S concentration might be negligible in such waste.

5.5 Environmental Condition

The environmental parameters as pH, Alkalinity, Temperature and COD are the major which give the information on the activities of microbes inside the reactors.

5.5.1 Alkalinity

Alkalinity is the found the most controlling parameters for chemical treatment during anaerobic digestion of such industrial waste. Addition of alkalinity enhances the biogas production and stop in shutting down the process which might be due to the brakeage of soluble organic compounds into the lower molecular compound by alkali hydrolysis and making it accessible of substrate to the microbes as described by Esposito et al. (2012). This helped to reach the RI system closer to steady state condition with 97% COD mass balance.

5.5.2 Substrate /inoculum ratio

The experiments have been done using the fresh inoculum but the recognition on its concentration and volume ratio has not been done. This might have affected the kinetic behavior of the anaerobic microbes. The concentration of inoculum should be high to the substrate ratio and the volume of inoculum required to digest this type of substrate needs to be pre analyzed. The amount of inoculum is directly related to the accumulation of VFA and acidic problem during anaerobic digestion (Angelidaki et al., 2009). The positive control assays were done, which showed the response of the inoculum towards these standard substrates. The dilution was carried out to lower the organic loading of feed and to prevent the system from inhibition. The result show that the maximum methane potential of 1 g COD is close to each other in both duplicate sample, which indicates that the inoculum is not overloaded, but overloaded substrate has less methane potential than the theoretical value, which is the indication that the inoculum to substrate volume ratio is not balanced and process gets inhibited by VFA accumulation and drop in pH.

5.5.3 pH

pH has no relationship with retention time, but it changes with increasing OLR as observed by Boe et al. (2010). The microorganisms are very active for the degradation of organic matter in pH between 6.5 to 7.5, so the system should be buffered at this pH to prevent the failure in the system (Jain et al., 2015), which also helps to prevent the quick accumulation of VFA. The anaerobic digestion for higher concentration of glycol containing waste water was

not feasible which might be due to pH inhibition even after balancing the alkalinity to 6000 mg/l as $CaCO_3$ (Stewart et al., 1995).

5.5.4 Temperature

Low temperature and short SRT might have imbalanced the system due to slow reaction rate. In general, long SRT, high reactor volume and low organic loading is recommended for low temperature operation. Furthermore, at low temperature when the room temperature goes down to 20^0C, there might be the problem of degradation of long chain fatty acid and accumulation of this acid inhibits the whole digestion process as explained by (Tchobanoglus et al., 2003).

5.5.5 OLR

Higher HRT leads to low OLR in the system but instead of increasing the HRT from 16 this research was carried with different dilution rate for lowering OLR which assumed to have higher biodegradability according to Elreedy et al. (2016). There is increase in volatile fatty acid with the increasing OLR in the middle of the degradation process, which might be due to increasing in H_2 production but further increase in OLR which led to decrease in H_2 production was unknow since the concentration of H_2 gas was not identified in this experimental work. The research finding of the Elreedy et al., (2016) at lower HRT, where occurs the maximum production of H_2 and ethanol and maximum methane production could be achieved only at the peak HRT, was also proven by this research data.

5.5.6 VFA

Only acetic acid was calculated for VFA analysis by the auto titration methods. Other products of VFA like propionic and butyric acid were not measured. The presence of H_2 concentration also results increase in VFA by the production propanoic and butyric acid (Boe et al., 2010). The increased amount of Propionic acid could cause problem in the stability of microbial process (Wellinger, 2013) because the higher concentration of hydrogen inhibits the conversion of propionic and butyric acids (Tchobanoglus et al., 2003). As long as the methanogenesis microbes adapted to the high OLR and changes in the operational condition than the decreased in VFA could have achieved in high OLR. The value of ratio between VFA and alkalinity also determines the stability of the system. Both Low and high ratio value could have caused the instability of the system.

6 CONCLUSIONS & RECOMMENDATIONS

This section summarizes the main conclusions on the carried out experimental work, as well as recommendations for further testing of chosen type of industrial waste.

6.1 Conclusions

Glycol is soluble and easily biodegradable substrate where acidogenesis reaction is faster at higher loading which results in the fastest accumulation of VFA concentration and quick drop in pH. The anaerobic digestion is not appropriate for highly concentrated glycol containing waste water due to pH inhibition. Batch test by AMPTS II shows that the saline waste water has 50 % of BMP and complete degradation time of 3 days. The SMY was 0.47 g COD/g COD. The biodegradability time for not-saline source was 5 days with 20% of BMP and SMY of 0.22 g COD/g COD. This concluded that there is presence of some inhibitory factor in not-saline waste water which gives lower methane production.

High organic load and short retention time results the VFA accumulation and pH drop causes the inhibition in the system. Failure in the system due to low buffer capacity with deficient of alkalinity is the good finding of this research. Use of fresh inoculum is enough source of nutrients for the digestion of given type of industrial waste. So, adding alkalinity and replacing inoculum add the economic cost. Optimal concentration of substrate to inoculum ratio, selection of dilution rate and alkalinity balance need to be done precisely for the stability in the system.

The results of CFSTR from optimized experimental condition showed the SMY of 0.45 g COD/g COD in saline waste water and 0.20 g COD/g COD for not-saline waste water. The removal efficiency, negative yield was high in the beginning due to wash out COD from the system, low COD loading, and high dilution rate. For the anaerobic digestion of saline waste water in the continuous system, maximum OLR of 2.43 g COD/l. d equivalent to 40 g COD/ d has found the optimum limits for the reactor size of 1000 ml. Whereas maximum COD loading for not-saline waste water was at OLR 1.53 g COD/l. d which is equivalent to 26 g COD/d was found the right loading to keep the mass balance, but further experiment with pretreatment is recommended for this type of waste water. Inhibition was observed as the pH dropped below

6.8 resulting in a VFA accumulation to 1068 mg/L and alkalinity concentration dropped to 813 mg/L and no production of biogas.

Batch system gives higher SMY as compared to CFSTR system, what is only due to low retention time and washout of biomass in continuous system. Both batch and CFSTR provides the similar results. So, batch reactor will be best choice for the methane production from both economical and operational point of view for small scale plant. CFSTR system requires more times, expertise and high cost to achieve mass balance in the system.

6.2 Further Recommendations

The following recommendations can be helpful for successful continuation of the experimental work on anaerobic digestion of given types of industrial waste:

1. The problem of wash out of bacteria due to low retention time in CFSTR can be maintained by the use of bio-membrane in the effluent point for the retention of biomass into the reactor.
2. High concentrated glycol containing waste water is not feasible for anaerobic treatment in short retention time due to pH inhibition.
3. The CSTR can be replaced by other high rate anerobic reactors.
4. The economic loss due to extra loaded of alkalinity, replacement of inoculum for nutrients can be achieved by co-digestion of industrial waste with domestic municipal waste which could be the good source for both alkalinity and nutrients.
5. Further experiments with detailed study on microbes are recommended for not -saline waste water.

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Appendices

Appendix A	Photo of Experimental Set up of CFSTR
Appendix B	Photo of Experimental Set up of Batch System
Appendix C	Biochemical Methane Potential Batch Test Data
Appendix D	Preliminary Experimental data in CFTSR
Appendix E	Optimized Experimental data in CFSTR
Appendix F	Methane Production and Yield Calculation in CFSTR
Appendix G	COD Mass Balance in CFSTR

APPENDIX A

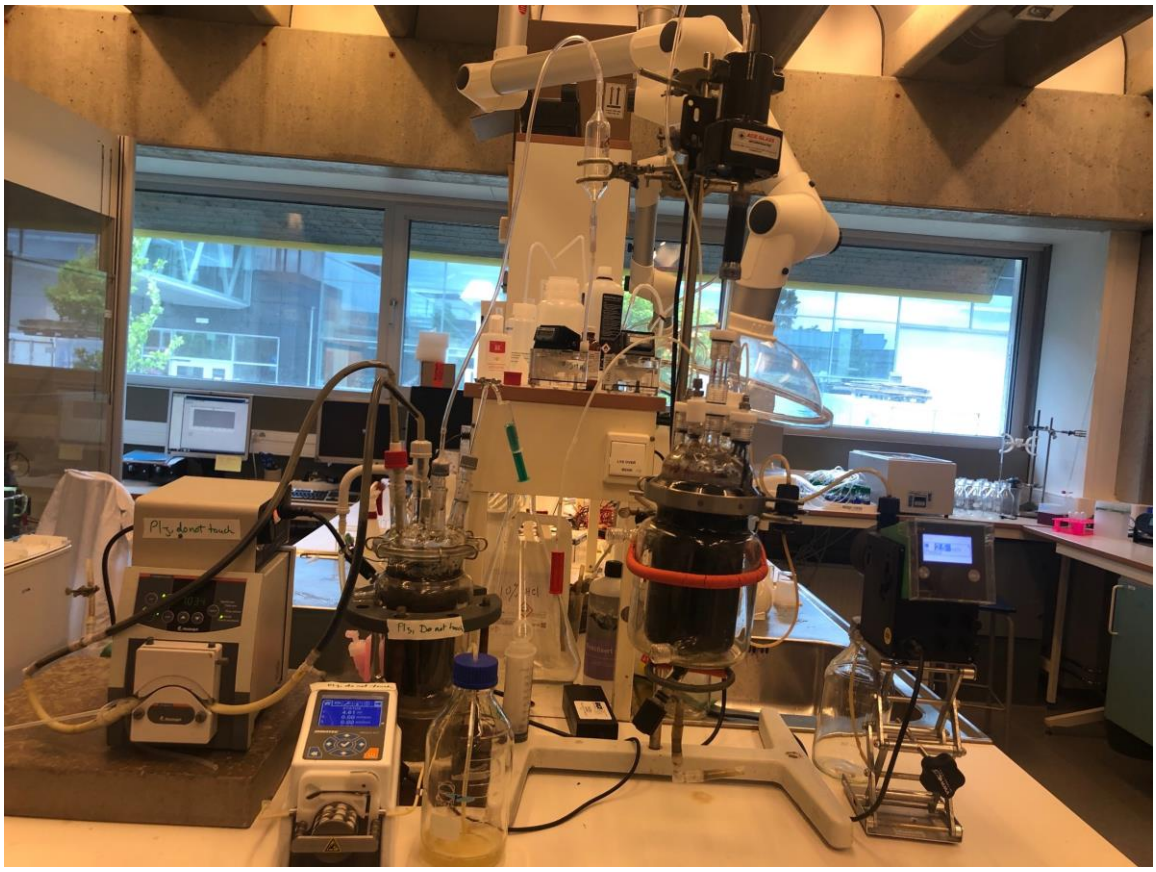

Figure A.1 Photo of Experimental Set Up of CFSTR, RI on The Right side and RII on the Left Side of The Picture

APPENDIX B

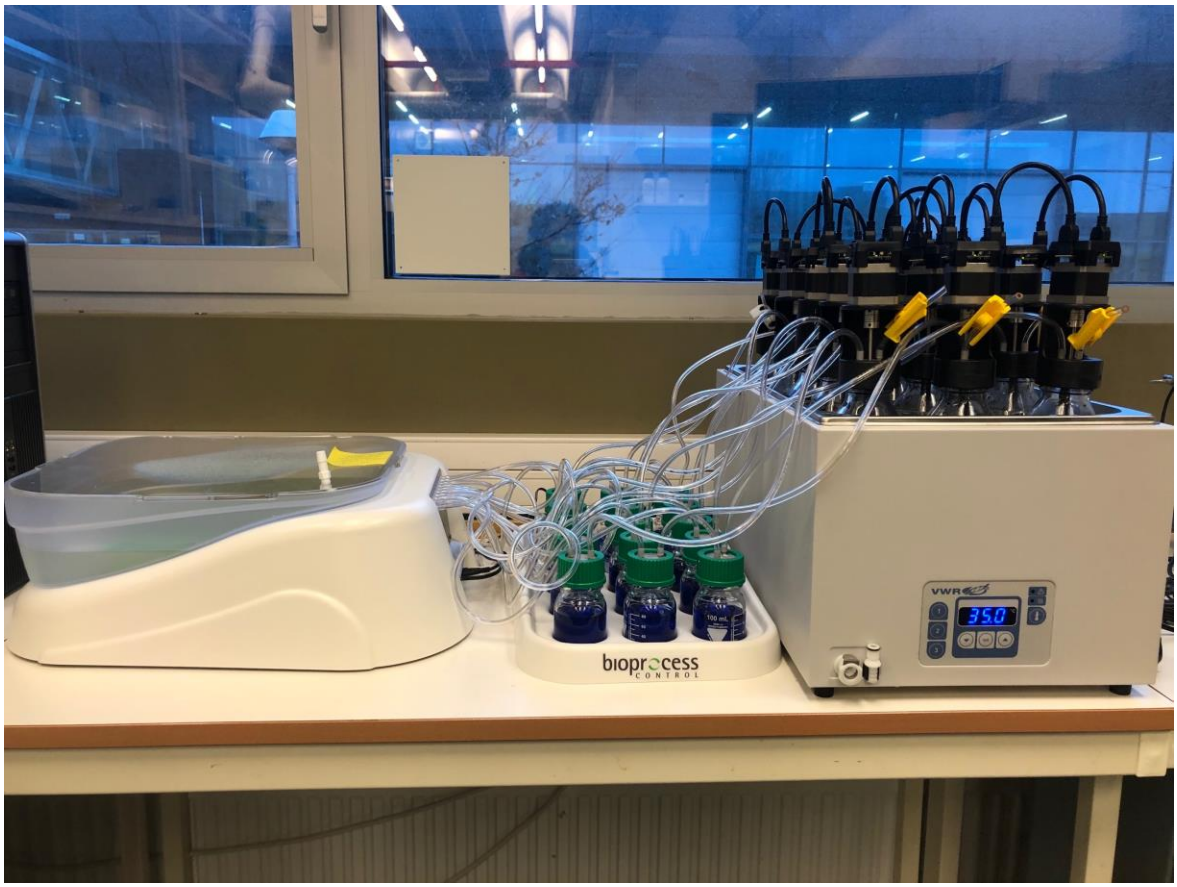

Figure B.1 Photo of Experimental Set Up of Batch Reactor (AMPTS II)

APPENDIX C

Biochemical methane potential test results from two Batch Reactor BRI and BRII are summarized in Tables C.1, C.2, C.3 and C.4.

Table C.1 Methane production in BMP test of saline waste water (BRI)

Day	Blank ($mlCH_4$)	Control ($mlCH_4$)	Methane Production at Different COD Loading ($mlCH_4$)				
			1 g	2g	3g	5g	8g
0	0	0	0	0	0	0	0
1	52±0.2	580±64	116±1.3	314	416±1.8	507±9.3	596±6.1
2	103±0.6	973±34	199±3.2	456	680±4.6	924±0.8	1054±6.1
3	145±0.8	1085±30	263±4.5	532	797±7.9	1047±1.7	1350±4.2
4	184±1.5	1168±30	316±3.3	582	877±10.1	1150±3.0	1452±5.9
5	219±2.3	1237±31	355±1.9	626	923±12.0	1233±6.0	1535±10.5
6	253±1.4	1291±32	396±0.6	658	957±13.1	1285±8.3	1589±12.1
7	288±1.3	1337±34	427±0.6	687	987±13.8	1320±5.2	1631±11.9
8	316±2.3	1378±35	451±2.1	717	1017±14.7	1353±5.3	1670±12.5
9	343±2.3	1419±35	468±1.2	740	1040±15.5	1377±7.3	1706±14.0
10	363±2.4	1448±31	489±4.7	756	1058±16.2	1396±7.9	1742±17.1
11	376±2.5	1464±28	500±4.6	771	1073±17.7	1411±9.0	1781±21.9
12	391±2.3	1480±26	510±4.4	783	1086±18.1	1423±9.5	1824±27.3
13	405±2.4	1495±25	520±4.8	795	1101±18.4	1436±11.2	1869±23.3
14	417±2.5	1507±23	526±3.1	799	1107±19.4	1441±10.8	1896±7.7
15	429±2.2	1517±21	526±2.7	805	1113±20.1	1448±10.9	1908±5.0
16	441±2.1	1524±15	544±0.7	812	1121±20.9	1455±11.1	1920±3.5
17	451±2.2	1528±12	558±3.6	819	1128±22.7	1462±11.3	1929±2.5
18	461±1.9	1537±12	669±0.1	825	1134±23.7	1467±11.5	1937±3.1
19	469±1.8	1544±12	673±0.6	830	1140±23.9	1472±11.4	1944±3.6
20	474±1.8	1548±14	678±2.0	836	1146±24.8	1477±11.2	1952±3.3
21	478±1.7	1551±16	685±2.3	842	1152±26.0	1483±10.9	1959±4.5
22	482±1.8	1555±15	688±3.0	847	1157±27.0	1487±11.0	1965±5.5

Table C.2 Flow rate of methane production of saline source (BRI)

Days	Blank ($mLCH_4$/day)	Control ($mLCH_4$/day)	Flow Rate of Methane Production per day (mL/day at different COD Loading				
			1g	2g	3g	5g	8g
0	0	0	0	0	0.0	0	0
1	51±0.6	580±64	152±0.1	314	416±1.8	507±9.3	596±6.1
2	51±0.5	393±31	141±2.9	142	264±2.8	417±8.6	458±0.0
3	42±0.2	112±4	75±3.2	76	117±3.4	123±1.0	297±10.3
4	40±0.5	83±1	68±2.9	50	80±2.2	103±1.3	102±1.7
5	36±0.2	69±1	55±1.1	45	46±1.9	83±3.0	83±4.7
6	34±1.1	54±1	52±0.4	32	34±1.1	51±2.3	54±1.5
7	35±0.8	47±2	43±3.1	29	30±0.8	35±3.1	42±0.1
8	27±1.9	41±1	27±0.9	30	30±0.9	33±0.1	39±0.6
9	25±1.8	42±0.1	25±1.4	23	23±0.8	24±2.0	36±1.6
10	18±1.8	28±4	20±0.3	17	19±0.8	19±0.6	37±3.1
11	12±1.8	17±2	13±0.8	15	15±1.5	15±1.2	39±4.9
12	13±2.1	16±2	13±0.2	12	13±0.4	13±0.5	42±5.3
13	12±1.5	15±2	12±0.0	12	15±0.4	13±1.7	46±4.0
14	11±1.7	13±1	11±0.4	4	6±0.9	5±0.3	26±15.6
15	10±1.6	11±1	11±0.5	6	7±0.7	7±0.0	12±2.7
16	12±0.1	10±1	10±0.8	7	8±0.8	7±0.3	12±1.5
17	10±1.1	9±1	9±0.8	7	7±1.8	7±0.2	9±1.0
18	9±0.8	7±1	8±0.4	6	6±1.0	6±0.2	8±0.6
19	6±0.4	6±1	7±0.5	5	6±0.3	5±0.0	7±0.6
20	5±0.0	6±1	5±0.2	5	7±0.8	5±0.2	8±0.4
21	4±0.0	5±1	4±0.1	6	6±1.3	6±0.3	7±1.3
22	3±0.5	3±	3±0.1	5	4±0.9	4±0.0	6±0.9

Table C.3 Methane production in BMP test of not- saline waste water (BRII)

Day	Blank ($mlCH_4$)	Control ($mlCH_4$)	Methane Production at Different COD Loading ($mlCH_4$)				
			1g	2 g	3g	5g	8 g
0	0	0	0	0	0	0	0
1	51	-1	-2	5	0	0	0
2	65	333	19	37	28	-42	53
3	86	581	37	62	78	-56	104
4	102	618	70	134	114	-75	174
5	117	633	63	153	203	-91	273
6	129	641	57	148	294	-105	343
7	137	649	55	145	292	-116	435
8	146	652	52	143	291	-124	573
9	149	656	54	143	289	-132	692
10	151	660	56	143	292	-135	700
11	152	662	58	144	295	-135	703
12	154	663	60	145	297	-136	704
13	155	664	62	146	299	-137	706
14	156	666	64	146	301	-137	704
15	157	664	66	148	303	-137	
16	155	668	72	152	305	-137	
17	153	671	77	156	310	-134	
18	152	674	81	159	315	-131	

Table C.4 Flow rate of methane production of not-saline source (BRII)

Days	Blank ($mLCH_4$/day)	Control ($mLCH_4$/day)	Flow Rate of Methane Production per day (mL/day at different COD Loading				
			8g	5g	3g	2g	1g
0	0	0	0	0	0	0	0
1	51.1	49.9	104.5	9.2	72.25	56.3	49.25
2	14.4	348.4	65.3	0.7	61.5	46.2	35.65
3	20.1	268.5	89.7	0.7	58.45	45.6	38.2
4	16.1	53.4	114.7	0.7	102.65	87.4	48.55
5	15.1	29.1	85.2	0.7	105.75	34	8.7
6	11.8	20.9	104.6	0.7	13.05	7	5.5
7	8.2	15.5	145.4	0.7	6.5	5.8	5.7
8	8.8	11.6	128.1	0.7	6.5	6.3	7
9	3.8	8.2	12.2	0.7	4.75	3.6	5.8
10	1.4	4.9	3.6	0.7	3.45	2.3	3.8
11	1.4	3.3	2.9	0.7	2.8	2.3	3.1
12	1.4	3.3	2.9	0.7	2.8	2.3	3.1
13	1.4	1.5	2.9	1.1	2.75	2	3.4
14	1.4	1.4	2.9	1.1	2.7	1.9	3.4
15	1.4	1.4	2.9	1.1	2.6	1.9	3.2
16	1.4	1.4	2.9	1.1	2.45	1.9	2.9
17	1.4	1.4	2.3	1.1	2.45	2.3	2.9
18	1.4	1.4	2.3	1.1	2.3	2.4	2.6
19				1.1	2	2.4	2.5
20				1.1	2	2.4	2.5

APPENDIX D

Results of Preliminary Experiments (I & II) from CFSTR (RI) are summarized in Tables D.1and D.2.

*Table D.1 Results on preliminary experiment **(Exp I)***

Date (March)	pH	Alkalinity mg/l	VFA mg/l	Biogas Production mL/d
06.03.2019	8.4	1753	4811	0
07.03.2019	6.3	269	5363	274
08.03.2019				334
11.03.2019	5.7	224	4993	39
12.03.2019				
13.03.2019	5.6	185	5957	
14.03.2019	6.8			
15.03.2019	7.0			161
18.03.2019	6.6			155
19.03.2019	7.1			60
20.03.2019	6.8	346	3792	143
21.03.2019	6.7	524	4518	80
22.03.2019	6.3	729	4601	
25.03.2019	5.9	608	5883	164
26.03.2019	6.3	495	5391	47
27.03.2019	6.2	293	5476	160

Notes: *Gaps in the tables represent no reading was taken on that days*
Dark shaded areas represent systematic problems in the Reactors

Table D.2 Result on preliminary experimental after adding alkalinity ***(EXPII)***

Date (April-May)	Alkalinity (mg/L)	VFA (mg/L)	pH	Biogas Production (ml/day)
09.04.2019	565	1493	7.5	147
10.04.2019	4372	685	7.5	254
11.04.2019	4406	641	7.3	224
12.04.2019	2685	2101	7.2	322
15.04.2019	2096	2619	6.7	140
16.04.2019	1863	2114	6.6	230
18.04.2019	1019	3565	6.4	250
19.04.2019				254
22.04.2019				304
23.04.2019				280
24.04.2019	1096	3792	6.4	153
25.04.2019	2843	4989	6.7	314
26.04.2019	3261	5813	6.8	30
29.04.2019	3734	6015	7.0	70
30.04.2019	4021	6501	7.1	137
1.05.2019	5025	6562	7.1	100
02.05.2019	4391	6907	7.1	175
03.05.2019	2131	3879	7.1	184
06.05.2019				83
07.05.2019				144
08.05.2019				26

APPENDIX E

Experimental Results after optimizing both operational and environmental parameters from RI (saline source) and RII (not-saline source) are summarized in Tables E.1 and E.2 respectively.

Table E.1 Optimized experimental results of saline source from RI

Days (May-June)	pH	Conductivity (mS / m)	Alkalinity (mg / l)	VFA (mg / l)	Effluent COD (g / l)	TS (g / l)	TVS (g / l)
1	7.5	1144	6662	0	33		
2	7.6	1130	2309	73	39	15	4
3	7.6	1130	2349	417	28	17	8
4	7.5	1119	2627	202	28	16	7
5	7.5	1124	2479	47	24	15	7
6	7.6	1092	2586	27	22	15	5
7	7.6	1074	2563	30	24	16	7
8	7.5	1080	3283	176	16	15	6
9	7.5	1081	2217	42	13	13	6
10	7.6	1071	2210	0	17	12	2
11	7.6	1111	1673	342	16	13	8
12	7.6	1060	2303	26	13	11	6
13	7.5	1037	1991	282	13	11	6
14	7.2	1007	1977	507	16	11	5
15	7.1	1009	1369	676		12	7
16	7.1	1019	1244	939	14	12	7
17	7.1	1031	1245	1145		11	5
18	6.9	1040	941	1500	19	12	5
19	6.7	1031	700	1640	18	12	5
20	6.9	1043	920	1378		11	6
21	6.7	1068	813	1650	16		

Table E.2 Optimized experimental results of not- saline source from RII

Days (April)	pH	Conductivity mS / m	Alkalinity (mg / L)	VFA (mg / L)	Effluent COD (g / l)	TS (g / L)	TVS (g / L)
1	7.9	1110	2093	290	13.6	6	2
2	7.8	1106	1302	302	15.5	9	4
3	7.8	1060	2018	53	11.1	5	2
4	7.8	1035	1832	5	7	7	3
5	7.8	1035	1663	354	9.1	9	4
6	7.7	993	2254	0	78.2	27	7
7	7.6	991	2025	0	41.2	0	0
8	7.6	1022	1735	0	25.3	12	6
9	7.6	1011	2618	18	15.6	14	5
10	7.6	1026	2036	111	8.4	10	4
11	7.6	1022	2318	317	25.2	7	3
12	7.4	1161	1952	86	18	17	7
13	7.4	1094	1807	297	17.7	14	6
14	7.4	1176	1897	304	15.7	13	4
15	7.6	1161	2183	122	12.4	8	3
16	7.4	1176	1932	113	14.4	9	3
17	7.4	1141	1844	286	18	7	3
18	7.4	1133	1766	373	12	0	0
19	7.3	108	1638	461	5.5	8	3
20	7.3	1055	1422	429		9	3
21	7.3	1039	1664	414		8	2
22	7.4	1032	1676	311			

APPENDIX F

Methane production and yield calculation in RI and RII are summarized in Table F.1 and F.2

Table F.1 Daily methane production and yield from saline waste water in RI

Days	Qin	COD_{in}	OLR	Biogas production	Calculated Methane as 62 %	Methane production	Removal Efficiency (%)	SMY
	l/day	g COD/L	g COD/l. d	L/d	L/d	g COD/d		
1	0.06	10	0.6	0.56	0.35	0.94	-233	-0.67
2	0.06	10	0.6	0.32	0.20	0.54	-291	-0.31
3	0.06	10	0.6	0.41	0.26	0.69	-184	-0.63
4	0.06	10	0.6	0.41	0.26	0.69	-182	-0.64
5	0.06	10	0.6	0.40	0.25	0.68	-137	-0.82
6	0.06	10	0.6	0.34	0.21	0.57	-121	-0.79
[redacted]								0.00
[redacted]								0.00
9	0.06	20	1.21	0.29	0.18	0.48	36	1.12
10	0.06	20	1.21	0.52	0.32	0.87	14	5.15
11	0.06	40	2.4	0.53	0.33	0.88	61	0.60
12	0.06	40	2.4	0.49	0.30	0.82	68	0.50
13	0.06	40	2.4	0.54	0.33	0.90	68	0.55
14	0.06	40	2.4	0.54	0.34	0.91	60	0.63
15	0.06	40	2.4	0.62	0.39	1.05	65	0.68
16	0.06	40	2.4	0.44	0.27	0.73	68	0.45
17	0.06	40	2.4	0.61	0.38	1.02	51	0.83
18	0.06	40	2.4	0.57	0.35	0.95	56	0.71

Table F. 2 Daily methane production and yield from not- saline waste water in RII

Days	Qin	COD_{in}	OLR	Biogas Production	Calculated Methane as 62 %	Methane production	Removal Efficiency (%)	SMY
	l/day	g COD/ L	g COD/l . d	L/d	L/d	g COD/d		
1	0.06	10	0.6	0.04	0.03	0.07	-36	-0.36
2	0.06	10	0.6	0.01	0.00	0.01	-55	-0.04
3	0.06	10	0.6	0.01	0.01	0.02	-10	-0.30
4	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
5	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
6	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
7	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
8	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
9	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
10	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	[redacted]	[redacted]
11	0.06	26	1.53	0.22	0.14	0.38	100	0.28
12	0.06	26	1.53	0.06	0.04	0.09	-62	-0.11
13	0.06	[redacted]	[redacted]	[redacted]	0.00	0.00	1	[redacted]
14	0.06	26	1.53	0.15	0.09	0.25	39	0.47
15	[redacted]	[redacted]	[redacted]	[redacted]	0.12	0.32	[redacted]	[redacted]
16	0.06	96	5.74	0.16	0.10	0.26	100	0.05
17	0.06	96	5.74	0.50	0.31	0.83	74	0.22
18	0.06	96	5.74	0.49	0.30	0.81	100	0.16
19	0.06	96	5.74	0.72	0.45	1.21	81	0.29
20	0.06	96	5.74	0.34	0.21	0.57	81	0.14
21	0.06	96	5.74	0.48	0.30	0.80	100	0.16
22	0.06	96	5.74	0.58	0.36	0.97	84	0.23
23	0.06	96	5.74	0.78	0.49	1.31	100	0.26
24	0.06	96	5.74	0.66	0.41	1.11	100	0.22
25	0.06	96	5.74	0.72	0.45	1.21	87	0.27
26	0.06	96	5.74	0.77	0.48	1.30	85	0.30

APPENDIX G

Calculation of COD mass balance in RI (saline) and RII (not-saline) reactors are summarized in Table G.1 and G.2 respectively.

Table G.1 COD mass balance in RI reactor

Days (April)	COD loading (g COD/l)	Flux of COD_{in} (g COD/d)	Flux of Methane Production (g COD/d)	Flux of Liquid COD_{out} (g COD/d)	COD Mass Balance (%)
1	10	0.6	1.1	1.9	510
2	10	0.6	0.6	2.3	492
3	10	0.6	0.8	1.7	415
4	10	0.6	0.8	1.7	413
5	10	0.6	0.8	1.4	364
6	20	0.6	0.6	1.3	329
[redacted]					
[redacted]					
9	20	1.2	0.5	0.8	109
10	40	1.2	0.9	1.1	167
11	40	2.4	1.0	0.9	81
12	40	2.4	0.9	0.8	70
13	40	2.4	1.0	0.8	74
14	40	2.4	1.0	0.9	82
15	40	2.4	1.2	0.8	85
16	40	2.4	0.8	0.8	67
17	40	2.4	1.2	1.2	97
18	40	2.4	1.1	1.1	89

Table G.2 COD mass balance in RII reactor

Days (May-June)	COD loading (g COD/l)	Flux of CODin (g COD/d)	Flux of methane production (g COD/d)	Flux of Liquid CODout (g COD/d)	COD Mass Balance (%)
1	10	0.6	0.002	0.8	136
2	10	0.6	0.000	0.9	155
3	10	0.6	0.000	0.7	110
[illegible]	[illegible]	[illegible]	[illegible]	0.4	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	0.5	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10	26	1.53	[illegible]	4.7	307
11	26	1.53	0.009	2.5	162
12	26	1.53	0.002	1.52	99
13	0	1.53	0.000	0.9	61
14	96	153	0.006	0.5	0
15	[illegible]	[illegible]	[illegible]		[illegible]
16	96	5.74	0.007		0
17	96	5.74	0.021	1.5	27
18	96	5.74	0.020		0
19	96	5.74	0.030	1.1	19
20	96	5.74	0.014	1.1	19
21	96	5.74	0.020		0
22	96	5.74	0.024	0.9	17
23	96	5.74	0.033		1
24	96	5.74	0.028	0.7	13
25	96	5.74	0.030	0.9	16
26	96	5.74	0.033	1.1	19