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Abstract

Oil and gas industries generate large volumes of wastewater during exploitation and processing of oil and gas. Industrial wastewater contains glycols along with other organic and inorganic compounds. It is essential to treat such wastewaters before discharge to the environment as these can have significant impacts on the environment. This research work has attempted to evaluate the feasibility of anaerobic treatment of glycol rich industrial wastewater for biogas production.

In the present study, three different laboratory scale experiments were conducted. The method used was the comparative assessments of results from two semi-continuous flow stirred tank reactors operating at same environmental conditions. One reactor was loaded with yeast extract solution while other reactor was operated with equal volumes of solution containing 50% COD load from Mono-ethylene glycol (MEG) while 50% COD load from co-substrate solution. The glycol solution used in the study was high strength laboratory manufactured MEG solution with COD of approx. 29,400 mg/L and 33,900 mg/L in experiments two and three respectively. The sludge collected from anaerobic digester of IVAR wastewater treatment plant was used as the source of microbes for anaerobic digestion.

Methane yields corresponding to the peak gas productions of 211 mL/g COD and 299 mL/g COD were determined from the glycol solution in the experiments two and three respectively even at partial inhibition states. There was a rapid drop in alkalinity and pH after introduction of glycol in the reactors leading to failure of the anaerobic process in experiment 2. pH inhibition was observed in experiment 3 as well but alkalinity was added this time to control pH. The results show that pH inhibition due to insufficient alkalinity in the reactor is most likely the prominent cause for failure of the process. Apart from this, analysis of bulk phase samples indicates that high organic loading and short solid retention time were most likely responsible for accumulation of volatile fatty acids causing pH reductions. With necessary improvements in the overall design and frequent monitoring and controlling of important parameters such as temperature, pH, alkalinity, nutrients, etc., it seems that reactor performance can be enhanced. From this study, it can be concluded that it is feasible to convert glycol rich industrial wastewater to biogas. However, due to lack of alkalinity production during fermentation and potential nutrient limitation, co-digestion with a complex substrate/sludge seems to be required for long term stable anaerobic digester performance.

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Abbreviations

ABR = Anaerobic Baffled Reactor

ACP = Anaerobic Contact Process

ADP = Adenosine diphosphate

AF = Anaerobic Filters

AM = Aceticlastic Methanogens

AMBR = Anaerobic Membrane Bioreactors

AMR = Anaerobic Membrane Reactor

ASBR = Anaerobic Sludge Bed Reactors

ATP = Adenosine triphosphate

BOD = Biochemical Oxygen Demand

C = Concentration

COD = Chemical Oxygen Demand

CSTR = Continuous Stirred Tank Reactor

DAF = Dissolved Air Flotation

DEG = Diethylene Glycol

EC = Effective Concentration

EGSB = Expanded Granular Sludge Bed

FB = Fluidized Beds

GLSS = Gas-Liquid-Solids Separation System

HAc = Acetic Acid

HRT = Hydraulic Retention Time

HM = Hydrogenophilic Methanogens

IUPAC = International Union for Pure and Applied Chemistry

k_h = Kinetics of hydraulics

K_{ow} = Octanol water partition coefficient

Ks = Half velocity constant

LC = Lethal Concentration

LCFA = Long Chain Fatty Acids

MEG = Monoethylene Glycol

MLSS = Mixed Liquor Suspended Solids

OIW = Oil in Water

PAHs = Polycyclic Aromatic Hydrocarbons

PTA = Purified Therephthalic Acid

PEG = Polyethylene glycol

Q = Hydraulic flow rate

SCFA = Short Chain Fatty Acids

SRT = Solid Retention Time

TEG = Triethylene Glycol

TPH = Total Petroleum Hydrocarbons

TREG = Tetraethylene Glycol

TS = Total Solids

TSS = Total Suspended Solids

TVS = Total Volatile Solids

UAF = Upflow Anaerobic Filter

UASB = Upflow Anaerobic Sludge Bed

VFA = Volatile Fatty Acid

VOL = Volumetric Organic Loading Rate

VS = Volatile Solids

VSS = Volatile Suspended Solids

Y = Growth Yield

μ_m = Growth rate of microorganisms

% = Percentage

List of Units

atm = atmosphere

°C = degree Celsius

d = day

°F = degree Fahrenheit

g = gram

K = Kelvin

kcal = kilocalorie

kg = kilogram

kJ = kilojoule

kPa = kilo Pascal

kWh = kilo watt hour

L = liter

m = meter

M = molarity

mg = milligram

MJ = Mega Joule

mol = mole

mS = milli Siemens

N = Newton

nm = nanometer

rpm = revolution per minute

s = second

1   INTRODUCTION

In the oil and gas industry, the term "industrial wastewater" denotes produced water which is generated in the exploitation and processing of oil and gas. Produced water is principally a mixture of formation water (the water which occurs naturally in the gas and oil reservoirs), injection water (any water injected to the formation) and also contains any chemicals added during the production processes [1, 2]. The produced water is composed of dissolved and dispersed oil compounds, dissolved formation minerals, production chemical compounds, production solids (including formation solids, corrosion and scale products, waxes and asphaltenes) and dissolved gases [1].

Gas industries use glycols such as ethylene glycol (MEG), diethylene glycol (DEG), triethylene glycol (TEG), and tetraethylene glycol (TREG) in multiphase pipelines that convey natural gas from remote gas fields to an onshore processing facility to inhibit hydrate formation. Hydrates can agglomerate and plug a flowline, damage valves and instrumentations leading to flow assurance failure which is highly detrimental [3]. Glycols are used for hydrate inhibition by dehydrating natural gas before transportation or by reducing temperature at which hydrate is formed. During use, glycol gets contaminated by dissolved salts from formation water along with scaling and corrosion products from the pipelines. Ethylene glycol can be regenerated from the natural gas and reused after purification treatment. In these processes, wastewater is produced which contains glycols.

Discharge of wastewater to the environment can have significant impacts on the environment. Dissolved hydrocarbons which are found naturally in formation water can be both toxic and bio-accumulative [2]. Produced water contains salt which is the major contributor of toxicity [1]. Moreover, dispersed and soluble oil increases the biochemical oxygen demand of the receiving water [1]. Consumption of dissolved oxygen from water can limit the availability of oxygen to aquatic lives. There is also possibility of toxic effects from additives, especially corrosion inhibitors. Furthermore, treatment chemicals can precipitate and accumulate in marine sediments [1]. However, long term consequences of such contaminants on the environment are not fully documented and understood [2]. A common legislation for produced water discharge to sea from offshore installations is 40 mg/l (ppm) oil in water (OIW) [2]. There are other national and international legislations which bind petroleum companies to treat produced water before discharge to the environment.

Different techniques such as dissolved air flotation (DAF), membrane separation, chemical precipitation, chemical oxidation, and biological treatment etc. are used to treat oily wastewaters for the removal of petroleum pollutants and chemical oxygen demand (COD) [4]. Conventional treatment includes oil water separators like API separators and coagulation/flocculation followed by clarification. Moreover, the wastewater may require further treatments depending on the effluent quality requirements or applicable regulations. However, the sludge generated in API separator and float from refineries are classified as hazardous which leads to high disposal costs [5]. Among these technologies, it is a common practice to employ biological treatment, typically activated sludge system for the treatment of oilfield produced water. Biological treatment systems are highly effective treatment systems which are economically feasible [4]. The dominant mechanism of hydrocarbon removal in biological treatment is biodegradation of organic particles by microorganisms and occluding particles by microorganisms similar to bio-flocculation [1]. However, this process has some disadvantages like high operational and energy cost due to oxygen supply by aeration, large sludge production etc. In addition, the ratio of biological oxygen demand (five-day) to chemical oxygen demand i.e. $(BOD_5)/COD$ is relatively low in oilfield produced water. It makes it difficult to reduce COD using a single aerobic biological treatment only [4]. Therefore, anaerobic processes may be used for the treatment of produced water [4].

In the anaerobic method, wastewater is degraded using microorganisms in the absence of molecular oxygen. There are several advantages of this process over traditional aerobic process such as very low sludge production, low energy requirement, low biomass yield which results in low nutrient requirements etc. Additionally, valuable byproduct in the form of methane can be generated by this process. Methane can be used as a fuel or energy as a renewable resource. However, anaerobic treatment process is a challenging process involving different microorganisms and very sensible operational stability. Heavy oil produced water is difficult to degrade because it contains large quantities of large-molecule non-biodegradation organics [4]. Moreover, low BOD_5/COD ratios, slow growth rates resulting in long start-up times, long retention times, poor solid-liquid separation and inhibition to the microbial population etc. are other potential problems associated with such treatments. Therefore, it is crucial to improve the biodegradability of produced water and optimize the anaerobic treatment conditions for optimal performance [4]. When it comes to glycol, it does not contain nutrients other than carbon which are vital for the growth of microorganisms. Co-digestion

with complex substrates/wastes can subsidize missing nutrients for long term stable anaerobic digester performance.

The main objective of this thesis is to investigate, in laboratory scale, the feasibility of anaerobic conversion of glycol rich industrial wastewater to biogas under mesophilic conditions. Besides, the study also aims to evaluate possibilities of co-digestion of glycol contaminated wastewater with other substrates and identify and assess different factors affecting the performance of anaerobic reactor. MEG is the solo glycol used in the study.

2 LITERATURE REVIEW

2.1 Anaerobic Digestion Overview

Anaerobic digestion refers to the anaerobic decomposition of organic matter, resulting in partial gasification, liquefaction, and mineralization [6]. In an anaerobic process, biodegradable organic matter, both soluble and particulate are converted to methane and carbon dioxide. Anaerobic digestion of solid waste and / or waste water sludge has long been used to stabilize organic wastes prior to final disposal of the organic wastes. However, due to the involvement of the complex microbial ecosystem and sensible operational stability, it has continued to be the subject of research and new process development [7]. Moreover, in our increasingly energy conscious society, generation of valuable by-product i.e. methane along with the waste treatment is very significant.

The production of biogas was discovered in the 17th century after scientists observed "marsh gas" burning on the surface of swamps [8]. Anaerobic treatment occurs naturally in any holding tank for wastewaters producing methane. This oldest form of wastewater treatment was not developed and was applied only circumstantially in ponds for high strength wastewaters. The first application of anaerobic treatment to raw wastewater was in the 1950s except for anaerobic ponds, when the anaerobic contact process was developed [8]. After that anaerobic process has been used to treat varieties of wastes in different scales. Anaerobic treatment proved to be successful for treating industrial wastewaters which usually have high organics concentrations.

Anaerobic digestion is used extensively for the stabilization of biodegradable particulate organic matter. Apart from this, destruction of pathogens is important when bio-solids are used. The measure of percentage of VS reduction can be used as a parameter to estimate the performance of the digesters. It is estimated that 80 to 90% of the influent biodegradable particulate organic matter will be converted to methane when an SRT of 15 to 20 days is provided [7]. This corresponds to destruction of about 60% of the VS contained in primary solids and 30 to 50% of the VS contained in waste activated sludge [7].

There are reports from various scholars about anaerobic treatability of produced water and various other issues and challenges. Dwyer and Tiedje proposed the metabolic pathway for anaerobic degradation pathway of EG in 1983 [9]. Stewart et al. [9] found Ethylene glycol at

concentration of 5,000 mg/L was suitable for fermentation. The same reference has indicated that EG concentrations of 15,000mg/L and 20,000 mg/L caused pH problems. Kawai [10] has mentioned that PEG with a molecular weight of 20,000 was metabolized by anaerobic bacteria. According to some authors, anaerobic treatment system will present a future viable treatment technology for handling of produced wastewater or wastewater containing glycol [11, 12]. However, literature indicates that in some oilfields, anaerobic treatment system has been already operated as a full-scale system coupling with aerobic treatment. For example, in the Jidong Oilfield of China, a full-scale anaerobic biological reactor succeeded by aerobic biofilm reactor has been used to treat produced water [13]. The anaerobic reactor effectively removes about 45% of the polycyclic aromatic hydrocarbons (PAHs) and 25% of the total petroleum hydrocarbons (TPH) from the influent produced water [13]. Similarly, in Liaohe Oilfield in China, oil produced water containing high concentrations of salts and low nutrient content has been treated with constructed wetland and anaerobic baffled reactor (ABR) systems [14].

There are several advantages of anaerobic waste treatments over aerobic treatment systems. Some are listed below [15].

a) A reduction in excess sludge production up to 90%.
b) Up to 90% reduction in space requirement when using expanded sludge bed systems.
c) High applicable COD loading rates reaching 20-35 kg COD per m^3 of reactor per day, requiring smaller reactor volumes.
d) No use of fossil fuels for treatment, saving about 1 kWh/kg COD removed, depending on aeration efficiency.
e) Improved sludge dewaterability
f) No or very little use of chemicals
g) Plain technology with high treatment efficiencies
h) Generation of a potentially valuable by-product (methane) which is nearly 13.5 MJ CH_4 energy/ kg COD removed.
i) Excess sludge has a market value.
j) No oxygen is required.

Disadvantages of the process are [16]:

a) Longer start-up time to develop necessary biomass stock.

b) May require alkalinity addition.

c) May require further treatment with an aerobic treatment process to meet discharge requirements.

d) Biological nitrogen and phosphorus removal is not possible.

e) Much more sensitive to the adverse effects of lower temperatures on the reaction rate.

f) May be more susceptible to upsets due to toxic substances.

g) Potential for production of odors and corrosive gases.

Three groups of bacteria viz. acidogens, acetogens and methanogens are involved in the biological anaerobic process and complex interactions of each species of bacteria are involved for the success of process. The process is generally considered to be four successive stages biological processes; i) hydrolysis, ii) acidogenesis iii) acetogenesis, and iv) methanogenesis involving waste conversion and stabilization. The end products are principally methane (CH_4), Carbon dioxide (CO_2), and stable organic residues. These processes are discussed in detail in the subsequent sections.
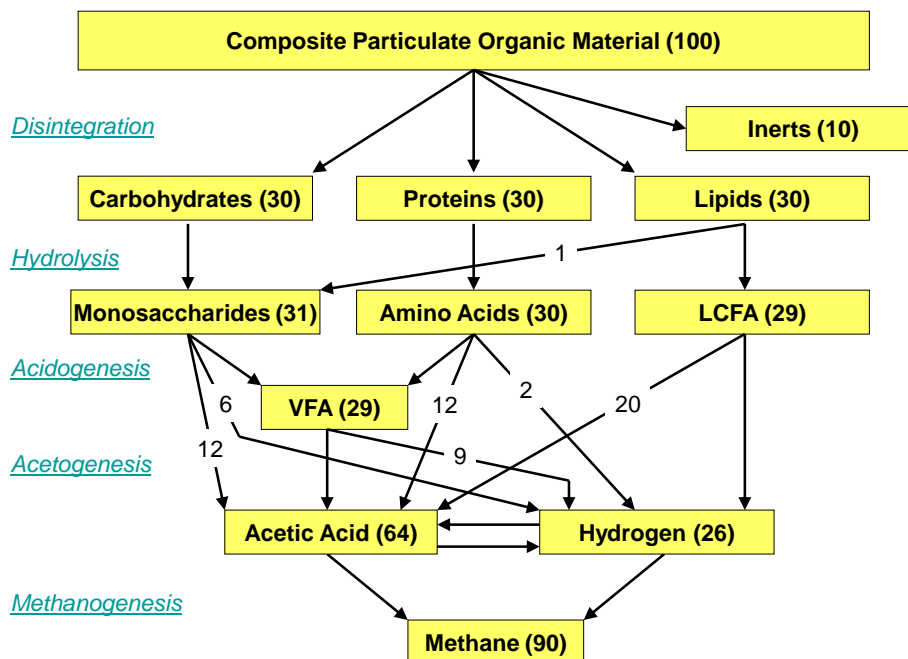
Figure 2.1: Reactive scheme for the anaerobic digestion of polymeric materials [17]

2.1.1 Disintegration

Disintegration is the initial step involved in an anaerobic process. In this step, the anaerobic degradation of complex polymers and particulates (i.e. colloidal 10 - 1000 nm or larger particles > 1000 nm) takes place mainly by physical shearing and dissolution while some extracellular enzymes may also be involved [17]. Composite particulate organic materials are disintegrated into its constituent products; carbohydrates, proteins, lipids and inerts. Disintegration follows a first order expression as a function of the total composite particulate material concentration, $X_{Composite}$, and thus the rate of change of its concentration, r_{dis} is given by [7]

$r_{dis} = -K_{dis} * X_{Composite}$ Where, first order disintegration rate coefficient (k_{dis}) = 0.4 and 1.0 1/d for mesophilic and thermophilic bacteria respectively [17]

2.1.2 Hydrolysis

Hydrolysis can be defined as a process in which complex polymeric substrates, particulates or undissolved particles are converted into monomeric and dimeric compounds which are readily accessible for the acidogenic bacteria [15]. In this process, hydrolytic and lytic extracellular enzymes are secreted by fermentative bacteria into the local environment for depolymerisation of organic polymers. Generally, extracellular enzymes are considered to be of two types; *Endo-* and *Exo-*hydrolytic enzymes. Endo-hydrolytic enzymes are responsible for cutting intrapolymeric bonds, while the other depolymerize polymer from one of the polymer ends. Even though the process is referred as hydrolysis, lytic enzymes also depolymerize in addition to hydrolases [17]. The key group consists of proteases (acting on proteins), cellulases, amylases, glucanases (all acting on polysaccharides), and lipases (acting on fats and oil, lipids). The end products of this process are the monomers i.e. monosaccharides, amino acids and long chain fatty acids resulting from their consecutive polymers; carbohydrates, proteins and lipids [17].

Anaerobic digestion models normally use first order kinetics due to the lack of information on biomass (fermenters). Kinetics of hydrolysis are often modeled as $r_{hyd} = -k_h \cdot X_{polymers}$ where, k_h is the hydrolysis coefficient for a given particulate biochemical component, $X_{polymers}$ [17]. The value of k_h ranges from 0.3-0.7d^{-1}.

2.1.3 Acidogenesis

During acidogenesis or fermentation process, the hydrolyzed products (amino acids, simple sugars, long chain fatty acids (LCFAs)), which are relatively small soluble compounds, are converted into volatile fatty acids (VFAs) by acidogens/fermenters. Acidogenesis is the most rapid conversion step in the anaerobic food chain, and the growth rate of fermenters (μ_m) is comparable to aerobic rates (2-7 1/d) [15, 17]. The acidification products are mainly short chain volatile fatty acids i.e. acetate, propionate and butyrate, as well as ethanol, formic and lactic acids, CO_2, H_2, NH_3 and H_2S [15, 17].

The process is strictly anaerobic implying that there are no external electron acceptors involved in this process. Thus, organic compounds serve as both electron donors and electron acceptors. The electrons from the substrate are captured in reduced organic compounds or H_2, originating from the substrate and is excreted from the cells as fermentation products. The large part of free energy associated with the excreted fermentation products cause the remaining energy for growth to be limited and thus the growth yield of acidogens is low (typically $Y_{X/S}$ of 0.1 - 0.2 g VSS/g COD) [17]. Acidogenesis is inhibited by free ammonia and LCFA accumulation [17].

Table 2.1: Stoichometries of product formation using Glucose as model substrate [18]

	Products	Reaction	ATP per mole glucose	Conditions	Note
(i)	Acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	4	low H_2	1
(ii)	Propionate	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$	~low	not observed	2
(ii')	Acetate, Propionate	$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$	4/3	any H_2	
(iii)	Butyrate	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	3	low H_2	1
(iv)	Lactate	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$	2	any H_2	
(v)	Ethanol	$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$	2	low pH	3

1. While thermodynamically possible at high H_2, may be limited by energetics of substrate-level phosphorylation
2. Not yet observed in cultured environmental samples. Coupling with substrate level oxidation is more common as in reaction ii'.
3. Energy yield taken from yeast pathway. Bacterial pathway may have 0 ATP/mole ethanol

The growth yield in acidogenesis is low and the remaining fraction of the substrate is converted into fermentation products, C_A (80 – 90 %).

Dissolved-COD (C_S) → Biomass (X_{Ac}) + Products (C_A)

The growth rate of acidogenic organisms is described by Monod equation as

$$\mu = \frac{\mu_{maxAc} \cdot C_S}{K_S + C_S}$$

$$\frac{dX_{Ac}}{dt} = \mu \cdot X_{Ac} = \frac{\mu_{maxAc} \cdot C_S}{K_S + C_S} \cdot X_{Ac}$$

The substrate removal rate can be expressed as

$$\frac{dC_S}{dt} = \frac{\mu \cdot X_{Ac}}{Y_{Ac}} = \frac{\mu_{maxAc} \cdot C_S}{K_S + C_S} \cdot \frac{X_{Ac}}{Y_{Ac}}$$

The product formation is proportional with growth rate and the fraction of substrate ending as products corresponds to $(1 - Y_{Ac})$

$$\frac{dC_A}{dt} = (1 - Y_{Ac})\frac{dC_S}{dt} = (1 - Y_{Ac})\frac{\mu \cdot X_{Ac}}{Y_{Ac}}$$

2.1.4 Acetogenesis

After acidogenesis process, acetogenic bacteria convert the short chain fatty acids (SCFA), other than acetate to acetate, hydrogen gas and carbon dioxide. This phenomenon is known as acetogenesis. Although propionate and butyrate are the most important substrates for acetogens, lactate, ethanol and even H_2 and CO_2 are also converted to acetate [15]. It is obligatory to keep the products (H_2 and formic acid) at a low concentration in order to favor thermodynamically their formation reaction (ΔG^o >0). This low concentration is maintained by the hydrogen utilizing methanogens. Thus, acetogens grow in close contact to the autotrophic methanogens in order to keep the intermediate concentration of electron carriers (H_2) at a low level [17]. The cooperation between the two different and very distant bacterial groups is called syntrophy. Interspecies hydrogen transfer is a process of direct transfer of the metabolic product (H_2) to the consumer in acetogenesis. The process is shown in figure 2.2, where $\Delta G'$ is associated to different hydrogen concentrations for the anaerobic oxidation of propionate, butyrate, and palmitate [17].
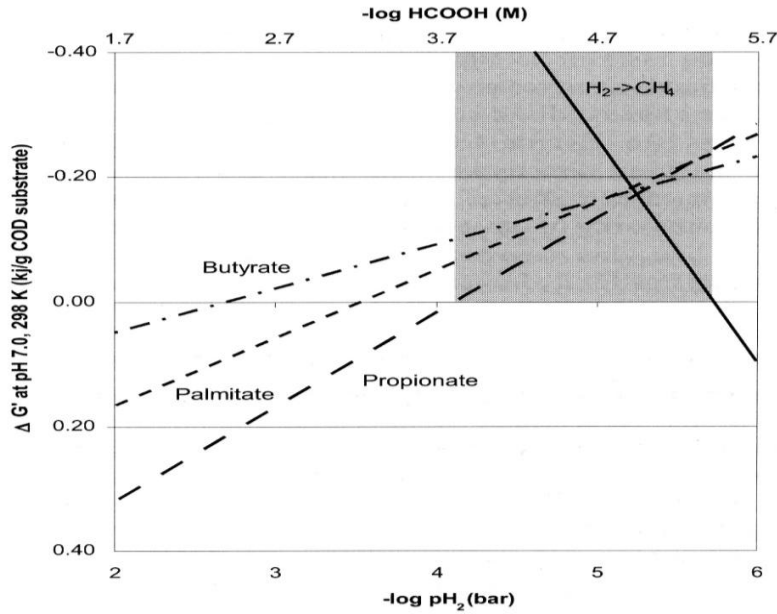
Figure 2.2: Interspecies hydrogen transfer [18]

In a properly functioning methane producing reactors, hydrogen pressure does not exceed 10^{-4} bars and is usually between 10^{-4} to 10^{-6} bars. If the H_2 concentration is not within the so called "hydrogen window", acetogenesis or autotrophic methanogenesis will be inhibited [17].

Table 2.2: Stoichiometry showing the product formation of the different substrates [18]

Substrate	Reaction	ΔG^0 (kJ gCOD^{-1})	$\Delta G'$ (kJ gCOD^{-1})
H_2, HCO_3^-	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−2.12	−0.19
Propionate	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$	0.68	−0.13
Butyrate	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	0.30	−0.16
Palmitate	$CH_3(CH_2)_{14}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2$	0.55	−0.16

$\Delta G'$ Calculated for T 298 K, pH 7, pH_2 1×10^{-5} bar, pCH_4 0.7 bar, HCO_3^- 0.1M, and organic acids 1mM.

The growth rate of acetogenic organisms is described by Monod equation:

$$\mu = \frac{\mu_{maxAce} \cdot C_p}{K_p + C_p}$$

$$\frac{dX_{Ace}}{dt} = \mu \cdot X_{Ace} = \frac{\mu_{maxAce} \cdot C_p}{K_p + C_p} \cdot X_{Ace}$$

The growth rate of acetogenic organisms is slightly higher than methane producing organisms, $\mu_m \sim 0.5 - 0.8$ d^{-1}, but lower than the acidogenic organisms.

The substrate removal rate is expressed as:

$$\frac{dC_p}{dt} = \frac{\mu \cdot X_{Ace}}{Y_{Ace}} = \frac{\mu_{maxAce} \cdot C_p}{K_p + C_p} \cdot \frac{X_{Ace}}{Y_{Ace}}$$

The product formation is proportional with growth rate and the fraction of substrate ending as products corresponds to (1 - Y_{Ace})

$$\frac{dC_{Ac}}{dt} = (1 - Y_{Ace})\frac{dC_p}{dt} = (1 - Y_{Ace})\frac{\mu \cdot X_{Ace}}{Y_{Ace}}$$

2.1.5 Methanogenesis

Methanogenesis is the final stage in the overall anaerobic conversion of organic matter to methane and carbon dioxide. In this step, the products formed by acetogens are utilized by methanogens which reduce carbon dioxide using hydrogen as electron donor and decarboxylate acetate to form CH_4. It is only in this stage, influent COD is converted to a gaseous form [15]. Methanogens exploit only certain specific substrates such as acetate, methylamines, methanol, formate, and H_2/CO_2 or CO. They are classified into two major groups depending upon their substrate needs; the acetate converting or aceticlastic methanogens and the autotrophic hydrogen utilizing methanogens [15].

Aceticlastic methanogens use acetate as substrate whereby a single carbon in the substrate molecule is reduced to methane, while the other is oxidized to CO_2 (a conversion mechanism sometimes referred to as intra molecular electron translocation). The overall reaction is slightly exothermic (one reaction equal approx. requirements for a single Adenosine diphosphate (ADP) to Adenosine triphosphate (ATP) phosphorylation), and provides chemical potential for growth of aceticlsatic methanogens. The dissimilative reaction is:

CH_3COOH → $CH_4 + CO_2$ ΔG^o = -31 kJ mol^{-1}

Autotrophic methanogens use H_2 as electron donor reducing CO_2 to CH_4 (electron acceptor). The overall free energy is significantly higher compared to aceticlastic methanogens, but their growth potential (i.e. required number of electron donor reactions per biomass formed) is still rather low.

$$CO_2 + 4\,H_2 \rightarrow CH_4 + H_2O \qquad \Delta G^o = \text{-135 kJ mol}^{-1}$$
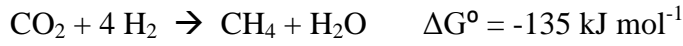
Energy generation in methanogens is not driven by substrate level phosphorylation, but reversed electron transport and ATPase [19]. As, the free energies for both aceticlastic and autotrophic methanogens are very low, these organisms are known to depend on proton or cation motive force energetic through reversed electron flow in the cell membrane [17]. The methanogenic growth rates ($\mu_{max} = 0.3 - 0.5$ 1/d) as well as the growth yield ($Y = 0.05 - 0.1$ g VSS/g COD) are low and thus methanogenesis is usually the limiting process during anaerobic sludge digester design [17]. It implies that long mean cell residence time is required in bioreactors for methanogenesis to take place. The optimal pH for both group of methanogens is around 7.0 and their activity drops to very low values when the pH falls outside of the range of $6.0 - 8.0$ [8].

The growth rate of aceticlastic methanogens (AM) is described by Monod equation as

$$\mu = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \qquad \frac{dX_{AM}}{dt} = \mu \cdot X_{AM} = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \cdot X_{AM}$$

The substrate removal rate of aceticlastic methanogens is expressed as

$$\frac{dC_{Ac}}{dt} = \frac{\mu \cdot X_{AM}}{Y_{AM}} = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{AM}}{Y_{AM}}$$

The methane formation is proportional with growth rate and the fraction of substrate ending as methane gas corresponds to $(1 - Y_{AM})$

$$\frac{dC_M}{dt} = (1 - Y_{AM})\frac{dC_{Ac}}{dt} = (1 - Y_{AM})\frac{\mu \cdot X_{AM}}{Y_{AM}}$$

For hydrogenophilic methanogens, CO_2 is the carbon source and H_2 the electron donor, forming CH_4 and H_2O as

Hydrogen (H_2) + $CO_2 \rightarrow$ Biomass (X_{HM}) + CH_4 + H_2O

The growth rate of hydrogenophilic methanogens (HM) is described by Monod equation:

$$\mu = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \qquad \frac{dX_{HM}}{dt} = \mu \cdot X_{HM} = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \cdot X_{HM}$$

The substrate removal rate of hydrogenophilic methanogens is expressed as:

$$\frac{dC_{H_2}}{dt} = \frac{\mu \cdot X_{HM}}{Y_{HM}} = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \cdot \frac{X_{HM}}{Y_{HM}}$$

The methane formation is proportional with growth rate and the fraction of substrate ending as methane gas corresponds to $(1 - Y_{HM})$

$$\frac{dC_M}{dt} = (1 - Y_{HM})\frac{dC_{H_2}}{dt} = (1 - Y_{HM})\frac{\mu \cdot X_{HM}}{Y_{HM}}$$

2.2   Factors influencing anaerobic treatment process

The performance of anaerobic treatment systems depends upon many factors. As many groups of bacteria are involved in this process, their activities are interrelated and affect each others' performance. Process factors such as the solids retention time (SRT), hydraulic retention time (HRT) and organic loading rate are important from the microbial kinetics point of view. Microbes are also sensitive to environmental factors such as pH, temperature, nutrient supply, and the presence of toxics and operational factors such as mixing and the characteristics of the waste being treated [7].

2.2.1   Solids Retention Time

Solids retention time (SRT) is a fundamental parameter which controls the types of microorganisms that can grow in the process and the extent to which reactions will occur. SRT equals to the HRT in flow through systems such as anaerobic digesters. SRT is increased relative to the HRT in some systems by recycling solids back to the system. Generally, SRT of pilot scale anaerobic treatment systems range from 30 to 40 days but it can range up to more than 100 days depending upon the system [7]. However, these values are significantly higher than required when it comes to treatment of wastewater. If long SRTs are used then very stable performance can be obtained in some anaerobic treatment systems. The increment of SRT is advantageous due to increased hydrolysis and stabilization of particulate organic matter. This can be significant for the stabilization of certain types of wastewater solids [7]. Nevertheless, it is possible that systems having high SRT may represent under loaded systems which are uneconomical [7]. Anaerobic systems can be shut down for extended periods of

time (up to several months) and that good performance can be restored shortly after they are restarted [7].

2.2.2 Volumetric Organic Loading Rate

Organic loading rate is the mass of COD added per unit volume per unit time. Volumetric organic loading rate (VOL) is related to the SRT through active biomass concentration in the bioreactor and it is used to characterize the loading on anaerobic treatment systems. Knowing VOL, we can know whether the bioreactor volume is used effectively or not. Volatile solids VOLs typically range from 2 to 6 kg volatile solids, VS/ (m^3day) [7].

Loading is an important factor for the design and operation of the anaerobic processes. If non-attached biomass reactor with long hydraulic retention time is hydraulically overloaded, it results in biomass washout leading to process failure. Similarly, for sewage sludge containing high protein, high loading can lead to toxicity problems. Such sludge release high concentration of ammonia which is toxic to microbes. Furthermore, organic overload is also not desirable in an anaerobic treatment system. If there is organic overload, VFA formed during the acetogenesis may get accumulated as the methanogens, due to its net slow growth, may not convert all of those VFA to methane [11]. It results in imbalance in the system due to pH reduction and can inhibit the activity of the methanogens [7].

The loading rate can be mathematically expressed as:

$L = \frac{Q \cdot Ci}{V}$ Where, L = loading rate (mg COD/L· d), Q = hydraulic flow rate (L/d), Ci = feed concentration (mg COD/L), and V= reactor volume (L).

Loading rate can also be related to the hydraulic retention time and the feed concentration as:

$$L = \frac{Ci}{HRT}$$

There are few reports regarding the changes in COD dynamics during anaerobic treatment of oily wastewater [4]. The overall influent COD content is not changed but it is transformed into volatile fatty acids (VFA), alcohol, hydrogen, and biomass [20].

2.2.3   Temperature

The operating temperature plays a significant role in all the biological processes including anaerobic treatment process. Generally, anaerobic reactors are operated in the mesophilic temperature range i.e. 30 to 40 ^{o}C or thermophilic range i.e. 50 to 60 ^{o}C. These two regions represent the optima for growth of the methanogens. On the other hand, it is possible to grow methanogens at lower temperatures by providing longer SRTs to compensate for the lower maximum specific growth rates. Even though, it is possible to sustain anaerobic activity at temperatures approaching 10°C, temperature ranges of 20 to 25°C is the lower limit from practical perspectives [7]. Different species of bacteria are involved in the anaerobic process. So, temperature effect is important in anaerobic systems because of the interacting populations. For example, different species of bacteria respond to changes in temperature in qualitatively similar but quantitatively dissimilar ways [7]. Operating temperature affects both hydrolytic and acidogenic reactions in addition to the methanogens. The activity and growth of methanogens decline by one half for each drop below 35 ^{o}C in the mesophilic region [21]. The effect of temperature on methanogenesis is the primary concern for wastewater consisting largely of simple, readily biodegradable organic matter. When it comes to the wastewaters containing largely of complex organic compounds or particulate materials, the effects of temperature is the major concern for hydrolysis and acidogens [7].

Temperature effect can be classified under different categories; psychrophilic (0 – 20 ^{o}C), mesophilic (20 – 42 ^{o}C) and thermophilic (42 – 75 ^{o}C) which is shown in figure 2.3 [17]. Thermophilic anaerobic digestion has additional benefits compared to mesophilic digestion such as high degree of waste stabilization, greater destruction of viral and bacterial pathogens, and improved post-treatment sludge dewatering (Lo et al., 1985, cited by [22]).   However, due to the high heating energy requirement, operation in thermophilic range is not generally practical [8].
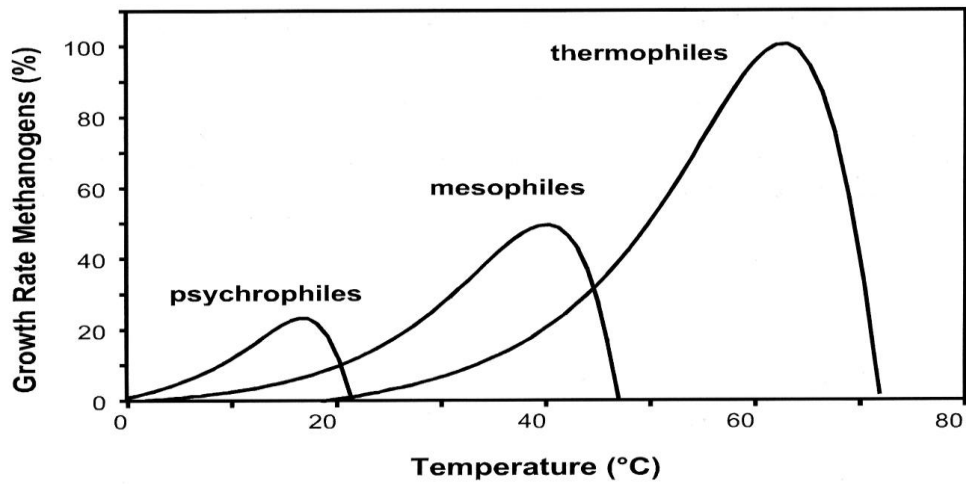
Figure 2.3: Effect of temperature on the growth rate of methanogens [17]

The effect of temperature on the growth rate can be expressed as

$$\mu_{m(20)} = \mu_{m(T)} \cdot \theta^{(T-20)}$$

Where, $\mu_{m(20)}$ = maximum specific growth rate at 20 ºC

$\mu_{m(T)}$ = maximum specific growth rate at temperature, T ºC

θ = temperature coefficient

Table 2.3: Average values of kinetic parameters for anaerobic enrichment cultures grown on various volatile fatty acids [7]

Volatile fatty Acid	35ºC		30ºC		25ºC	
	μ (day^{-1})	Ks (mg/l as COD)	μ	Ks (mg/l as COD)	μ	Ks (mg/l as COD)
Acetic	0.36	165	0.26	356	0.24	930
Propionic	0.31	60	-	-	0.38	1145
Butyric	0.38	13	-	-	-	-

2.2.4 pH

pH is the most vital process control parameter in an anaerobic treatment system. The optimal pH range for all methanogenic bacteria is between 6 and 8 but the optimum pH for the group as a whole is near 7.0 [8]. It is important to operate the process at pH conditions most favorable to the methanogens due to the fact that methanogens are affected to a greater extent than other microorganisms in the microbial community due to their lower growth rates [7, 8].

Although the effect is less significant compared to methanogens, pH will also affect the activity of the acidogenic bacteria [7]. The primary effect of pH upon the non-methanogenic population is based on the types of products formed. This affects the rate at which aceticlastic and hydrogenophilic methanogenic bacteria operate due to the changes in availability of substrates. In the single stage reactor system, production of higher molecular weight VFA, predominantly propionic and butyric acid is higher, at the expense of acetic acid due to a decrease in pH [7]. Conversely, in the acidogenic reactor of a two phase system, lower pH values do not favour the production of propionic or butyric acid over acetic acid [7]. Hydrolytic microorganisms tolerate pH deviations from neutrality the most as compared to other bacteria [7]. Sometimes, a reactor may be running in an ''inhibited steady state'', a condition where the process is stable but methane production is low due to the interaction between free ammonia, VFAs and pH ([23], Angelidaki and Ahring, 1993; cited by [22]).

There must be excess alkalinity or ability to control pH in a reactor to neutralize the effects of accumulation of excess volatile acids and carbon dioxide. The process is more stable at a constant pH. The alkalinity requirement varies with the waste, system operation, and type of process [8]. For an anaerobic process functioning within the acceptable pH range, bicarbonate buffering system is largely used to check the pH in the system [7].

2.2.5 Nutrients

Nutrients are necessary components for biomass build up in an anaerobic process like all other biochemical operations. On the other hand, nutrient requirements is less in the anaerobic processes than aerobic processes due to lower biomass yields in such processes [7]. While the nutrient requirements in table 2.4 are appropriate for anaerobic processes, only about 4 to 10 % of the COD removed is converted onto biomass, and thus the nutrient quantities required will be much lower [7]. The COD:N:P requirement ratio for a typical activated sludge process

is 100:5:1 on a mass basis [8]. The phosphorus requirements can be approximated as one-fifth of the nitrogen requirement [21]. Carbon rich industrial wastes deficient in the macronutrients, nitrogen and phosphorus require the addition of nutrients. Nickel and cobalt are particularly significant for growth of methanogens while the concentrations of micronutrients such as iron, nickel, cobalt, sulphur, and calcium may also be limiting [7].

Table 2.4: Approximate nutrient requirements [7]

Nutrient	Approximate requirement	
	g/Kg of VSS wasted	**g/Kg of TSS wasted**
Nitrogen	125	104
Phosphorus	25	21
Potassium	14	12
Calcium	14	12
Magnesium	10	8
Sulphur	8.5	7
Sodium	4.3	3.6
Chloride	4.3	3.6
Iron	2.8	2.4
Zinc	0.3	0.2
Magnesium	0.1	0.2

2.2.6   Inhibitory and toxic materials

In an anaerobic process, different groups of microorganisms are involved. The activity or inactivity of one group of bacteria affects the activity of other group. This makes anaerobic processes sensitive to inhibition by chemicals present in the wastewater or substances produced as process intermediates. Inhibitory materials are those materials which causes an adverse shift in the microbial population or inhibition to bacterial growth. A decrease of the steady-state rate of methane gas production and accumulation of organic acids can be taken as an indicator of inhibition (Kroeker et al., 1979, cited by [22]). The maximum specific growth rate of microorganisms is reduced by inhibition which results in increment in the SRT of a biochemical operation to maintain the same effluent quality prior to the inhibition.

Nevertheless, if the inhibitor concentration increases sufficiently, toxic effects can be seen. It can cause total process failure due to the death of microorganisms [7]. Literature has not made a clear distinction between inhibition and toxicity [7]. However, generally, inhibition precedes toxicity as the concentration of compound is increased. Several inorganic materials like light metal cations, ammonia, sulphide etc. can also cause an inhibitory response except the organic materials [7]. Some major inhibitors and toxic materials are discussed below.

2.2.6.1 Light metal cations

The light metal cations such as sodium, potassium, calcium and magnesium etc. must be present in an anaerobic reactor for anaerobic digestion to occur. Like other nutrients, microbial growth depends upon these cations and influence specific growth rate of microbes [7]. These cations may be present in the influent, released by the breakdown of organic matter (such as biomass), or added as pH adjustment chemicals. They exhibit a complex interaction; moderate concentrations stimulate microbial growth, excessive amount slows it, and it can cause severe inhibition or toxicity if the concentration is very high enough [7]. When two light cations are present at their moderately inhibitory concentrations, inhibition increases. This is known as a synergistic response because the combined effects of the two light metal cations exceed that of either individually. Secondly, the inhibition caused by one light metal can be amplified if the other light metal cations are present at concentrations below their stimulatory concentrations.

Table 2.5: Stimulatory and inhibitory concentrations of light metal cations [7]

Concentrations, mg/l			
Cation	**Stimulatory**	**Moderately Inhibitory**	**Strongly Inhibitory**
Sodium	100-200	3500-5500	8000
Potassium	200-400	2500-4500	12,000
Calcium	100-200	2500-4500	8000
Magnesium	75-150	1000-1500	3000

Ultimately, the presence of one light metal cation at its stimulatory concentration can reduce the inhibition of another. This phenomenon is known as antagonism, since the effect is reduced [7].

Table 2.6: Antagonistic response for light metal cations and ammonia [7]

Inhibitor	Antagonist
Na^+	K^+
K^+	$Na^+, Ca^{2+}, Mg^{2+}, NH4^+$
Ca^{2+}	Na^+, K^+
Mg^{2+}	Na^+, K^+

2.2.6.2   Ammonia

Ammonia-N is an essential nutrient which stimulates bacterial growth at low concentrations. The anaerobic degradation of wastes with high protein content releases nitrogen either in the form of ammonium ion (NH^{4+}), or dissolved free ammonia (NH_3) depending upon the pH of the system. Ammonia combines with carbon dioxide and water to form ammonium bicarbonate which acts as natural pH buffer [24]. For anaerobic processes, ammonia concentrations between 50 and 200 mg/l as N are generally within the stimulatory range [7]. However, ammonia is inhibitory at higher concentrations and toxic if the concentration is high enough. Ammonia is a weak base and dissociates in water as

$$NH_3 \quad + \quad H_2O \quad \leftrightarrow \quad NH^{4+} \quad + \quad OH^-$$
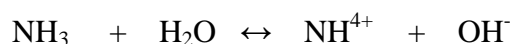
There are several mechanisms proposed for ammonia inhibition such as a change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction [25]. Free ammonia (NH_3) is the primary inhibitory species and can cause a toxic response at concentrations of about 100 mg/l as N [7]. Ammonium concentrations as high as 7000 to 9000 mg/l as N have been successfully treated without a toxic response with an acclimated culture, although concentrations as low as 1500 mg/l as N can be toxic [7]. Ammonia is present primarily as the ionized species at the pH values typically occurring in anaerobic processes as the pKa for the dissociation of ammonia is approximately 9.3. However, if the total ammonia ($NH_3 + NH_4^+$) concentration is high enough, an adequate concentration of free ammonia can be present to cause an inhibitory or toxic response [7]. Ammonia inhibition can be more severe to the methanogens among the four types of anaerobic microorganisms, affecting their growth (Kayhanian,1994, cited by [22]). There is conflicting information in the literature about the sensitivity of aceticlastic and

hydrogenotrophic methanogens [22]. Some researchers reported that aceticlastic methanogens are inhibited more than the hydrogenotrophic based on the comparison of methane production and growth rate while, some others observed that aceticlastic methanogens resisted more to high total ammonia nitrogen levels than autotrophic methanogens [22].

Ammonia inhibition can be reduced in anaerobic processes either by reducing the temperature or reducing pH or reducing the total ammonia concentration [7]. The addition of Hydrochloric acid in bioreactors can be useful to reduce pH as chloride ion has little or no impact on anaerobic biomass. It is possible to reduce the total ammonia concentration by dilution of the wastewater or solids with clean water [7].

2.2.6.3   Sulphides

Wastes may contain oxidized sulfur compounds such as sulphate, sulfite and thiosulfate. Sulphides may be generated by the degradation of sulphur-containing organic matter such as proteins and by the reduction of sulphate in an anaerobic process. Only soluble sulphides are inhibitory as only they are available to bacterial cell. If concentrations of sulphides rise over 200 mg/l, metabolic activity of methanogens is strongly inhibited leading to process failure, while concentrations up to 100 mg/l can be tolerated with little or no acclimation [7]. Sulphide inhibition can be prevented by the addition of iron which reduces the concentration of sulphide in a bioreactor.

Hydrogen sulphide is sparingly soluble in water, so it will partition between the liquid and gas phases. Hydrogen sulphide forms sulphur oxides, the corrosive gases when the product gas is combusted. Combustion products formed from sulphur oxidation are considered air pollutants. Hydrogen sulphide can also be controlled by adding iron to the bioreactor to precipitate the sulphide anion as iron sulphide [7].

Sulphate can serve as electron acceptors for sulphate reducing bacteria and produce H_2S which are toxic to methanogens. This has a number of adverse effects such as inhibition due to release of sulphide, lower methane production, reduction in the value of gas and also lesser removal of COD from the wastewater being treated [7]. The problem could be tackled by designing an anaerobic reactor receiving a wastewater containing sulphate in such a way that the methanogens outnumber the sulphate reducing bacteria. However, methods for doing this are currently not possible [7].

2.2.6.4 Heavy metals

Heavy metals have strong effects on anaerobic processes. These are required for the function and structure of enzymes in bacteria but can be inhibitory or toxic at high concentrations. However, only the soluble metal ions are inhibitory and the metal sulphides are exceedingly insoluble, giving residual heavy metal concentrations much less than the concentration in the table 2.7 [7]. If concentration of copper, nickel, zinc, cadmium, and mercury is less than 1 mg/l, then it can be inhibitory to anaerobic microorganisms [24].

Table 2.7: Soluble heavy concentrations exhibiting 50% inhibition of anaerobic digesters [7]

Cation	Concentration (mg/l)
Fe^{2+}	$1-10$
Zn^{2+}	10^{-4}
Cd^{2+}	10^{-7}
Cu^{+}	10^{-12}
Cu^{2+}	10^{-16}

The sulphide produced in an anaerobic process aids to avoid heavy metal inhibition. Sulphur can be added where inadequate sulphide is produced. For the precipitation of one mg of heavy metal, nearly 0.5 mg of sulphide is needed [7]. Table 2.7 shows that Ferrous iron is much less inhibitory than other heavy metals. So, it can be used to provide supplemental sulphide in a reactor[7]. Moreover, the sulphide precipitates formed by the more inhibitory heavy metals are more insoluble than ferrous sulphide itself. Ultimately, inhibition by soluble iron is checked as any excess iron will precipitate as iron carbonate as long as the pH is 6.4 or above [7].

2.2.6.5 Volatile Fatty Acids (VFA)

Volatile fatty acids (VFA) accumulation is the major cause of pH drops in anaerobic reactors with insufficient alkalinity. VFA concentrations above 2000 mg/l can be inhibitory to methanogens. However, it has been observed that at pH closer to neutral, neither acetic nor butyric acid at concentrations up to 10,000 mg/l were inhibitory to methanogens [7]. Propionic acid was inhibitory at a concentration of 6000 mg/l at neutral pH [7]. It implies that propionic acids are more inhibitory to methanogens than the acetic and butyric acids at neutral pH [7]. Some authors have suggested that non-ionized form of the VFAs is essentially

inhibitory and concentrations on the range of 30 to 60 mg/l have effects [7]. Volatile acids are weak acids that are largely dissociated at neutral pH. As long as the pH remains within the normal range for the growth of methanogens (6.8-7.4), inhibition caused by VFAs is not significant since high concentration of VFAs is tolerated. When pH gets lower than this range, pH exerts considerable impacts which will be compounded by any inhibition by non-ionized VFAs [7].

2.2.6.6 Other Organic Compounds

Anaerobic processes can be inhibited by different kinds of organic compounds. Like aerobic processes, these chemicals can be degraded significantly at sufficient acclimation [7]. The responses of anaerobic cultures upon initial exposure to the different concentrations of these compounds are presented in table 2.8. However, it has been found that, with acclimation, anaerobic cultures can tolerate concentrations of 20 to 50 times those values while successfully metabolizing the compounds [7]. The response of both aerobic and anaerobic processes to inhibitory organic chemicals is an area of continued research [7].

Table 2.8: Concentrations of organic compounds reported to be inhibitory to anaerobic processes [7]

Compound	Inhibitory concentration, mg/l
Formaldehyde	50-200
Chloroform	0.5
Ethyl benzene	200-1000
Ethylene dibromide	5
Kerosene	500
Linear ABS (detergent)	1% of dry solids

2.2.7   Mixing

Mixing system is a vital tool in an anaerobic process which ensures homogenous mass distribution in every point of a reactor. It provides close contact between the microorganisms and their substrates, reduces resistance to mass transfer, minimizes the buildup of inhibitory reaction intermediates, and stabilizes environmental conditions. Mixing is an important factor in pH control as well. In contrast, inefficient mixing reduces the effective volume of the bioreactor and unfavourable microenvironments can develop. Many high-rate systems such as Upflow Anaerobic Sludge Blanket (UASB) integrate mixing as a fundamental part of their design. However, loading is limited due to less effective mixing on other high-rate anaerobic processes such as Anaerobic filter (AF) [7].

2.2.8   Waste Type

Anaerobic process is significantly affected by the nature and composition of wastewater. Approximately 70% of the organic matter in municipal primary solids, measured as either COD or VS, is biodegradable in an anaerobic environment [7]. The relative amounts of soluble and particulate organic matter determine the choice of treatment systems for the particular waste. Some anaerobic processes can effectively treat wastewaters constituting primarily particulate organic matter, while others are useful to remove soluble substrates. For example, particulate matter can be effectively treated using anaerobic digesters and solids fermentation systems. In contrast, UASB and hybrid UASB/AF systems suit better for soluble wastes since they do not retain particulate organic matter.

2.3   Co-digestion

Co-digestion is an anaerobic process of digesting a mixture of two or more different types of materials simultaneously. Anaerobic digestion is now no longer a single substrate, single purpose treatment. Co-digestion has become a standard technology due to growing knowledge and understandings of the limits and possibilities of the process [26]. It is a very common practice to mix a main basic substrate (e.g. sewage sludge) with minor amounts of a single, or a variety of additional substrates for simultaneous digestion. The term co-digestion is independent to the ratio of the respective substrates used concurrently [26]. However, the performances of anaerobic treatment systems depend upon the blend ratio between the main

substrate and co-substrate. It is important to choose the best blend ratio which favours the positive interactions, avoids inhibition and optimizes methane production [27].

In a co-digestion process, it is very important to balance appropriate C/N ratio along with the right combination of other parameters like nutrients, pH/alkalinity, inhibitors/toxic compounds, biodegradable organic matter, and dry matter etc [27]. The optimum values in the anaerobic digestion ranges from 20 to 70 for the carbon-to nitrogen ratio (C/N) [27]. There is more balanced operation by co-digestion resulting in more stable process and enhancement of biogas production as it provides missing nutrients [27]. Generally, biogas production from co-digestion is higher than the sum of the biogas production from individual substrates independently [27]. Depending upon the operating conditions and the amount and characteristics of co-substrates used, methane production from co-digestion can enhance up to 200 % [27].

Co-digestion has several advantages like [26, 28]
- improved nutrient balance for an optimal digestion and a good fertilizer quality
- homogenization of different types of particulate, floating or settling wastes
- increased recovery of biogas production throughout different seasons
- higher income
- additional fertilizer
- renewable biomass production for digestion as a potential new income of agriculture
- economic benefit due to sharing of equipments
- easier handling of mixed wastes, usage of common access facilities etc

The disadvantages of the process are high slurry transport costs and the problems arising from the harmonization of different policies of the waste generators [28].

Co-digestion is mostly employed to digest the sewage sludge along with the organic fractions of municipal solid wastes, agricultural wastes etc. while co-digestion of industrial wastes accounts the least [27]. When it comes to the co-digestion of glycol wastes, it is important to consider that glycol waste primarily does not contain macronutrients and micronutrients which are indispensable during the growth of anaerobic microorganisms. So, co-digestion may be beneficial for the degradation of glycols in anaerobic conditions.

2.4   Glycols

The International union for pure and applied chemistry (IUPAC) name for Ethylene glycol (MEG) is ethane-1, 2-diol and its molecular formula is $C_2H_6O_2$. It is an organic compound which is colorless, odourless and syrupy liquid at room temperature. Its molecular mass is 62.07 g/mol and specific gravity is 1.1132. It has a boiling point of 387°F and viscosity of 1.61×10^{-2} N.s / m^2. Ethylene glycol easily partitions into the aqueous phase with a log K_{ow} of -1.36, and results in high mobility and rapid dispersion through the biosphere [29].

The IUPAC name for Di-Ethylene Glycol (DEG) is (2-hydroxyethoxy) ethan-2-ol and its molecular formula is $(HOCH_2CH_2)_2O$. It is an organic compound which is a colorless, odorless, poisonous, and hygroscopic liquid with a sweetish taste. Its density is 1.118 g/mL while molecular mass is 106.12 g/mol. It has a boiling point of 244-245°C and melting point of -10.45°C. It is highly miscible in water.

Tri-Ethylene Glycol (TEG) is a straight-chain dihydric alcohol aliphatic compound terminated on both ends by a hydroxyl group. It is a clear, practically colorless and odorless, hygroscopic liquid at room temperature and stable liquid with low viscosity. This liquid is miscible with water, and at a pressure of 100 kPa has a boiling point of 285°C and a melting point of -7°C. Its molecular formula is $HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$ and has a molecular mass of 150.20 g/mol.

Polyethylene glycol (PEG) is a synthetic water soluble polymer whose structural formula is $H(OCH_2CH_2)_nOH$. Ethylene glycols and its polymers i.e. PEGs of various molecular weights from 106 to 20,000 are widely used in the manufacturing of pharmaceuticals, lubricants, anti freeze agents hydraulic brake fluids, some stamp pad inks, ballpoint pens, solvents, paints, plastics, films, and cosmetics [30, 31]. These are also used in oil and gas industry. Ethylene glycol is used to inhibit hydrate formation in long multiphase pipelines that convey natural gas from remote gas fields to an onshore processing facility. These uses result in various amount of EG entering into the environment.

EG is not persistent in air, surface water, soil or ground water implying that it is highly biodegradable in both aerobic and anaerobic environments. Thus, nearly 100 % of EG is removed within 24 hours to 28 days of exposure in the environment [32]. EG has a half life

time of about 2 days in air as it reacts with photo-chemically produced hydroxyl radicals [32]. For fish and aquatic invertebrates, acute toxicity values (lethal concentration, LC_{50} and effective concentration, EC_{50}) are generally higher than 10,000 mg/l [32]. EG is almost non-toxic to aquatic organisms, and does not bio-accumulate in aquatic organisms [32]. Although the toxicity is low and its lethal dose (LD_{100}) is 1.4 ml kg^{-1} for humans, acute exposure can result in kidney and brain damage and poses a risk of teratogenesis [29].

2.4.1   Anaerobic degradation of Glycols

The anaerobic conversion of MEG to CH_4 is assumed to follow the pathway as shown in figure 2.4. The first step of this conversion takes place by conversion of EG into acetaldehyde which further splits into acetate (reaction 2) and ethanol (reaction 3) [33]. Furthermore, ethanol is converted into acetate and methane which is shown by the reaction 4. Although reactions 1, 2 and 3 are energetically favourable, the oxidation of ethanol to acetate, is only possible if the hydrogen concentration is very low [33]. Acetates thus formed are converted to methane by aceticlastic methanogens. Moreover, autotrophic methanogens consume H_2 and CO_2 to produce methane. It is unlikely that any other degradation pathway is followed based on the stiochiometry and sequence of the products. Other less likely route is the hydrogenation of EG into ethanol ($\Delta G° = -21$ kcal) with the hydrogen derived from acetaldehyde oxidation to acetate in the presence of noble enzymes [33].

Figure 2.4: Anaerobic degradation pathway for Ethylene glycols [33]

Stoichiometry of the reaction is:

$$4C_2H_6O_2 \longrightarrow 4CH_3CHO + 4H_2O$$

$$4CH_3CHO + 2H_2O \longrightarrow 2CH_3COOH + 2CH_3CH_2OH$$

$$2CH_3CH_2OH + 2H_2O \longrightarrow 2CH_3COOH + 4H_2$$

$$4CH_3COOH \longrightarrow 4CH_4 + 4CO_2$$

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$
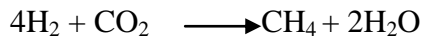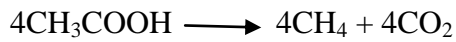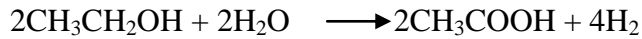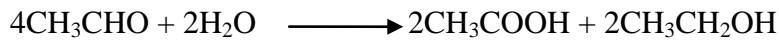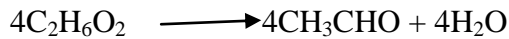
The overall equation can be written as:

$$4C_2H_6O_2 \longrightarrow 5CH_4 + 3CO_2 + 2H_2O$$

The anaerobic degradation pathway for conversion of PEGs to CH_4 follows the paths as shown in figure 2.5. In the first step, terminal hydroxyl group of PEG is shifted to the sub terminal carbon atom by the PEG degrading enzyme [10, 31, 34]. After this reaction, ether bond in PEG is broken leading to splitting off of acetaldehyde as product [34]. This reaction forms a PEG molecule which is shorter by one unit. The whole PEG chain is degraded in the same manner and the remaining residue splits off to acetaldehyde and water by the action of diol dehydratase [34].
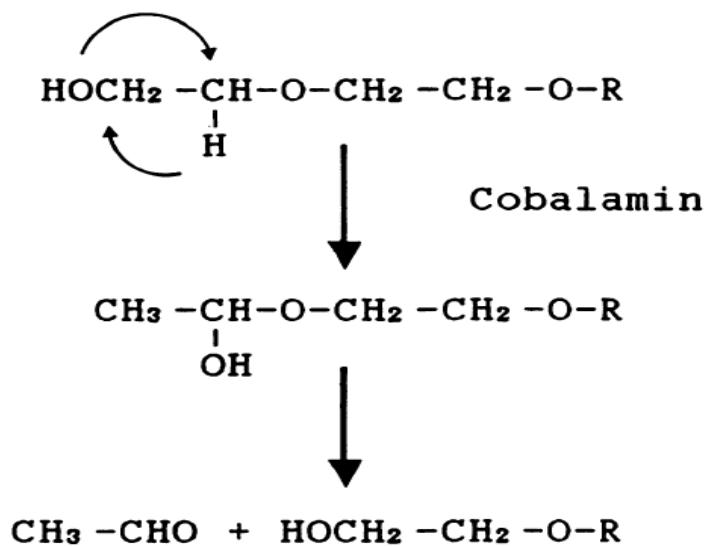

Figure 2.5: Anaerobic metabolism of polyethylene glycol (PEG) [10, 34]

2.5   Anaerobic Reactor Systems

Mouras and Cameron developed automatic scavenger and septic tank in the 19th century which are considered to be the earliest anaerobic reactors [15]. The reactors were used to reduce the amounts of solids in the sewerage systems. The first anaerobic stabilization processes occurred at a very slow rate in the tanks that were designed for intercepting the black water solids. Imhoff tank, the first anaerobic reactor was developed in 1905 by Karl Imhoff in which solids sediments are stabilized in a single tank. The actual controlled digestion of entrapped solids in a separate reactor was developed by the Ruhrverband, Essen-Relinghausen in Germany [15].

In low rate or conventional anaerobic systems, no special features were included on the design to enhance the anaerobic catabolic capacity. Anaerobic bioreactors can be treated as a continuous stirred tank reactor (CSTR) in which the SRT and HRT are alike and digester is well mixed with no liquid-solids separation. Digesters are normally provided with an SRT of 15 to 20 days, although SRTs as low as 10 days have been used successfully and longer SRTs are preferred when greater waste stabilization is essential [7]. Generally, in low rate anaerobic systems such as anaerobic ponds, a well mixed condition is not provided as mixing is provided simply by the addition of influent wastewater and by gas evolution. Consequently, suspended solids settle and accumulate in the bioreactor [15].

Most anaerobic digesters are cylindrical concrete tanks with cone-shaped bottoms and steel or concrete covers. Their diameters vary from 10 to 40 m and sidewall depths from 5 to 10 m. Digesters employ internal mechanical mixers, external mechanical mixers that re-circulate the tank contents, gas recirculation systems of various types, or pumped recirculation of the tank contents for mixing [7].

To overcome the problems encountered in low rate anaerobic digesters, high rate anaerobic wastewater treatment has been developed in which biomass retention and liquid retention are uncoupled. In an anaerobic process, the maximum permissible load is determined by the amount of viable anaerobic bacteria which are in full contact with the wastewater constituents. Bioreactors in anaerobic high rate systems are designed in such a way that reactors retain active biomass which allow the application of high COD loading rates, while maintaining long SRTs at relatively short HRTs [15]. Bioreactors employ three different mechanisms to retain biomass; (1) the formation of settleable particles that are retained by

sedimentation, (2) the use of reactor configurations that retain suspended solids, and (3) the growth of biofilms on surfaces within the bioreactor [7]. High rate anaerobic system can achieve BOD_5 removal of 80 to 90% despite being compact and small in area [15]. There are different types of high-rate anaerobic reactors ranging from suspended growth to attached growth and even hybrid ones which utilize both suspended and attached biomass. Some commonly used high rate anaerobic reactors comprise of anaerobic contact process (ACP), anaerobic filters (AF), the upflow anaerobic sludge blanket (UASB), fluidized bed (FB) and expanded granular sludge bed (EGSB), the anaerobic baffled reactors (ABR) and anaerobic membrane reactor (AMR).

The typical performance of high-rate performance processes are presented in the table 2.9.

Table 2.9: Typical high-rate anaerobic process performance [7]

Parameter	Value
BOD_5 removal %	80% - 90%
COD removal, mass	$1.5 \cdot BOD_5$
Biogas production	0.5 m^3/kg COD removed
Methane production	0.35 m^3/kg COD removed
Biomass production	0.05-0.10 g VSS/g COD removed

2.5.1 Anaerobic Contact Process (ACP)

The anaerobic contact process (ACP) is a process which utilizes external settlers and sludge return [15]. Anaerobic contact process consists of a completely mixed suspended growth bioreactor, a vacuum degassifier, and a liquid-solid separation device. Liquid-solids separation devices such as conventional clarifiers or plate settlers are used to separate the bioreactor effluents into liquid effluent and concentrated slurry of bio-solids which is recycled back to the bioreactor. Recycling of anaerobic facilitates further contact between biomass and influent waste. Mechanical mixing systems are used to obtain completely mixed conditions which are similar to those used in conventional anaerobic systems.

First generation of ACP faced major problem due to poor sludge settlement arising from gas formation by anaerobic bacteria in settling tank. Gas bubbles attach themselves to the solids

and hinder their settling and subsequent recycling to the bioreactor if the gas is not removed [11]. However, the development of knowledge and understanding of the process paved the way for newer efficient versions which use different methods for sludge separation. Newer versions employ vacuum degasification in conjunction with sedimentation, the addition of organic polymers and inorganic flocculants, centrifugation or even aeration. These days, ACP may reach organic loading rates of 10 kg COD/m^3.day [15].
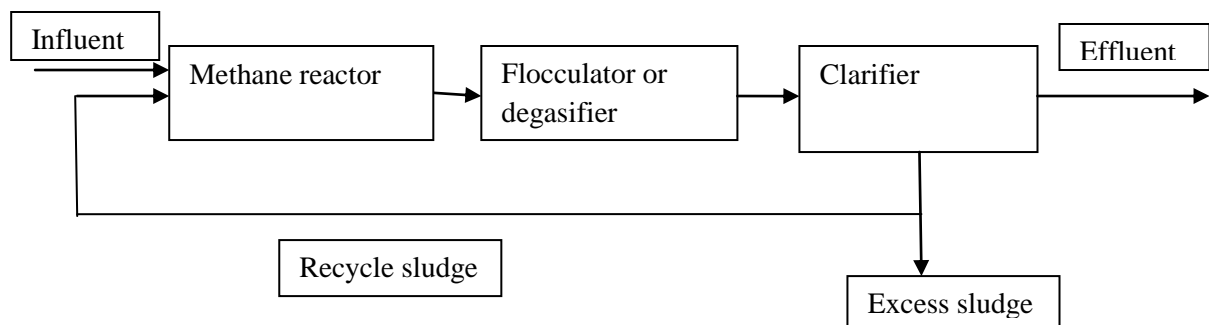

Figure 2.6: Anaerobic contact process, equipped with flocculator of a degasifier unit to enhance sludge sedimentation in the secondary clarifier [15]

2.5.2 Anaerobic Filters (AF)

In the late 60s, Young and McCarty from USA developed the modern version of upflow anaerobic filter (UAF) [15]. Anaerobic filter (AF) is a fixed-film biological wastewater treatment process in which a fixed matrix provides an attachment surface that supports the growth of anaerobic microorganisms in the form of a biofilm. Different kinds of synthetic and natural packing materials such as gravel, coke, bamboo segments are used [15]. Anaerobic filter employs both suspended and fixed biomass retained by media for treating wastewater. Influent wastewater and recirculated effluent are fed at the bottom of the reactor which gets distributed across the bioreactor cross section and flow in upward mode through the media. Effluent is discharged from the top of the media section. Likewise, gas is collected at the top of the bioreactor and is conveyed to subsequent use. Uniform loading on the bioreactor is ensured typically by recirculation of the effluent.

The performance of bioreactor is determined by SRTs but accurate measurement is not possible [7]. So, bioreactors are designed on the basis of HRTs and VOLs. Generally,

bioreactors are designed with HRT of 0.5 and 4 days, along with VOLs in the range of 5 to 15 kg COD/ (m^3.day) [7].

The major drawback of the UAF is the clogging of the bed which results in difficulty to maintain the required contact between sludge and wastewater. Other limitations are the higher costs and short-circuiting flows leading to disappointing treatment efficiencies [15].
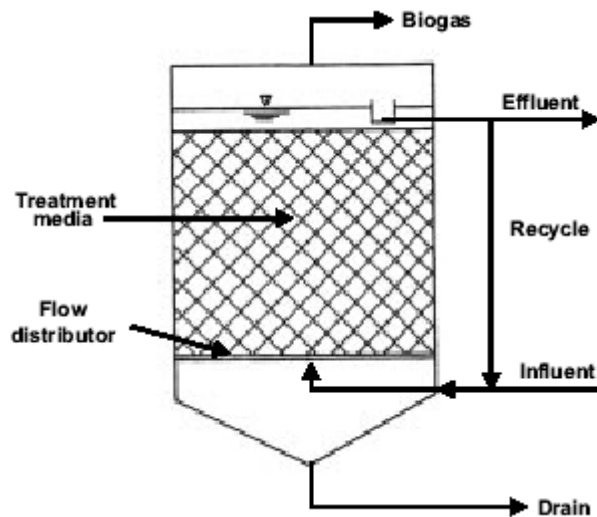

Figure 2.7: Anaerobic Filter

2.5.3   Upflow Anaerobic Sludge Blanket (UASB)

Upflow Anaerobic Sludge Blanket (UASB) was developed in Netherlands in the early 70s and is the best known example of the anaerobic sludge bed reactors (ASBR) [15]. Retention of sludge in ASBR depends upon the application of an internal gas-liquid-solids separation system (GLSS) and on the formation of readily settleable, dense sludge mass called flocs or granules [15].

UASB is the most widely used high rate anaerobic technology which is applied in the treatment of various types of wastewater [15]. The good retention of biomass in UASBs, enable them for treating wastewaters with relatively low substrate concentrations. Unlike UAF, UASB does not require any packing material in the reactor vessel. In UASB, the wastewater moves in an upward direction at a velocity that matches the settling velocity of the biomass through the reactor. In the lower portion of the bioreactor, a dense slurry of granules is formed which gets mixed with the influent wastewater by the joint effects of the influent wastewater distribution and gas production. Wastewater is treated as it moves within the

dense blanket of granules. The biomass which is in the form of compact granules comprises of mixed cultures of methanogenic and acidogenic bacteria [7]. This type of granular sludge allows high concentrations of suspended solids on the order of 20 g/L mixed liquor suspended solids (MLSS) to 30 g/l MLSS [7, 35]. A special zone is provided in the reactors that allow the gas formed to escape without attaching with sludge particles. Usually, depending upon the wastewater characteristics and whether granular or flocculent solids develops, bioreactors are designed with the HRTs of 0.2 to 2 days along with volumetric loading rates of 2 to 25 kg COD/(m^3·day) [7].
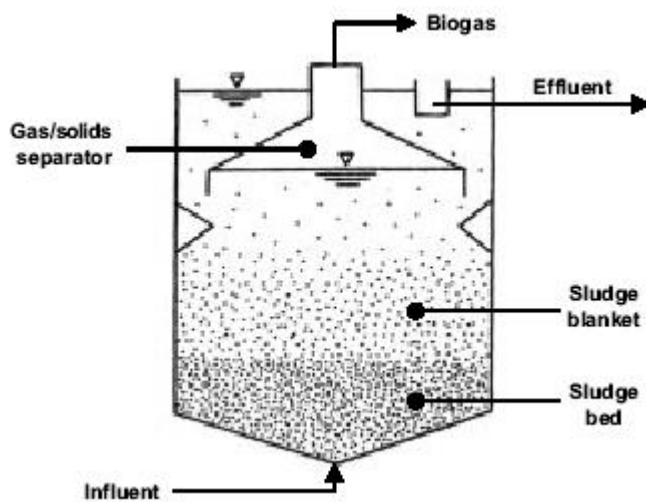

Figure 2.8: Upflow Anaerobic Sludge Blanket (UASB)

2.5.4   Hybrid Upflow Anaerobic Sludge Blanket/Anaerobic Filter

Hybrid upflow anaerobic sludge blanket is a merger between UASB and UAF reactors [7]. The hybrid UASB/AF process principally uses suspended biomass for the anaerobic treatment of wastewater. The solid removal systems and process loadings are similar to those applied with the UASB process [7].

Influent wastewater and recirculated effluent are introduced at the bottom of the reactor which is distributed uniformly across the bioreactor cross section. Wastewater is treated as it flows upward through granular and flocculent sludge. Like AF systems, there is a section of media where gas-liquid-solids separation occurs when effluent exits from sludge blanket. After the

separation, treated effluent is collected for discharge from the bioreactor while collected gas is conveyed to storage.

The comparison of Hybrid systems with UASB reveals that it possess better treatment efficiencies for some specific chemical wastewaters such as the treatment of purified therephthalic acid (PTA) wastewater [15]. After prolonged use, filter section of the reactor starts to deteriorate which is the major drawback of the hybrid reactors [7].
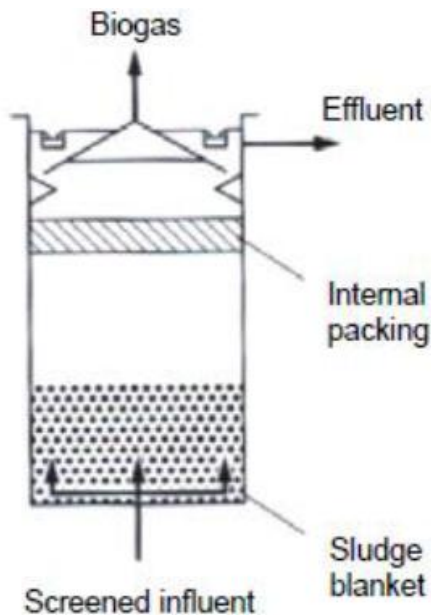

Figure 2.9: Hybrid reactor: UASB with AF process

2.5.5   Anaerobic Expanded Granular Sludge Bed (EGSB) and Fluidized Bed (FB)

Expanded granular sludge bed (EGSB) bed systems are an extension of the UASB process and are considered as the second generation of sludge bed reactors [7, 15]. EGSB and FB systems are similar to UASB, AF, and hybrid UASB/AF processes when it comes to the use of upflow bioreactors. However, in EGSB and FB systems, the granular sludge bed is expanded by the use of a deeper and narrower bioreactor for achieving high upflow velocities resulting in minimal retention of suspended biomass [7, 15]. The net result is increased mass transfer which allows extreme organic loading rates exceeding 30 to 40 kg COD/m^3·d [15]. These systems use specialized solids and gas separators to accommodate the higher hydraulic and organic loading rates.

Furthermore, the fluidized bed (FB) is regarded as an advanced anaerobic technology where loading rates may reach up to 50-60 kg $COD/m^3 \cdot d$ [15]. FB process depends upon the development of uniform biofilm attached to mobile carriers such as basalt, pumice, fine sand or plastic. The techniques used for maintaining a stable biofilm development include avoiding dispersed matter in the feed and a high degree of pre-acidification is also essential [15]. The major disadvantage is that in many situations, a segregation of different types of biofilms over the height of the reactor occurs due to difficulty in maintaining an even biofilm thickness and long term stable operations are problematic [15].
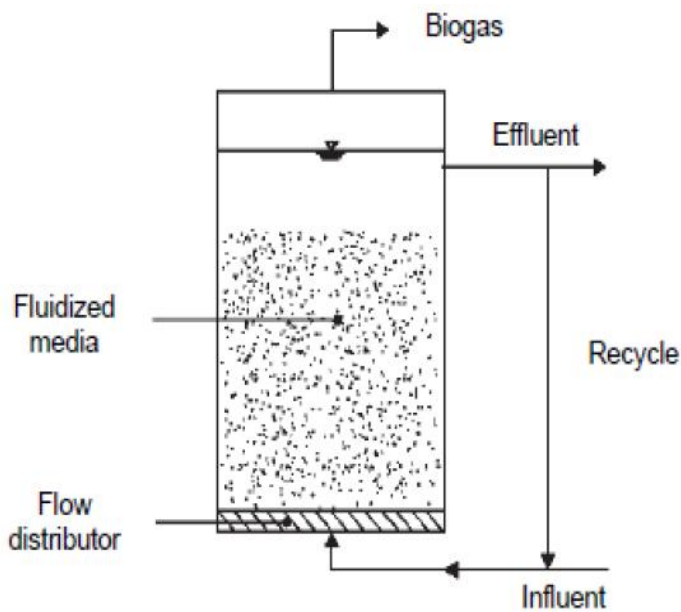

Figure 2.10: Fluidized Bed reactor

2.5.6   Anaerobic Baffled Reactor (ABR)

Anaerobic baffled reactor (ABR) employs staging of the various phases of anaerobic treatment and can be characterized as a series of UASB reactors connected in series [15]. ABR is simple in design and does not require additional sludge or gas separation equipments. There are various compartments within a reactor separated by baffles. Due to such configuration, ABR functions as two stage process where the acidification step and methanogenic step are completely isolated from each other [15]. ABR retains high biomass content and can recover quickly from high hydraulic and organic shock loads [11]. Wastewater is treated as it moves over and under the vertical baffles from inlet to outlet. The

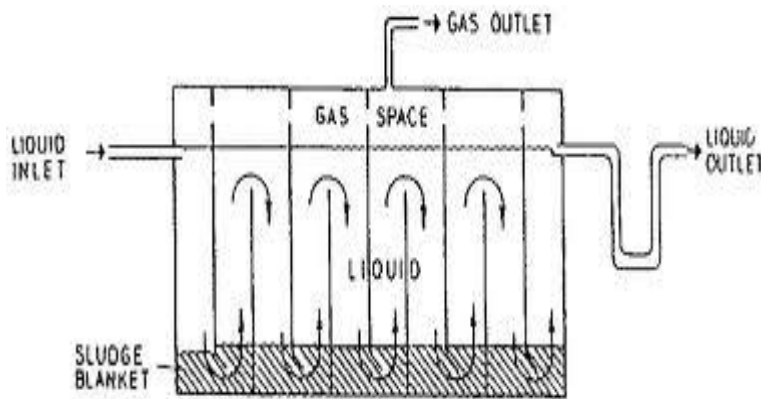major drawback is the hydrodynamic limitation giving constraints to the achievable SRT in the system [15].

Figure 2.11: Anaerobic Baffled Reactor (ABR)

2.5.7   Anaerobic Membrane Bioreactors (AMBR)

Anaerobic membrane bioreactor (AMBR) is a recent technology which may be possible to apply where other technologies fail. AMBRs are ideal options to treat wastewaters with refractory and/or toxic compounds and when extreme conditions prevail, such as high temperatures and high salinity [15]. MBR systems retain high biomass concentration which makes it possible to downsize the reactors. Moreover, long SRT coupled with high biomass concentration enable them for increased organic loadings and removing refractory or organic compounds with higher effluent quality [11, 15]. Membrane fouling caused by accumulation of colloidal particles and bacteria on the surface of membrane surface are the major problems in MBR systems. The solution for membrane fouling could be high liquid velocities across the membranes and gas agitation systems.

3 MATERIALS AND METHODS

In the present study, three different laboratory scale experiments were conducted in order to achieve the objectives of this research. The method used was co-digestion of glycol along with other substrates in anaerobic conditions. The first experiment was carried out from 19th of January to 27th of February feeding yeast extract solution as substrate solution in a single reactor. The second experimental setup was modified somewhat and was run from 2nd of March to 12th of April with two different reactors operating simultaneously in same environmental conditions. One reactor performed as a control reactor (reactor A) with substrate solution other than glycol while the other reactor acted as a test reactor (reactor B) where glycol was introduced and co-digested along with other substrate solution after reaching nearly stable gas production. It was assumed that the comparison of gas production and other parameters between two reactors would reveal the actual effect of MEG on the anaerobic process. Ultimately, third experiment was operated from 20th of April to 23rd of May which was identical to experiment 2. However, different substrate was used.

3.1 Experimental Setup and Procedures

A semi-continuous flow stirred tank reactor was operated at a HRT of 10 days by fill and draw procedures in order to establish an anaerobic consortia adapted to yeast solution. The reactors were operated in mesophilic conditions. Glass flask having a sludge volume of one liter was used as a reactor throughout the experiments.

The first experimental setup commenced by sparging a clean and empty flask with nitrogen gas carefully. It was then filled up with 1025 gm (approx. 1 liter) sludge from anaerobic digester from IVAR wastewater treatment plant as inoculums as well as initial substrate. The reactor was closed with a rubber stopper containing three different holes to facilitate the reactor for feeding substrate, withdrawal of digested sludge, and collection of the gas. The reactor was placed on a hot plate magnetic stirrer with heat and speed control. Glass tubes were already fitted in the holes and were connected with additional silicon tubes for facilitating its closure and opening at the tips/ends or wherever required. One of the tubes was connected to an inverted volumetric cylinder filled with 0.1 M $Ca(OH)_2$ solution and dipped in a container for gas collection. Moreover, the cylinder was supported by an iron stand. The container contained 0.1 M $Ca(OH)_2$ solution for CO_2 absorption which acted as a barrier solution. Furthermore, phenolphthalein solution was added in the barrier solution.

Phenolphthalein imparts pink colour in alkaline solutions and change in colour indicates whether $Ca(OH)_2$ is available to absorb CO_2 or not. The system was ensured to be air tight before starting the experiment so that there was no gas leakage or air introduction into the reactor. The reactor was operated on mesophilic conditions i.e. average temperature of 34.7 °C was maintained throughout the experiment. Syringes were used for feeding and withdrawal purposes.

Figure 3.1: Experimental setup in experiment 1

Experiment 2 was identical to experiment 1 with some modifications. This time two reactors were operated simultaneously placing inside a water bath for better temperature control. Temperature of the water bath was maintained to be constant at about 38 °C. The water bath was itself placed above two hot plate magnetic stirrers directly and positioned in such a way that each reactor in the water bath reclined just above the plates for stirring purposes. Experimental setup was similar to experiment 1 but at this time, both reactors were filled with 900 ml sludge from anaerobic digester from IVAR wastewater treatment plant and 100 ml yeast extract solution (total volume = 1 liter) from the very initial day. After then, both reactors were loaded with yeast extract solution for almost 13 days to obtain a stable gas production. However, from 14th day of operation i.e. from 16th of March, one of the reactors (control reactor A) was fed with yeast extract solution only while other (test reactor B) was

fed with equal volumes (50 ml each) of solution containing 50% COD load from yeast extract solution while 50% COD load as MEG.
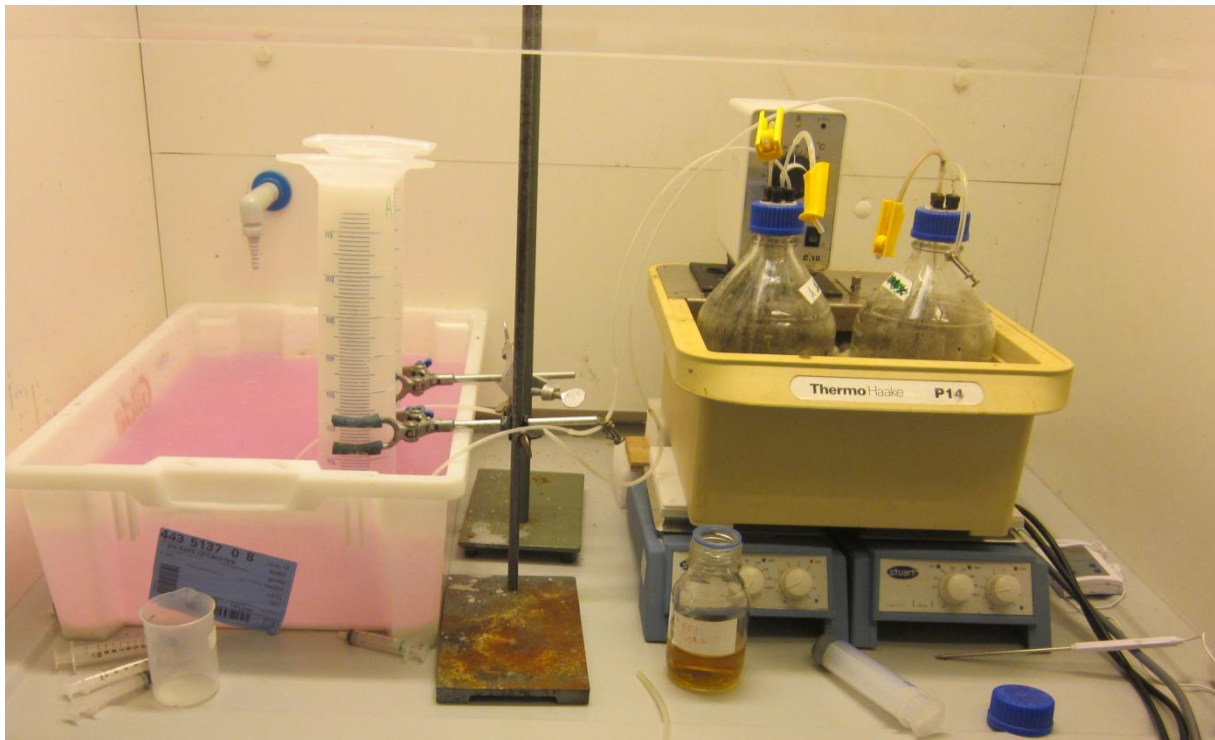

Figure 3.2: Experimental setup in experiments 2 and 3

Experiment 3 was identical to experiment 2. It was different in a sense that different substrate was used for microbes. The substrate was composed of nutrient broth, starch and yeast extract solutions. Experimental setup procedure was exactly a replica of 2nd experiment. From 2nd of May, one of the reactors (control reactor A) was fed with the substrate solution only while other (test reactor B) was fed with equal volumes (50 ml each) of solution containing 50% COD load from substrate solution and remaining 50% COD load as MEG.

3.2   Substrate Solutions

The granulated yeast extract solution used for experiments 1 and 2 was prepared by using 6.66 grams of granulated yeast extract in 200 ml solution of tap water which is equivalent to 33.3g/l. Freshly prepared solutions were used in experiment 2 while the solution lasted for 2 days in the experiment 1. The yeast extract was manufactured by Merck, Germany. The pH of the solution was found to be neutral along with COD of 30.67 g COD/L, TS of 0.94 g TS/g yeast and TVS of 0.795 g TVS/g yeast.

In experiment 3, different substrate was used than that used in previous experiments. The solution constituted of 18 g of nutrient broth, 17 g of starch and 5 g of granulated yeast extract solution in one liter of tap water. The solution was stored at 4 °C in darkness. The COD, TS and TVS of the solution were determined to be 34.01 g COD/L, 34.94 g/L and 32.12 g/l respectively.

Monoethylene glycol solution manufactured by Laboglass, Norway was used to feed the digester. The stock solution was prepared dissolving 24.2 gm glycol solution in one liter of distilled water for the experiment 2 while it was prepared dissolving 26.4 gm in a liter of distilled water for the experiment 3. In experiment 3, glycol solution containing 3.8 gm/L of Na_2CO_3 was loaded as glycol solution when pH dropped below 6. COD of the glycol solution was determined to be 29.4 g COD/L and 33.9 g COD/L in experiments 2 and 3 respectively.

3.3 Operation

The reactors initially comprised of 90% - 100% sewage sludge collected from digester of IVAR wastewater treatment plant as substrate. As the experiment proceeded, the sludge was gradually replaced by other substrate solutions day by day. Glycol solutions were introduced for co-digestion with other substrate solutions after reaching nearly stable gas productions. 100 ml sludge was withdrawn from the reactor using a syringe on daily basis while equal volume of substrate solution was fed directly to the reactor using next tube. Withdrawal process always preceded the loading process as analysis of digested sludge reveals changes in a day.

Temperature and pH of the sludge were monitored immediately after withdrawal. Volume of the generated gas was also recorded from the graduated cylinder daily. The sludge that was withdrawn from the reactor was used to analyze different chemical parameters on the reactor. On every second day, Total Solids (TS), Total Volatile Solids (TVS) and COD were measured in experiments 1 and 2. Moreover, some samples were diluted, filtered and stored by freezing those in freezers for VFA determination using Ion Chromatography. On the experiment 3, COD and VFA measurements were discontinued. However, some samples were evaluated at the end of the experiment. However, alkalinity and short chain fatty acids (SCFA) were determined using TITRA 5 software on every alternative day.

Temperature was maintained more or less constant throughout the experiment. The temperature was monitored daily so that it could be adjusted if necessary. In experiments one and two, pH was monitored daily but no efforts were made to adjust it. However, Na_2CO_3 was added to replenish alkalinity in experiment 3 (see details for dosage in appendix A7)

3.4 Analytical Procedures

The mixed liquor which was withdrawn from the reactors was sampled for analytical purposes.

3.4.1 Temperature and pH

Hand held digital thermometer was used for measuring temperature. The temperature was measured immediately after withdrawing the sample from the reactor.

pH was measured using a Metrohm 744 pH meter. Measurement was done as soon as the sludge was withdrawn from the bioreactor. Electrode of the pH meter was standardized using standard buffer solutions of pH 4 and pH 7 before measuring the pH. Beaker containing the samples was shaken gently and pH was recorded when the stable reading was shown in the pH meter.
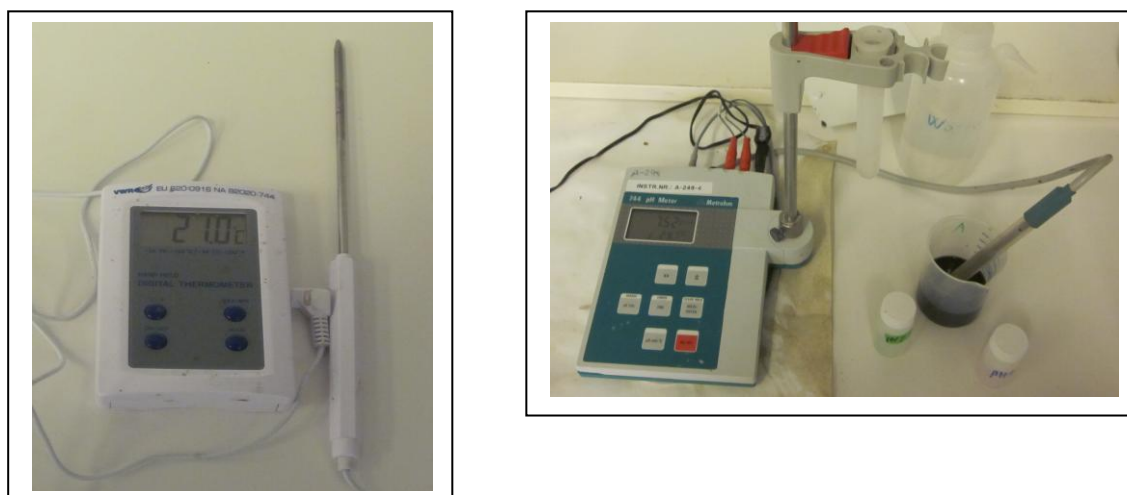

Figure 3.3: Digital thermometer (left) and pH meter (right)

3.4.2 TS and TVS

For the determination of TS, at first porcelain dish was pre-combusted and cooled in dessicator. Then tara weight of the dish was measured on an analytical balance. 10 ml of homogenized sample was transferred into the dish using a syringe. The dish was placed in an oven at 95-97°C, and left there to evaporate overnight. Next day, the dish along with residual was dried for 1 h at 103-105°C and it was cooled in a dessicator. The dish containing residual was weighed on analytical balance and the weight was noted. It was cooled for another 15 minutes in dessicator. The measurements were repeated until the dish + residual reached constant weight.

TS of sample was calculated as

$$TS \left[\frac{mg}{l}\right] = \frac{m_{dish+residual} - m_{dish}}{V_{sample}}$$

For determining TVS, the porcelain dish was placed in muffle oven and the sample was combusted for 20-30 minutes at a temperature of 550°C. The dish was cooled for short time in air before transferring it to a dessicator. The dish containing residual was weighed on analytical balance and the weight was noted. It was cooled for another 15 minutes in dessicator. The measurements were repeated until the dish + residual reached a constant weight.

TVS of the sample was calculated as

$$TVS \left[\frac{mg}{l}\right] = TS - \frac{m_{dish+ignited\,residual} - m_{dish}}{V_{sample}}$$

Finally, porcelain dish was soaked in soap water for cleaning after removing fixed solids residual.

3.4.3 COD measurement

The measurement of COD was based on the "Closed reflux, colorimetric method" described in Standard Methods [36]. At first, high range digestion solution was prepared by adding 10.216 g of $K_2Cr_2O_7$, previously dried for 2 hours at 150°C, 167 ml of concentrated H_2SO_4 and 33.3 g of $HgSO_4$ to 500 ml of distilled water. The mixture was then left to cool to room temperature before diluting to 1000 ml. Sulphuric acid reagent was prepared by adding 5.5 g

Ag_2SO_4 to one kg of H_2SO_4. It was left to stand for 1 to 2 days to dissolve forming a mixture. Potassium hydrogen phthalate, KHP ($HOOCC_6H_4COOK$) was used to prepare standard solutions. KHP was lightly crushed and then dried to constant weight at 110°C. 425 mg of KHP was dissolved in distilled water and diluted to 1000 ml. KHP has a theoretical COD of 1.176 mg COD/mg and this solution has a theoretical COD of 500 microgram O_2/ml.

Total COD and filtered CODs were determined from the effluent samples. The effluent sludge was homogenized by stirring to prepare a test solution. One ml of the homogeneous mixture was diluted by 50 times using distilled water in a volumetric flask. 2.5 ml of this diluted solution was used for determining total COD. For determining filtered COD, the diluted samples were transferred into centrifugation vials and centrifuged at 10,000 rpm for ten minutes in a centrifugal machine. The supernatant was filtered using Cronus syringe filter retaining 0.45 μm particle size. Then, 2.5 ml samples were placed in COD vial tubes and 1.5 ml of digestion solution was added using hand held pipette. Then 3.5 ml of Sulphuric acid reagent was carefully run down inside the tube so that an acid layer was formed under the sample/digestion solution layer. The vial tubes were tightly sealed and inverted several times to mix the contents properly. A reagent blank was prepared by repeating above stated procedure, substituting 2.5 ml of distilled water instead of the sample. The mixtures were then digested in a thermo reactor (Merck Spectroquant Thermoreactor TR 620) at 150°C for 2 hours. The vials were removed from the thermo reactor and allowed to cool in a metal test tube rack and were vented to relieve any pressure generated during digestion. The vials were swirled couple of times during cooling. After cooling, the samples were analyzed on a pre-calibrated and programmed Spectrophotometer (Spectroquant Pharo 300 by Merck company). The spectrophotometer was calibrated beforehand using standard KHP solution as sample at a wavelength of 600 nm and using the same procedure described above.

Figure 3.4: Spectroquant Pharo 300 - (left) and Merck Spectroquant thermoreactor (Model TR620) - (right)

3.4.4 Volatile Fatty Acid and Alkalinity

For VFA determination, effluent sludge was diluted by 50 times using distilled water. The diluted samples were filtered using Cronus syringe filter retaining 0.2 μm particle size and the filtered samples were transferred to 1.5 ml glass vials. Those samples were stored by freezing those in freezers for VFA determination. Ultimately, VFAs were determined using Ion Chromatography.

TITRA 5, a 5-point titration was used for determining short chain fatty acids and alkalinity in experiment 3. The sample was titrated from its initial pH to further four pH points [37]. The first step in the procedure was filtration of approximate volume of the sample. Centrifugation was carried out before the filtration. 5 ml of the filtered sample was diluted to 50 ml on a volumetric flask and put on a magnetic stirrer at low rotation to avoid or at least minimize CO_2 input or loss [38]. Normally, the second step involves measurement of conductivity. However, conductivity was not measured and a constant conductivity value of 488 mS/m was used while finding the SCFA and alkalinity. The third step was the measurement of temperature. After then, initial pH of the sample was measured. Then each sample was consecutively titrated to pH 6.7, 5.9, 5.2 and 4.3 (+/-0.1) with 0.1008 M HCl. The volume of the acid consumed for reaching each pH was recorded. These values were entered into a

computer program TITRA 5 for determining SCFA as mg/l of Acetic acid (HAc) and alkalinity as mg/l CaCO$_3$.

4. RESULTS AND DISCUSSIONS

This chapter presents the results obtained from Experiment 1, Experiment 2 and Experiment 3 along with discussions.

4.1 Experiment One (19th of Jan – 27th of Feb 2012)

This experiment was conducted for about 39 days with yeast extract solution as substrate in a single reactor. The organic loading was 3.07 g COD/L·d.

Temperature and pH

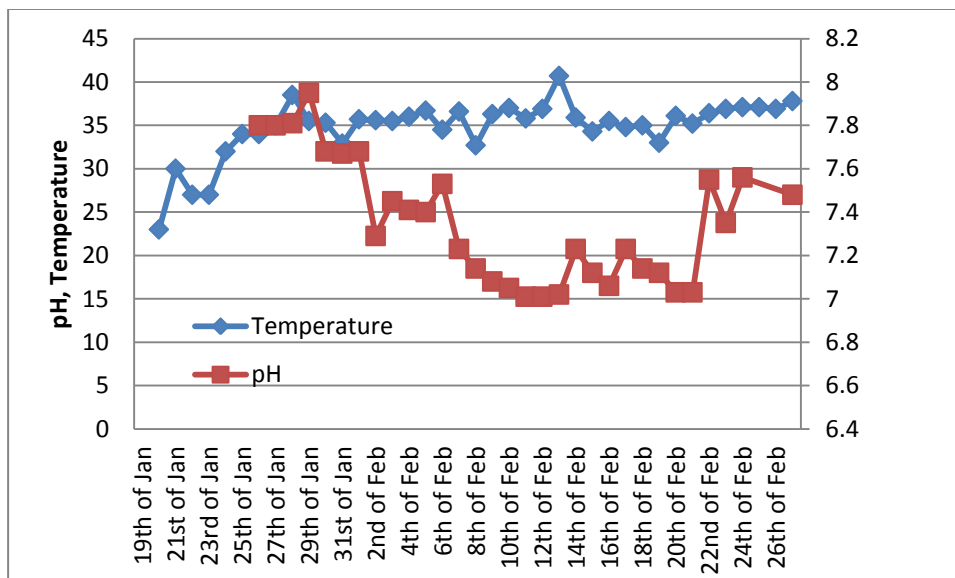Temperature and pH profiles during the experiment 1 are illustrated in figure 4.1.

Figure 4.1: pH and temperature profiles in experiment 1

Reactor was operated at mesophilic conditions. When the experiment was setup, initial temperature of the reactor was 23°C which was later increased to maintain about 35°C, the sludge temperature. It was assumed that the reactor was operating approx. at 37°C assuming some reductions in temperature during measurements. The observed data showed that the temperature range was fluctuated from 23°C to 40°C with an average temperature of 34.8°C.

pH varied from lowest value of 7.01 to highest value of 7.95 with an average value of 7.35. There was no requirement to adjust pH as it was almost stable near neutral. As pH of 6.5 to

7.5 is considered as the most favourable pH range in an anaerobic process [35], it can be said that the reactor was operating at optimum pH conditions.

Gas Production

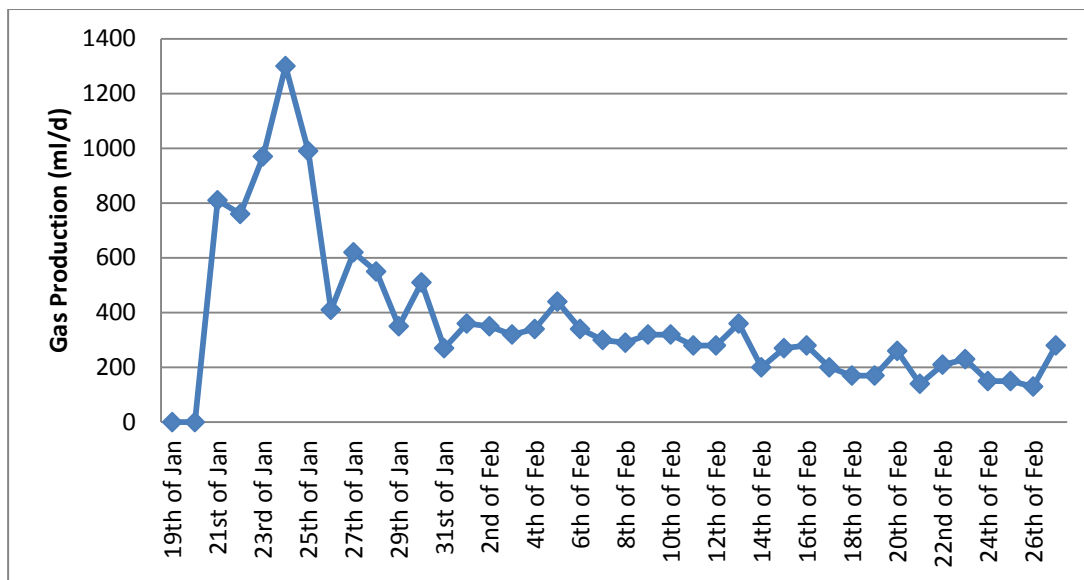Daily gas production during the experiment is exhibited in figure 4.2.

Figure 4.2: Methane production variation along time during the experiment 1

Qualitative and quantitative gas analyses were not done for determining the fractions and compositions of the gas collected in all three experiments. It was assumed that the measured gas primarily consisted of methane since $Ca(OH)_2$ solution was used as a barrier solution for absorbing CO_2.

Figure 4.2 shows that there was a short lag phase at the beginning of the experiment. Gas production increased sharply from 2nd day and continued with slight fluctuations until it reached a peak value of 1300 mL/d on 5th day. The gas production decreased drastically after that and reached 410 mL/d on the 7th day of operation. After that, there were few fluctuations on gas production until it reached approximately stable state producing 200 mL/d.

The CH_4 equivalent of COD converted under anaerobic conditions at standard conditions (0°C and 1 atm) is 0.35 L CH_4/g COD [16] which is equivalent to 0.38 L CH_4/g COD at 20°C. The organic loading of 3.07 g COD/L·d solution had to produce about 1167 mL/d CH_4 theoretically. So, 200 ml/day gas production shows that there was an inhibition. Ion

chromatography analysis revealed high accumulation of VFA in the reactor suggesting inhibition to methanogens which caused lower gas production. When sludge was withdrawn from the reactor, there was foul smell suggesting production of toxic gases like H_2S, ammonia etc. So, it can be said that the reactor was running in "inhibited steady state" as discussed in the section 2.2.4 where the process is stable but gas production is low due to interaction between ammonia, VFAs and pH.
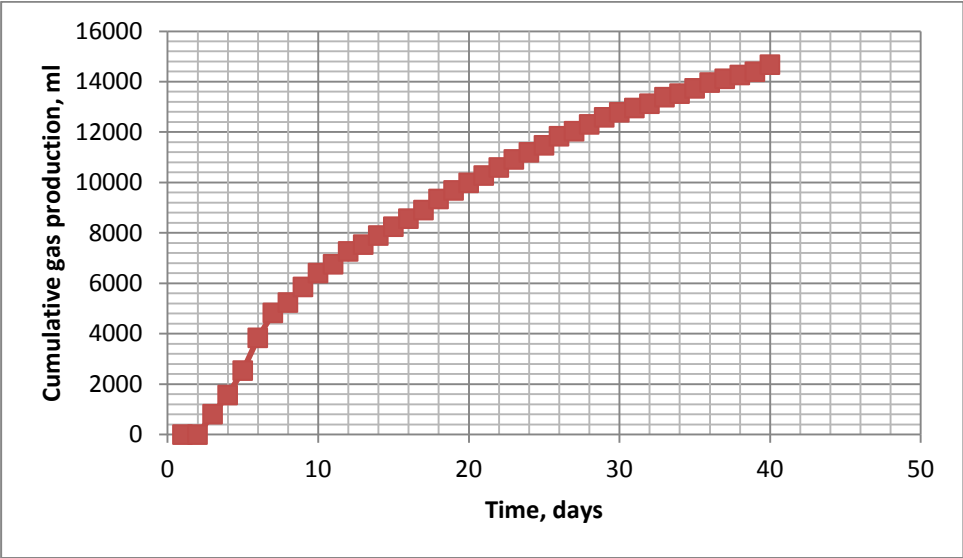

Figure 4.3: Cumulative gas production during the experiment 1

Figure 4.3 shows the cumulative gas production which is simply the sum of daily gas productions was 14680 mL ≈ 14.7 L.

TS and TVS

TS and TVS profiles during experiment 1 are depicted in figure 4.4.
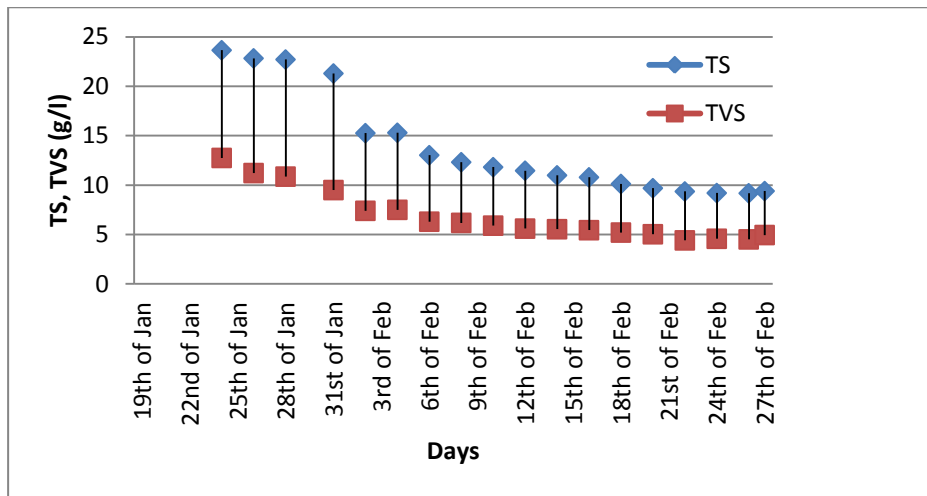

Figure 4.4: TS and TVS profiles in experiment 1

Figure 4.4 shows that there was a continual decrement in TS and TVS in the reactor during the experiment. The experiment began with the sludge from IVAR digester, Stavanger which had relatively high concentrations of TS and TVS. However, the sludge was gradually replaced with yeast solution having TS of 0.94 g/g yeast and TVS of 0.795 g/g yeast which caused the reduction of TS and TVS in the reactor. There was also biomass loss during sludge withdrawal process. Generally, biomass can be retained using membrane or recycled back with other means.

COD

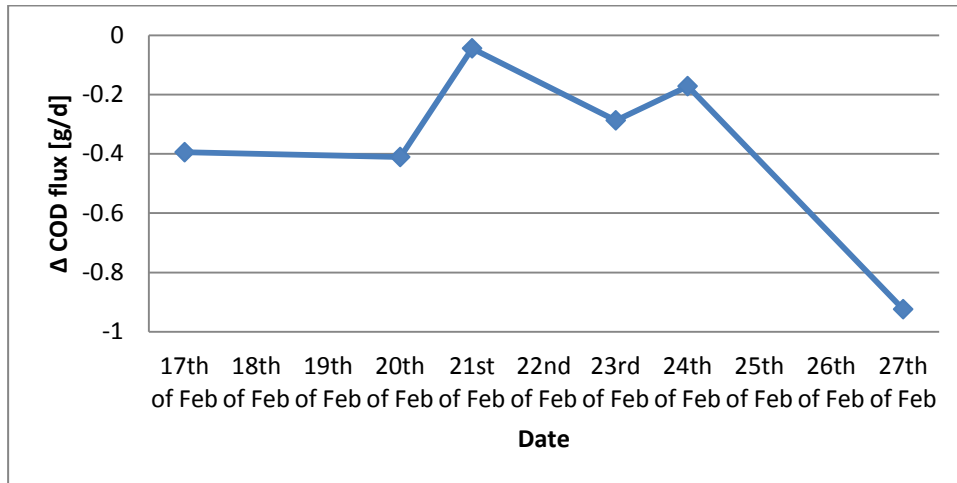COD mass balance deviation during the last session of the experiment is presented in figure 4.5.

Figure 4.5: COD mass balance deviation along time

(N.B: COD of CH_4 was determined by dividing measured CH_4 volume by a factor of 376.86 to adjust the measurements in room conditions (20°C and 1 atm) using theoretical value of $4gCOD/gCH_4$ along with ideal gas equation; PV= nRT i.e. to say that $1gCOD \approx 376.86$ ml CH_4)

ΔCOD is the difference between inlet and outlet COD concentrations per day.

Mathematically, ΔCOD in a day = COD of influent in that day – (COD of solution withdrawn in that day + COD of CH_4 in that particular day)

Figure 4.5 shows that total outgoing COD in the form of CH_4 and solution withdrawn was slightly higher than the COD input in the particular day. This COD mass balance deviation is most likely due to the fact that COD mass balance is valid only for steady states. For assuming steady state it would require much longer time (at least 5 SRTs, or 50 days) [39]. Moreover, there could be some unaccounted COD already present in the reactor during setup and some errors as well during COD measurements.
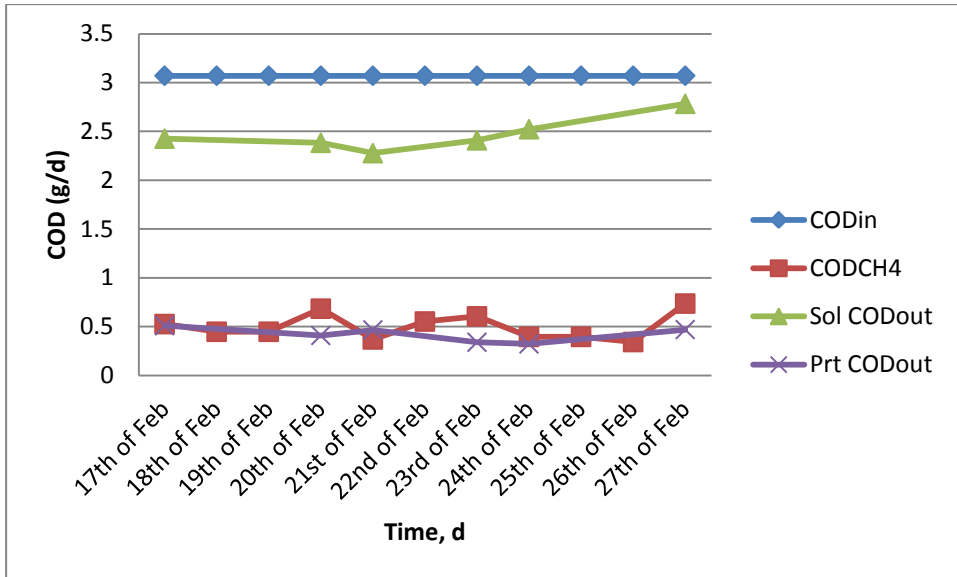
Figure 4.6: COD dynamics at the last session during the experiment 1 where, COD_{in} = influent COD per day, COD_{CH4} = COD of methane per day, Sol COD_{out} = effluent soluble/filtered COD per day and Prt COD_{out} = effluent particulate COD per day

Figure 4.6 exhibits COD dynamics during the last days of the experiment. COD_{in} was constant due to fact that the reactor was loaded with uniform amount of solution containing fixed concentration of substrate. COD_{CH4} fluctuated slightly due to fluctuations in gas production. Prt COD_{out} diminished slowly while, Sol COD_{out} rose during the experiment since influent solution contained no particulates and no means were applied to retain or recycle biomass on the reactor. Slight rise in at the ending day Prt COD_{out} matches with the increment in TS and TVS.

VFAs

Table 4.1: VFA concentrations in experiment 1

Date	Lactic acid	Propionic acid	Formic acid	Acetic acid	Butyric acid	Valeric acid	Total VFA
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
21- Feb	0	1783.53	243.53	5669.92	1894.13	1288.79	10879.9
23- Feb	0	1936.01	266.04	5956.70	1949.96	1368.48	11477.2
24- Feb	0	2121.95	292.87	6222.84	2111.95	1488.17	12237.8
27- Feb	0	2421.63	312.41	6395.61	2737.26	1678.87	13545.8

Table 4.1 demonstrates VFA concentrations during the last days of the experiment. The analysis shows that all the acids were increasing slowly indicating that more and more VFA was being accumulated day after days. Rise in the total VFA concentration shows that methanogens were affected more than the acidogens causing imbalance in the system. pH was close to neutral suggesting that there was sufficient alkalinity in the system although it was not determined. So, it is most likely that there were higher organic loadings coupled with short SRT which caused VFA accumulation. In these conditions, acidogens complete their task, but due to lower growth kinetics, methanogens are not able to convert acetates or hydrogen into methane resulting VFA accumulation and lower gas production.

4.2 Experiment Two (2nd of March – 12th of April)

Two identical reactors (reactor A and reactor B) were operated in parallel during the experiment. Initially, both reactors were loaded with yeast extract solution for almost 13 days to obtain a stable gas production. However, from 14th day of operation i.e. from 16th of March, one of the reactors (control reactor A) was fed with yeast extract solution only while the other was fed with equal volumes (50 ml each) of solution containing 50% COD load from yeast extract solution while 50% COD load as MEG. Hence, glycol was co-digested along with yeast extract solution in reactor B from 16th of March. The organic loading was approx. 3.07 g COD/L·d in both reactors. The results observed from the experiments are presented with comparisons and discussions.

4.2.1 Control Reactor A (Yeast extract solution)

Temperature and pH

Temperature and pH profiles in reactor A during the experiment 2 are shown in figure 4.7
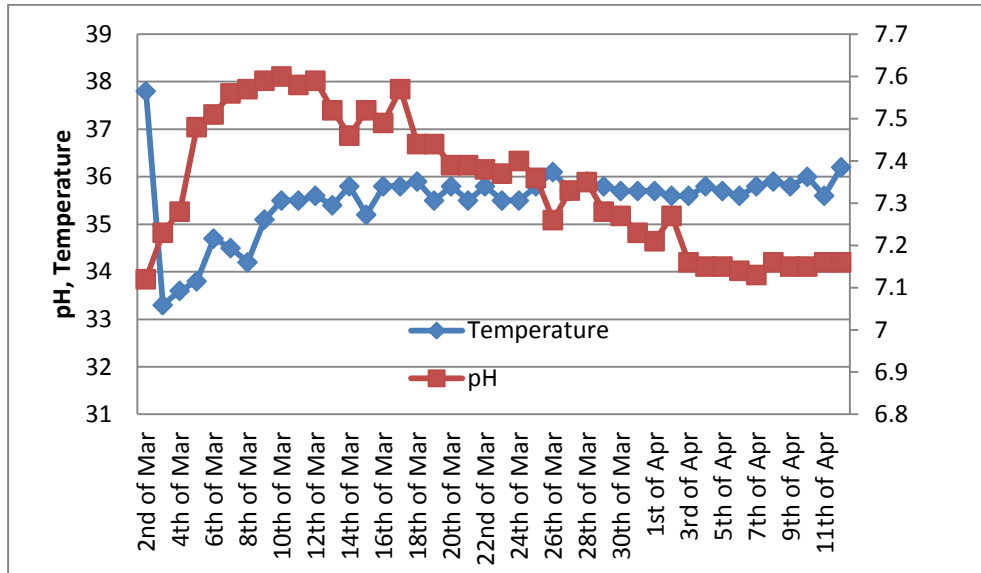

Figure 4.7: Temperature and pH profiles in reactor A during experiment 2

Figure 4.7 shows that there were slight variations in temperature of the reactor ranging from 33.3°C to 37.8°C with an average value of 35.5°C. It is to be noted that the constant temperature of about 38.3°C was maintained in water bath during the experiment. Although the temperatures of the samples were measured instantaneously, reduction of the temperature can be attributed to the heat absorption by syringe, beaker etc. and also heat loss due to contact with air.

pH of the reactor varied from 7.12 to 7.59 with an average of 7.4. There were very nominal changes in pH during the operation which is normal. The reactor was operating in optimum pH conditions.

Gas Production

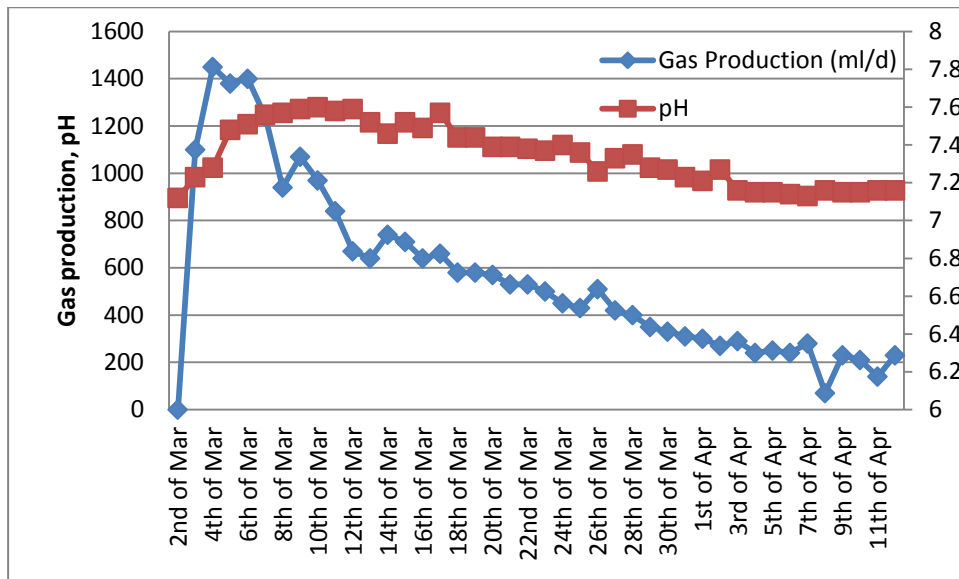Gas production fluctuation is exhibited in figures 4.8 and 4.9.

Figure 4.8: Gas production variation and pH changes during the experiment 2

Unlike, the experiment 1, no lag phase was observed in this experiment. This might have happened because relatively large amount of sludge was brought from IVAR at this time and microbes used in the reactor were probably undisturbed. Moreover, the experiment was setup with 100 ml yeast extract and 900 ml IVAR sludge from the very initial day. The feed composition might have supplied substrates and nutrients for microbes in no time. The gas production surged right away after setting up the experiment, reached a peak value of 1450 ml on 2nd day of operation and achieved almost stationary phase for some days. The production declined gradually with some fluctuations and achieved stable state later. Lower gas production than the theoretical values may be due to inhibition. This reactor can also said to be running in inhibited steady state as discussed in section 2.2.4.
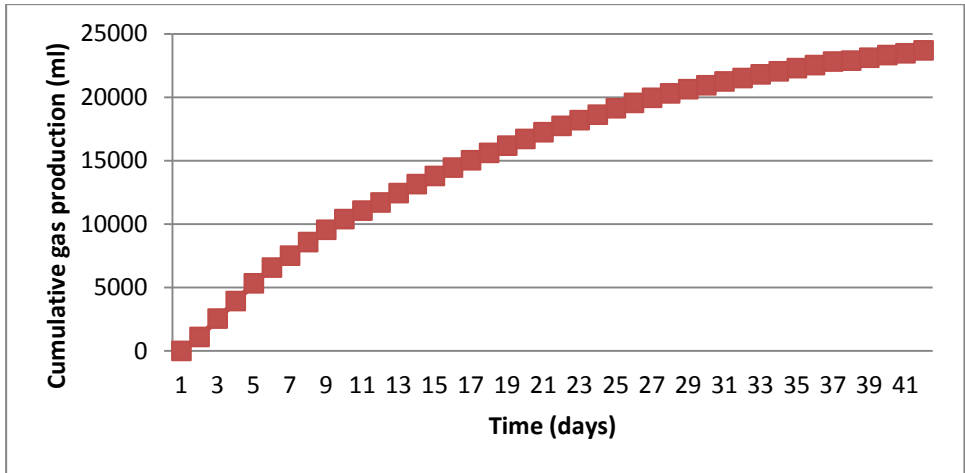
Figure 4.9: Cumulative gas production in reactor A during the experiment 2

Figure 4.9 shows the cumulative gas production in reactor A during the experiment. Total gas production was 23690 mL i.e. 23.7 liters.

TS and TVS

TS and TVS profiles in reactor A during the experiment 2 are shown in figure 4.10.
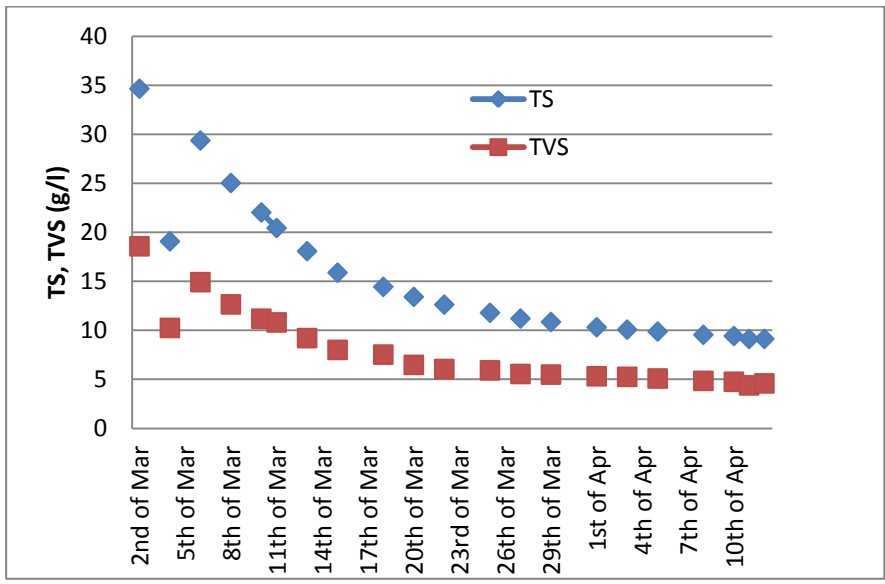

Figure 4.10: TS and TVS concentrations in reactor A during experiment 2

Figure 4.10 shows that TS and TVS continually declined from initial values of 34.64 g/L and 18.57 g/L to 9.12 g/L and 4.58 g/L respectively during 41 days of operation. The value was more or less stable after 20th of March. The value observed on 2nd day i.e. 4th of March can be

considered as an outlier compared to the overall trend of the data series. It can be attributed to the heterogeneous sample withdrawn from the reactor due to stirring problem that occurred sometimes due to the defects of magnetic plate stirrer. Decline in TS and TVS can be attributed to the gradual replacement of sludge containing high amounts of TS and TVS from IVAR with yeast solution devoid of particulates. In addition, there was loss of biomass during the sludge withdrawal process.
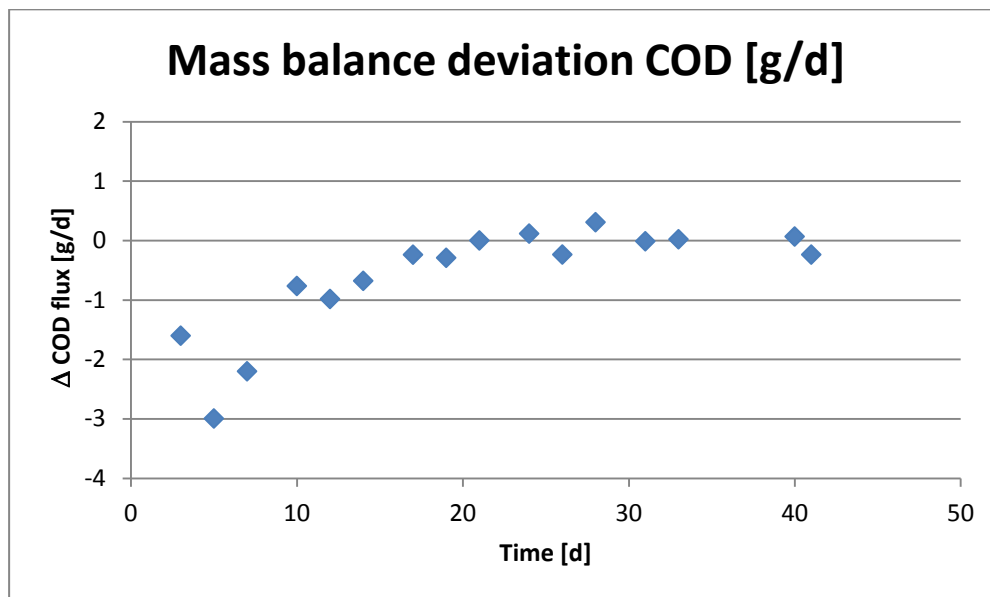
COD

Figure 4.11: COD mass balance deviation i.e. ΔCOD against time (days)

Figure 4.11 clearly indicates that initially, outgoing COD per day which is the sum of COD of effluent + COD of CH_4 per day was larger than inlet COD per day. It shows that there was already large amount of unaccounted COD present in the reactor which can be attributed to COD of sludge from IVAR. Meanwhile, as the operation continued, the deviation declined gradually indicating that the process was approaching to steady state. Ultimately, the COD deviation was approximately 0. During the last days, COD of CH_4 per day was low while COD of effluent per day was high showing that large fraction of COD was going out through sludge without being converted to methane.
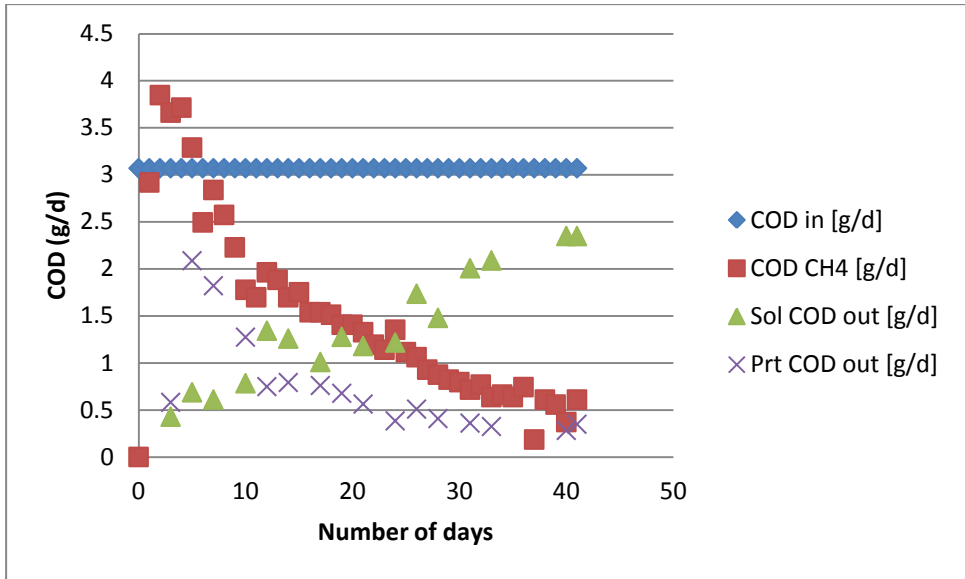
Figure 4.12: COD dynamics during the experiment 2 where, COD_{in} = influent COD per day, COD_{CH4} = COD of methane per day, Sol COD_{out} = effluent soluble/filtered COD per day and Prt COD_{out} = effluent particulate COD per day

Figure 4.12 demonstrates COD dynamics during the experiment 2. COD_{in} was constant due to fact that the reactor was loaded with uniform amount of solution containing fixed concentration of substrate. COD_{CH4} declined gradually due to decrease in gas production. Prt COD_{out} diminished slowly while Sol COD_{out} rose during the experiment since, influent solution contained no particulates and no means were applied to retain biomass in the reactor. The COD value of day 3 may be taken as an outlier resulting from error in measurements or use of heterogeneous sample due to stirring problem in reactor.

VFAs
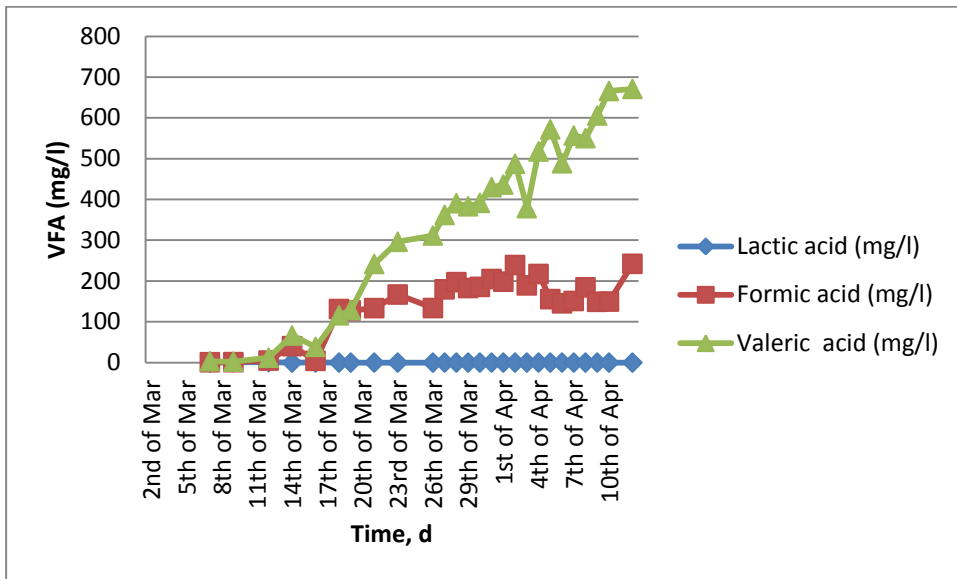
Figure 4.13: VFA profiles in reactor A during experiment 2

Figure 4.13 depicts that there was too little development (< 1mg/l) in lactic acid concentration initially. Its concentration was almost 0 mg/l after 16th of March. Formic acid concentration slowly rose day by day till 28th of March. It was nearly stable afterwards. Valeric acid continually rose up during the experiment.
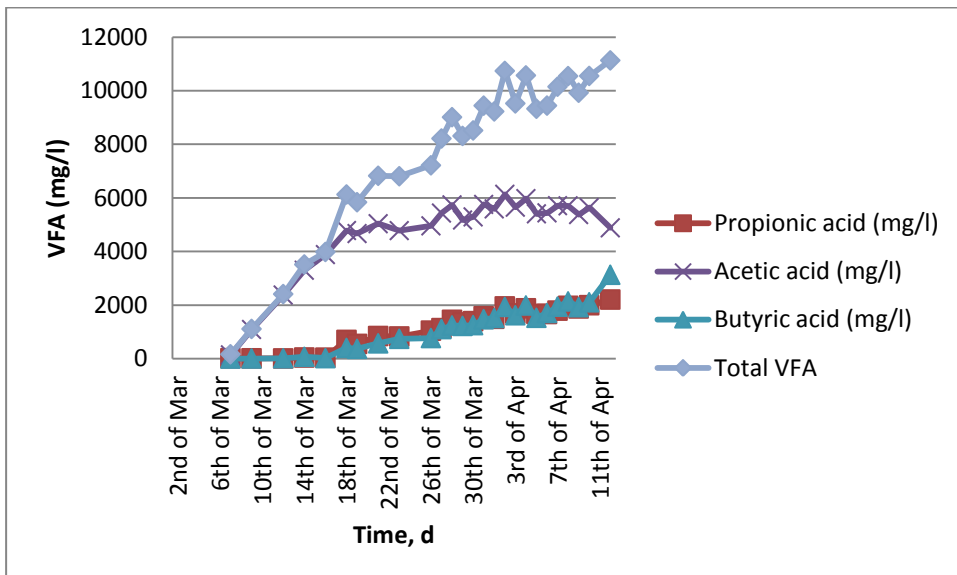

Figure 4.14: VFA profiles in reactor A during experiment 2

The concentration of acetic acid increased till 18th of March and after that it seems to be fairly constant with minor fluctuations. Propionic and butyric acids gradually started to get accumulated and their concentration was getting higher day by day indicating possibility of inhibition. The rise of VFA concentration is most likely due to higher organic loadings or insufficient SRT for methanogens. If there is higher organic loading or short SRT, acids formed by acidogens begin to accumulate as methanogens are not able to utilize it. During the last days of operation, VFA concentration has risen more suggesting more imbalances in the system. Charts 4.13 and 4.14 show that methanogens were partially inhibited as they can tolerate high concentration of VFAs as long as pH remains within the normal range for the growth of methanogens (6.8 to 7.4) [7]. So, the effects of VFA accumulation did not completely fail the process.

4.2.2 Test Reactor B (Yeast extract solution + glycol)

The vertical line on the following figures drawn on 16th of March indicates when glycol was introduced to the reactor for co-digestion along with yeast extract solution. The contribution to COD load and volume was 50% from each solution. Before that day, only yeast extract solution was loaded on the digester with equal COD.
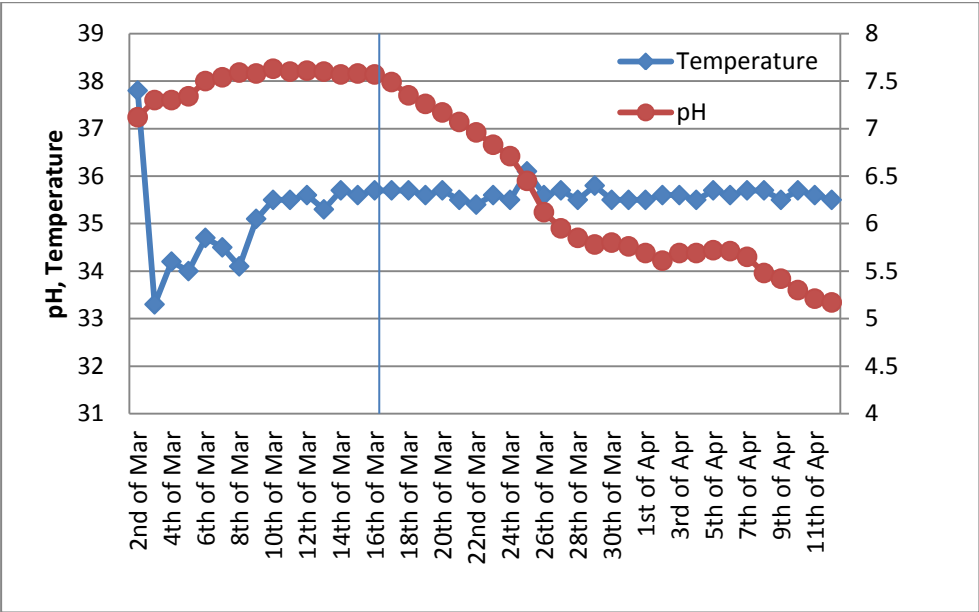
Temperature and pH

Figure 4.15: pH and temperature profiles in reactor B during experiment 2

The temperature varied from a range of 33.3°C to 37.8°C with an average temperature of 35.4°C.

pH varied from 5.17 to 7.63 with an average value of 6.6. Figure 4.15 clearly shows that approximately neutral pH was maintained until glycol solution was added in the reactor. After addition of glycol solution, pH started to descend slowly day by day and finally reached 5.17 during the experiment. Consequently, the process ceased with no gas production at all indicating a full pH inhibition. No attempts were made to control pH in order to observe the effects of co-digestion of glycol. It shows that there was insufficient alkalinity in the reactor to counteract pH change. If there is no sufficient alkalinity present in the reactor then slight rise in VFA concentration can lead to reduction in pH. In case of substrates containing protein, alkalinity is produced by the breakdown of protein and amino acids to produce NH_3, which combines with CO_2 and H_2O to form alkalinity as $NH_4(HCO_3)$ [15, 16, 40]. Alkalinity contributes positively to maintain neutral pH encountering VFA accumulation and pH drop. However, glycol solution does not contain any protein implying that there was no generation of alkalinity in the reactor to check pH drops. The methanogenic activity is inhibited when pH drops below 6.8 [16]. Thus, there was pH inhibition in the reactor which slowly diminished the gas production until there was no production at all.
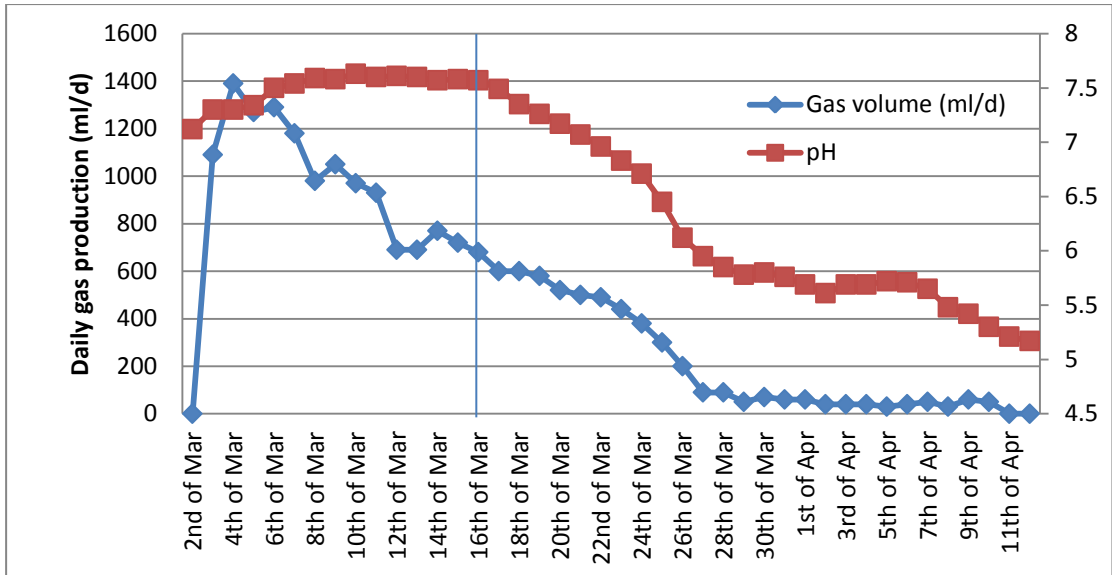
Gas Production

Figure 4.16: pH effects on methane production

Figure 4.16 shows that initially, the trend of gas production in reactor B was similar to reactor A (refer figure 4.24). Besides, pH was almost stable at about 7.5. However, after the addition of glycol, gas production declined slowly day after days as pH started to decrease. Gas production reduced until it reached approx. stable value of about 50 mL/day for some days. Meanwhile, pH was more or less stable at about 5.7. After that, pH plunged down rapidly until the gas production dropped to nil. The process was entirely pH inhibited.
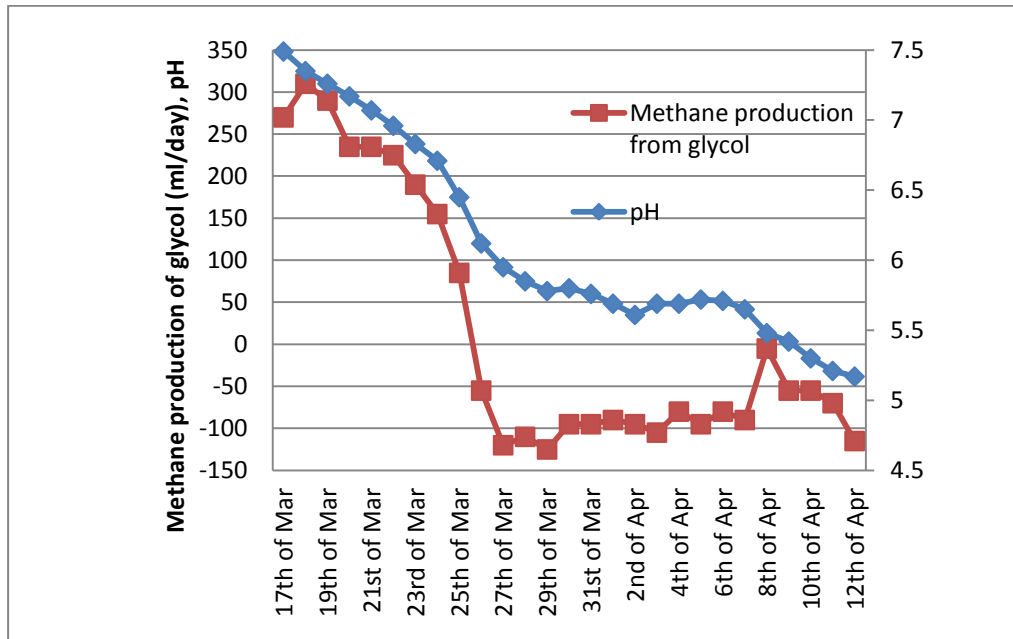

Figure 4.17: Effect of pH on methane production from glycol

Methane production from glycol is the difference between gas production from test reactor containing glycol and yeast solution and half the gas production from control reactor containing yeast solution only. It was assumed that the contribution of glycol and yeast extract solutions to the measured gas production in the test reactor B was 50% each owing to 50% COD contribution from each solution.

Mathematically, methane production from glycol = methane from reactor containing glycol and yeast solution – ½ methane from reactor containing yeast solution only

From the results obtained, glycol produced peak value of 310 mL/d which is equivalent to 210.9 mL/g COD of glycol at 20°C and 1 atm. The lowest positive value was 85 mL/d ≈ 57.8 mL/g COD of glycol at 20°C and 1 atm. During the experiment, pH declined gradually as no measures were taken to control it. So, it was not possible to obtain steady value for methane

production due to pH inhibition. Besides, when pH descended below 6.45, methane production of glycol was determined to be negative. As the value cannot be negative, it should be taken as zero indicating pH inhibition.
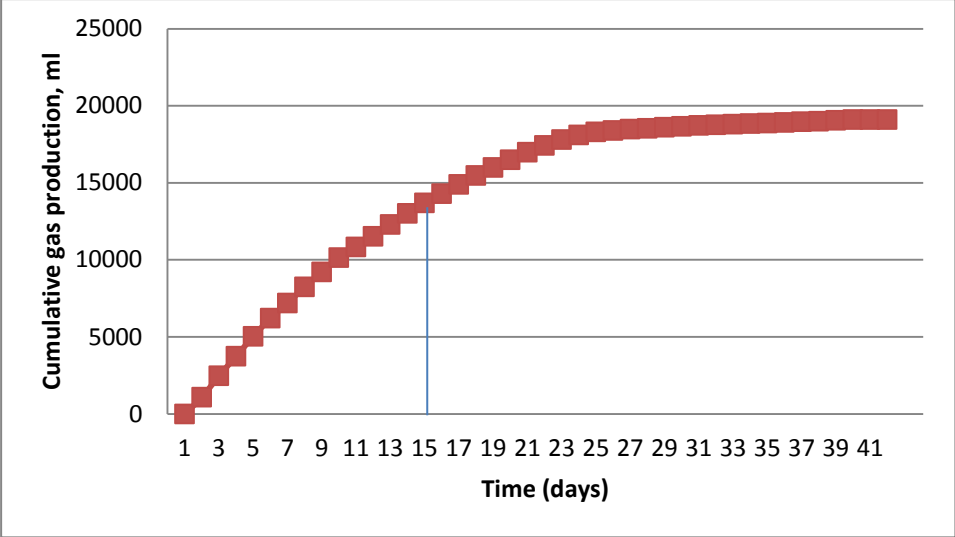

Figure 4.18: Cumulative gas production in reactor B during experiment 2

Figure 4.18 shows cumulative gas production in reactor B during the experiment. The total gas production was 19,110 mL i.e. 19.11 liters which was lower by 4.58 L compared to the control reactor A.
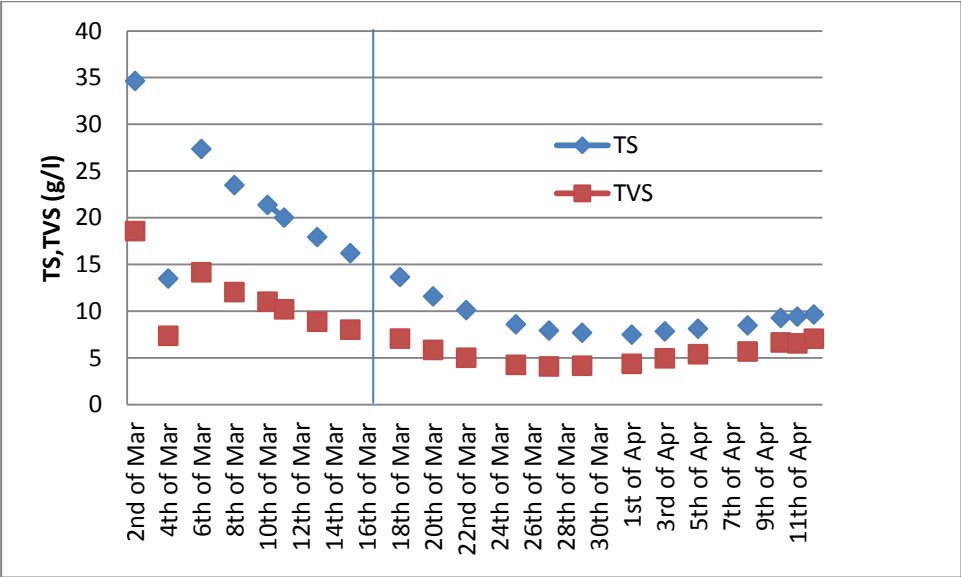
TS and TVS

Figure 4.19: TS and TVS profiles in reactor B

Figure 4.19 demonstrates that TS declined gradually from initial value of 34.64 g/l to 7.49 g/l till 1st of April then it slowly increased and reached 9.64 g/l eventually. Like TS, TVS decreased slowly from 18.57 g/l to 4.08 g/l till 27th of March after that it replenished again and reached 7.05 g/l finally. The value observed on 2nd day i.e. 4th of March can be considered as an outlier compared to the overall trend of the data series. It is most likely due to the heterogeneous sample withdrawn from the reactor due to stirring problem that occurred sometimes due to the defects of magnetic plate stirrer. Like other reactors, sludge containing high concentration of TS and TVS from IVAR was replaced by supply of solution containing dissolved solids only which caused the reduction in TS and TVS. A slight increase in TVS after 27th of March is most likely due to the fact that during the later stages of this test, less and less organic material got converted to methane, and accumulated as VFA and monomers. These organic dissolved contributed to relative increase in TVS. In the other hand, it could also be due to undigested substrate due to inhibition.

COD
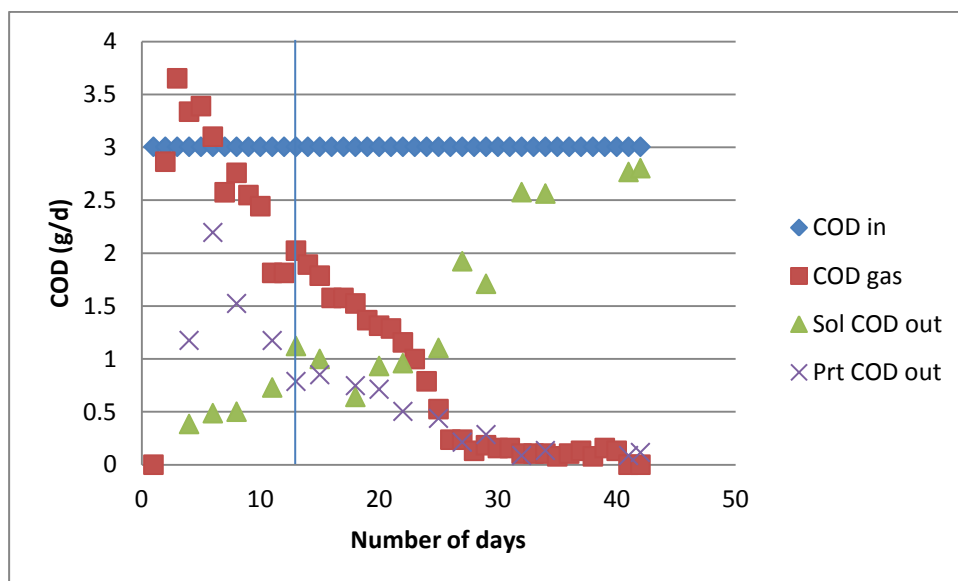
Figure 4.20: COD profiles in reactor B during the experiment 2 where COD$_{in}$ = influent COD per day, COD$_{CH4}$ = COD of methane per day, Sol COD$_{out}$ = effluent soluble/filtered COD per day and Prt COD$_{out}$ = effluent particulate COD per day

Figure 4.20 shows COD dynamics in the test reactor during the experiment 2. Initially, COD$_{CH4}$ was zero as gas production was nil. With increment in gas production, it surged instantaneously and it was larger than COD$_{in}$ for some days indicating that there was already

large amount of unaccounted COD present in the sludge in the reactor. Then COD_{CH4} started to decline day by day with the decrement in gas production and it became zero when there was no gas production. Prt COD_{out} was also continually decreasing in the reactor from its initial value, as inlet substrate was devoid of any particulates and there was also biomass loss from the reactor in withdrawal process. It was almost negligible at the end of the experiment. Meanwhile, Sol COD_{out} continually rose as only soluble COD was supplied in the reactor. We can see in the graph that when the gas production completely ceased, Sol COD_{out} was nearly equal to COD_{in}.
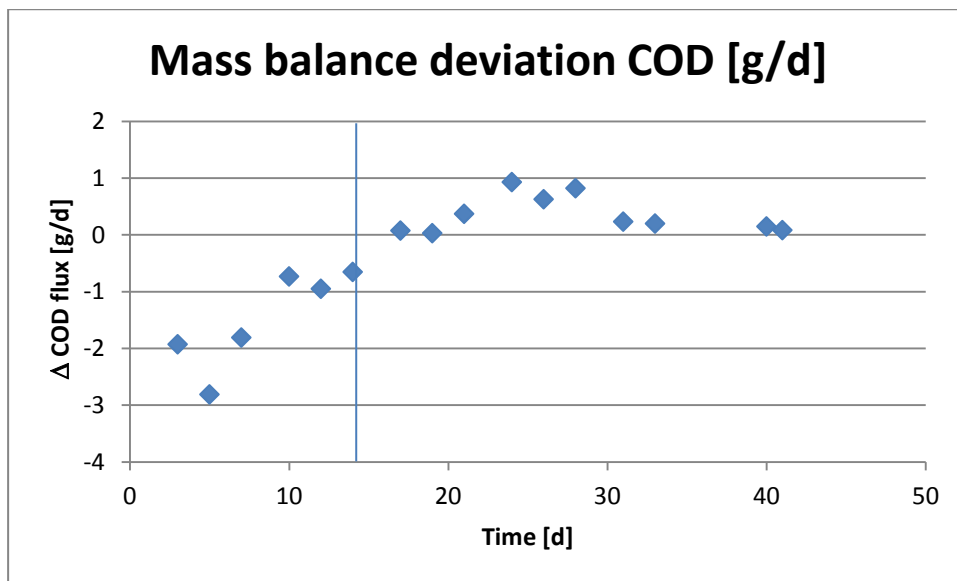
Figure: 4.21: COD mass balance deviation i.e. ΔCOD against time (days)

Figure 4.21 depicts that initially, outgoing COD per day was larger than inlet COD per day. It shows that there was already large amount of unaccounted COD present in the reactor which can be attributed to COD of sludge from IVAR. In addition to this, COD mass balance is not valid in unsteady states and it requires at least 5 SRTs i.e. 50 days to reach a steady state [39]. Meanwhile, as the operation continued, the deviation declined gradually which is most likely that reactor was approaching to the steady states. Finally, when the gas production ceased, inlet COD per day was nearly equal to effluent COD per day while, COD per day of methane was zero.

VFAs
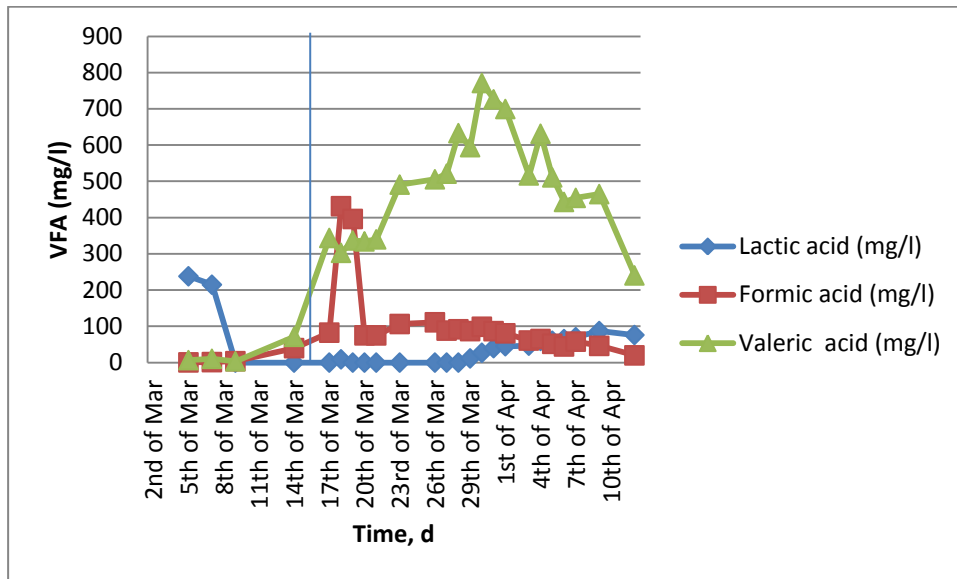
Figure 4.22: VFA profiles in test reactor B during experiment 2

Figure 4.22 depicts that there was slight development of lactic acid initially, which fell down to almost negligible concentration later. However, after introduction of glycol solution on 16th of March, lactic acid's concentration also increased once again although not to the same extent. Formic acid concentration was slowly rising day by day but it suddenly surged high to 432 mg/l on 18th of March most likely due to the effect of glycol solution and again plunged down to original level after 2 days. After 31st of April, the concentration of formic acid declined. Valeric acid concentration was rising slowly before the addition of glycol but after introducing glycol, its concentration also increased exponentially to about 344 mg/l on 17th of March. The value steadily rose and reached its peak value of 771 mg/l on 30th of March. Like other acids, its concentration also diminished after that.

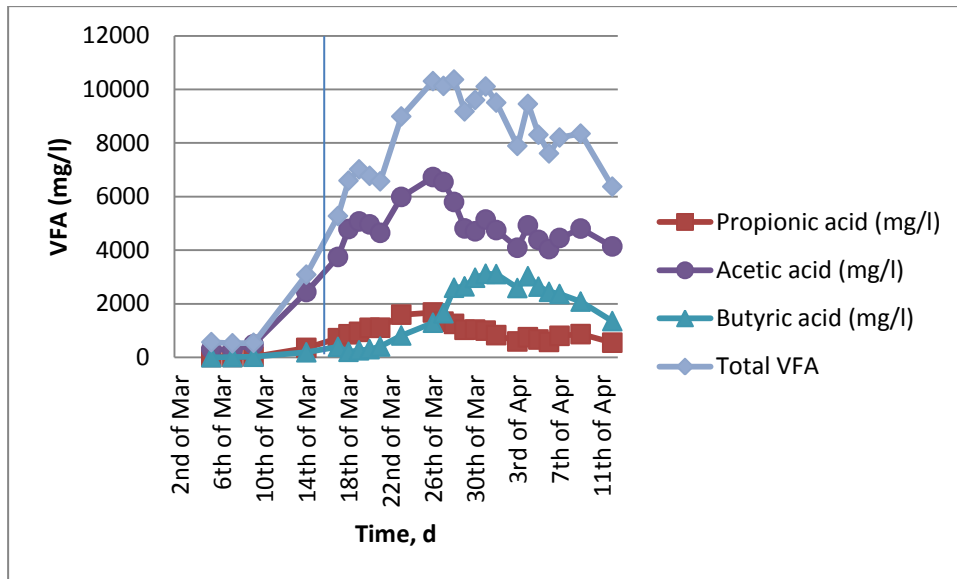**Figure 4.23**: VFA profiles in test reactor B during experiment 2

From figure 4.23, it can be observed that acetic acid was more or less constant initially. However, it increased sharply after 9th of March and reached peak value of 6727 mg/l on 26th of March. The value declined slowly and was almost steady at about 4500 mg/l after that time. Propionic acid steadily rose after addition of glycol and then it fell down like other acids after 27th of March. Butyric acid rose to a greater extent and time than propionic acid. It also fell down after 3rd of April.

The figures show that initially, methanogens were inhibited. Rise in propionic and butyric acid concentrations are inhibitory to the methanogens. However, as pH declined below 6, it is most likely that even acidogens might have been inhibited as the concentration of hydrogen rose high enough as there was decline in production of acids. Unlike in the reactor A, pH was too low in this reactor after addition of glycol suggesting lack of sufficient alkalinity in the reactor. In low pH, non-ionized forms of VFA are inhibitory even at lower concentrations [7]. So, the process was entirely pH inhibited as pH dropped down slowly and reached to 5.17 at the last day of the experiment.

Comparison between two reactors

Figure 4.24 presents the comparative performances of two reactors. Reactor containing yeast extract can be taken as a control reactor which helps to distinguish the effects of co-digestion of glycol with yeast solution.
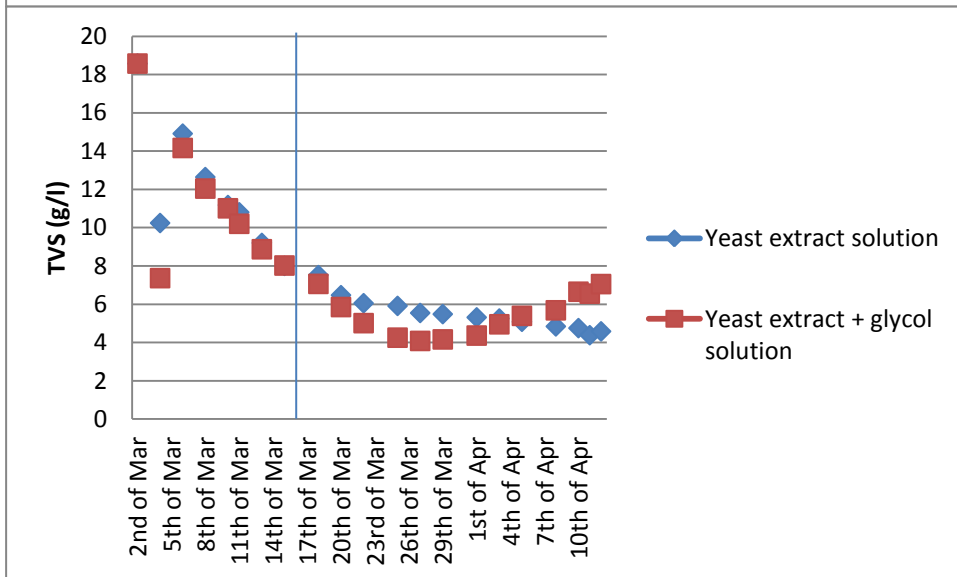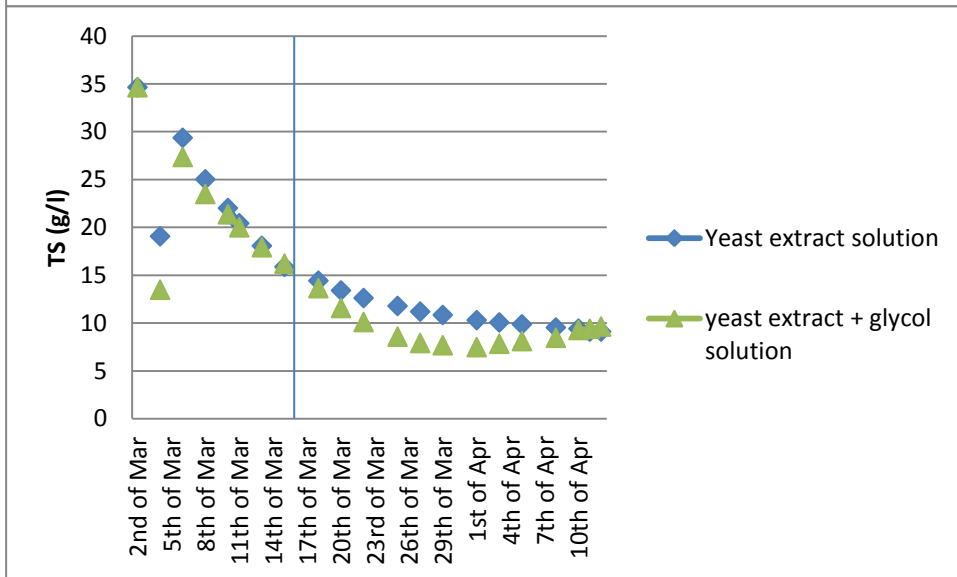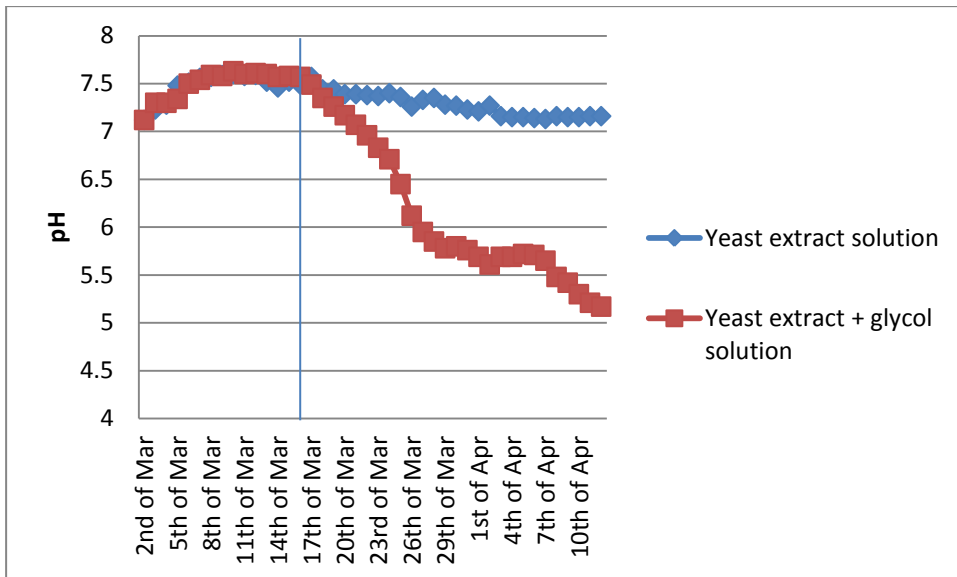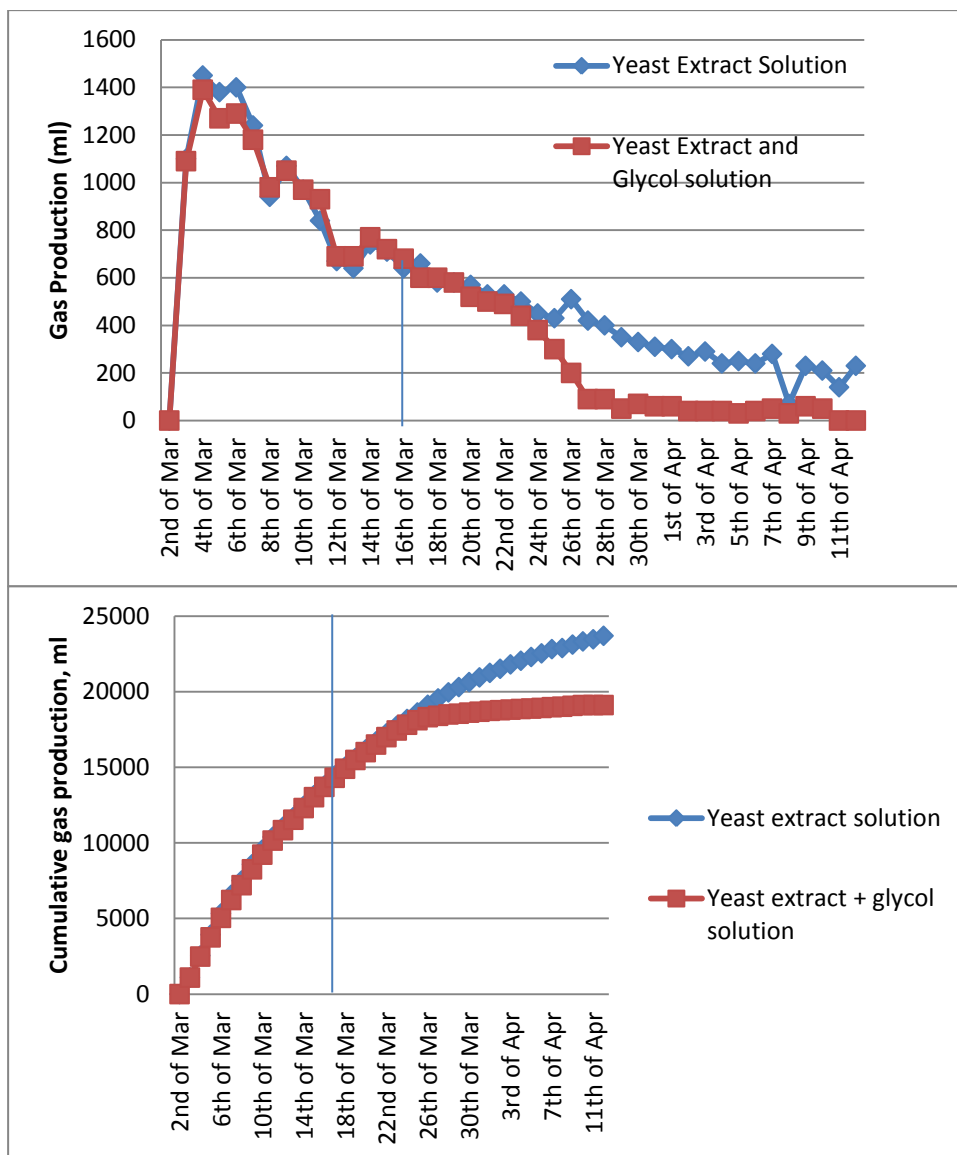
Figure 4.24: Plots showing the comparative performances of two reactors

Figure 4.24 depicts that there was negligible pH drop in control reactor while pH reduction was very significant in test reactor indicating that the buffering capacity of glycol solution is very low. TS and TVS followed more or less parallel trends. There were some differences predominantly due to the differences in the substrate composition. Gas production was lower in test reactor due to higher inhibition caused by greater pH drops.

4.3 Experiment Three (20th of April – 23rd of May)

Two identical reactors (reactor A and reactor B) were operated in parallel during the experiment. Initially, both reactors were loaded with a solution comprised of yeast extract, starch and nutrient broth for almost 11 days to obtain approx. stable gas production. However,

from 12th day of operation i.e. from 2nd of May, one of the reactors (control reactor A) was fed with the solution constituting yeast extract, nutrient broth and starch only while the other (test reactor B) was fed with equal volumes of solution (50 ml each) containing 50% COD load from the solution containing yeast extract, nutrient broth and starch solution while 50% COD load as MEG. Hence, glycol was codigested along with solution containing yeast extract, nutrient broth and starch in reactor B from 2nd of May. The organic loading was about 3.4 g COD/L·d in both reactors during the experiment. The results observed from the experiments are presented with comparisons and discussions.

4.3.1 Control Reactor A (Yeast extract + Starch + Nutrient Broth solution)

Temperature and pH
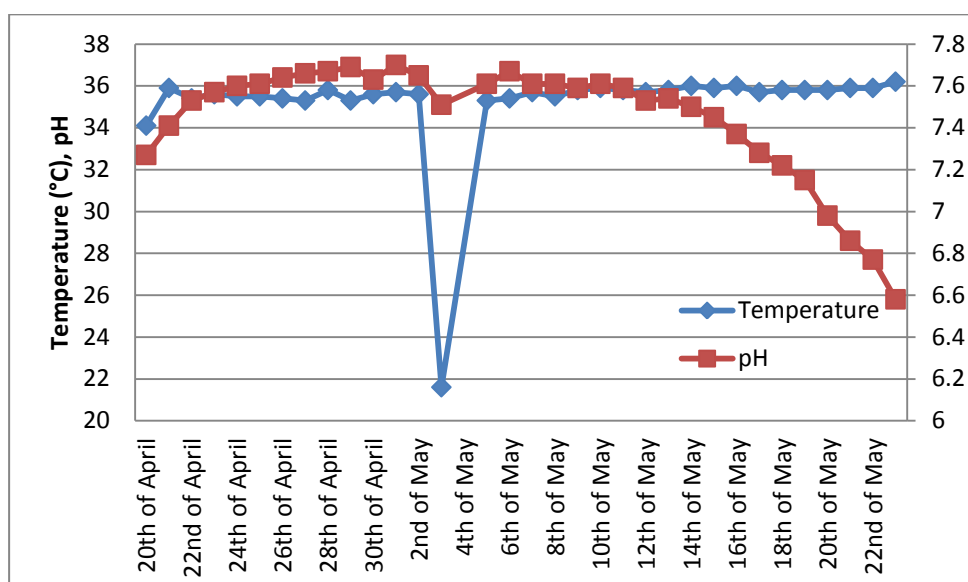
Figure 4.25: Temperature and pH profiles in reactor A during experiment 3

During the experiment 3, the temperature of water bath was maintained to 38.1°C which was constant during the experiment. However, due to a technical problem, the experiment was operated in room temperature ($\approx$ 22°C) from 2nd of May till 3rd of May for about 18 hours. Some changes in the performance of reactors were observed due to decrement in temperature. From 3rd of May, the temperature was again increased to about 38.1°C and maintained constant till the end of the experiment. The plot shows that there was a variation in measured temperature of the reactor ranging from 21.6°C to 36.2°C with an average value of 35.2°C. Although the temperature of the sample was measured instantaneously, the reduction of

temperature can be attributed to the heat absorption by syringe, beaker etc. and also heat loss due to contact with air.

pH of the reactor varied from 6.58 to 7.7 with an average of 7.4. The optimal pH range for all methanogenic bacteria is between 6 and 8 [8] which means that the reactor was operating within optimal range. The plot shows that there was a slight rise in pH and then it was almost steady later until it started to drop gradually at the final stages of the experiment. The fall of pH at last stages is most likely due to decline in alkalinity in the reactor (refer fig 4.36) and accumulation of VFA. The fall of pH on 3rd of May is most likely caused by the decline in temperature on 2nd/3rd of May. During the event, it can be assumed that methanogens were more affected than acidogens and VFA might have risen slightly. Moreover, lower gas production supports this assumption. With increase in temperature once again, pH was recovered to original value indicating methanogens were working effectively once again.

Gas Production
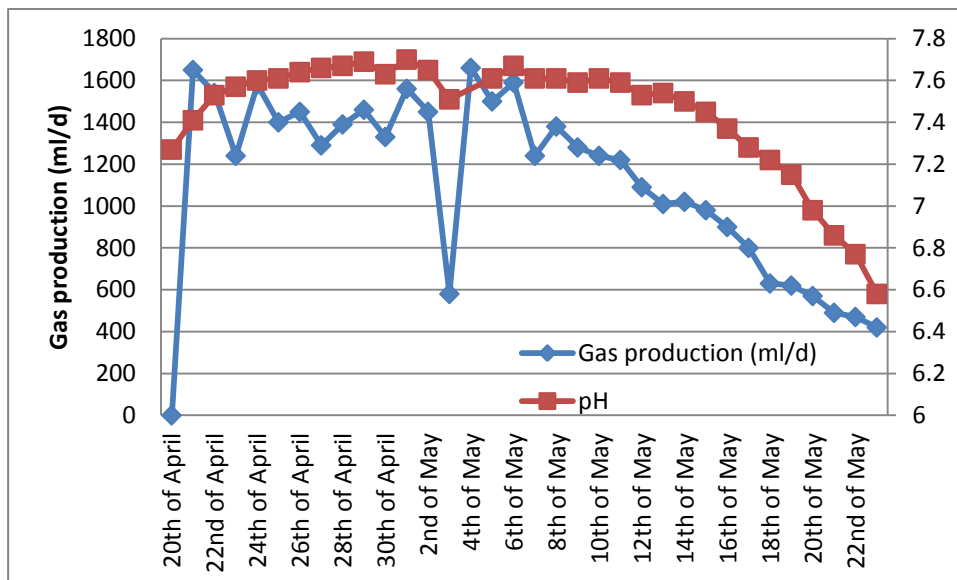
Figure 4.26: pH effects on daily gas production in reactor A during experiment 3

Figure 4.26 shows that lag phase was not noticeable in the experiment. The gas production rose sharply within 24 hours of operation and reached peak value of 1650 mL/d. Owing to the lowering of temperature, the gas production plunged down to 580 mL/d on the next day. However, after restoration of temperature to original value, gas production also replenished once again to its initial level. It remained almost steady for some days and then started to decline gradually. The figure exhibits that gas production reduces due to reduction in pH.

Decline in pH and gas production indicate some inhibition. VFA analysis revealed high concentration of accumulated VFA which is most likely the prime cause for inhibition. In addition, some foul smelling gases were smelled after withdrawing sludge which were most likely ammonia, H_2S etc. which are toxic. This reactor can also be said to be running in inhibited steady state due to the interactions between free ammonia, VFAs and pH as discussed in section 2.2.4.

This time daily gas production was relatively higher than that produced in experiment 2. It shows that substrate composition plays a significant role for gas production. Microorganisms most likely have got better nutrients condition than the former by the addition of nutrient broth and starch along with yeast extract solution.
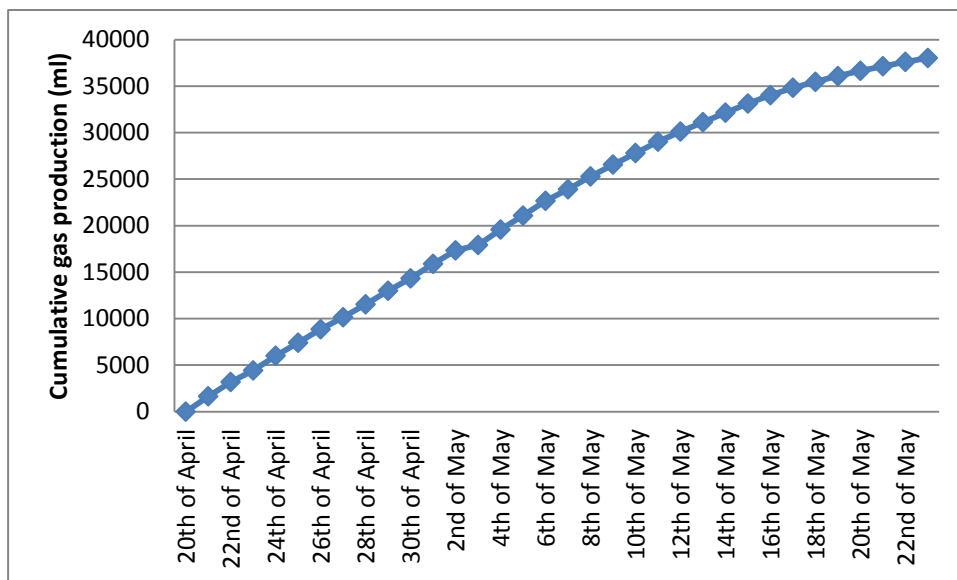
Figure 4.27: Cumulative gas production in reactor A during experiment 3

The total gas production in control reactor A during the experiment was 38,030 mL i.e. 38.03 liters.

TS and TVS
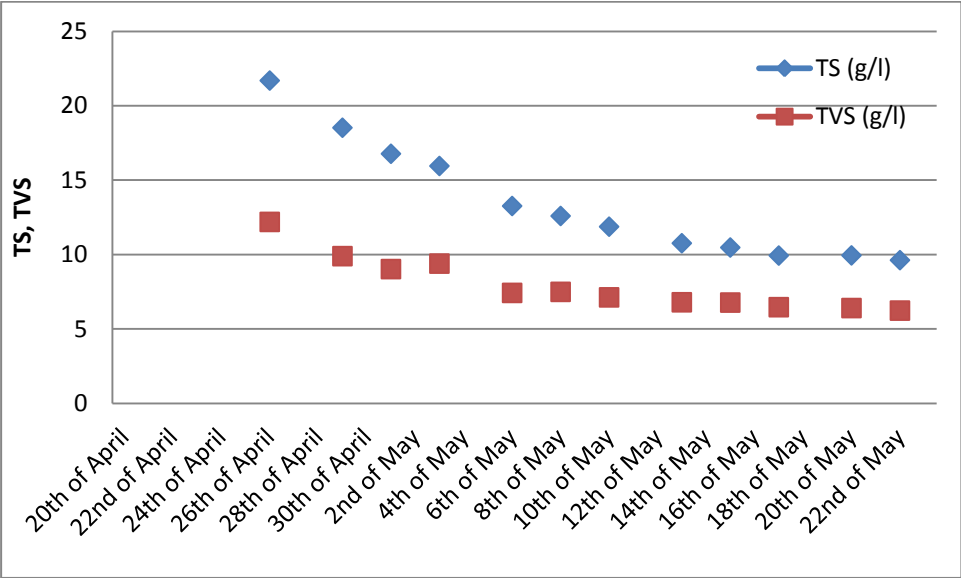
Figure 4.28: TS and TVS profiles in reactor A during experiment 3

According to the figure 4.28, TS and TVS diminished continuously with some fluctuations during the experiment indicating a change in reactor solids concentration attributed to change in inlet solids loading and biomass loss as no means were used to retain those.

COD

Total and dissolved CODs of the effluent sludge were determined to be 25.34 g/L and 23.74 g/L respectively on 23rd of May, the final day of the experiment. The values indicate that most of the COD was in the form of soluble COD as the particulate COD was low in the reactor.

Alkalinity and SCFA
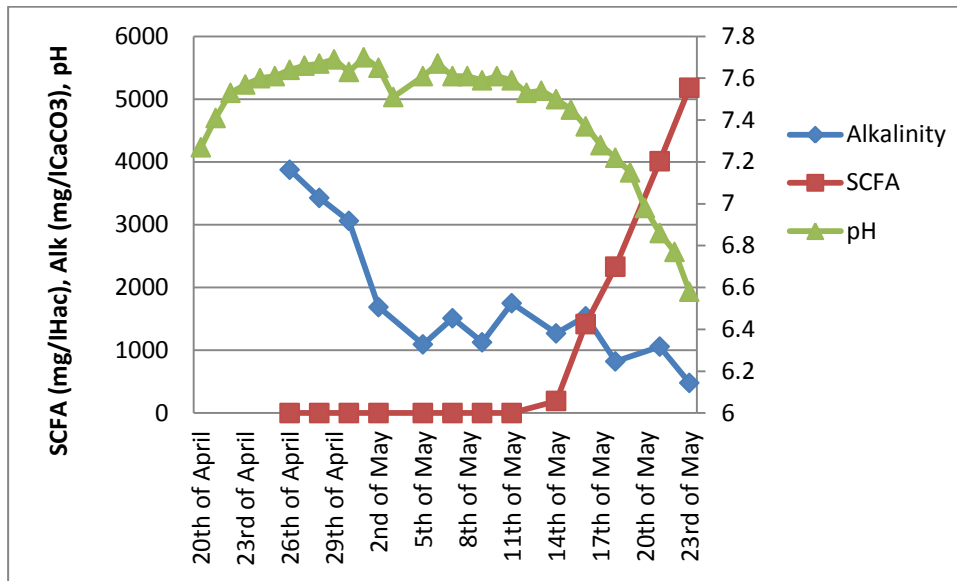
Figure 4.29: pH, SCFA and Alkalinity variations in reactor A during experiment 3

Figure 4.29 shows that the alkalinity was descending initially from 3875 mg/l as $CaCO_3$ to 1094 mg/l till 5th of May. After that it was more or less stable with some fluctuations until it finally diminished to 480 mg/l. SCFA value remained constant at about 0 mg/l as Acetic acid (HAc) up to 21 days of operation i.e. 11th of May. After that, SCFA increased significantly and reached 5181 mg/l on the final day of operation. The trend is comparable to changes in pH. Anaerobic processes are stable when the concentration of fatty acids is minimum indicating that sufficient methanogenic population exists and sufficient time is available to minimize hydrogen and VFA concentrations [16]. With lowering of alkalinity, excess VFA starts to accumulate and pH starts to drop down due to lack of buffering capacity in the reactor.

Using Ion chromatography, the concentrations of lactic, propionic, formic, acetic, butyric, and valeric acids were determined to be 3.53 mg/L, 2730.03 mg/L, 248.21 mg/L, 4299.96 mg/L, 3386.07 mg/L and 1294.62 mg/L respectively on 22nd of May and 2.06 mg/L, 2974.89 mg/L, 265.45 mg/L, 4435.99 mg/L, 3567.34 mg/L and 1313.67 mg/L respectively on 23rd of May. The results show that high concentrations of VFAs were accumulated in the reactor causing inhibition. There was accumulation of acids of higher molecular weights like butyric, valeric etc. indicating process instability and inhibition on methanogenic activity.

SCFA concentration from TITRA 5 and total VFA concentration from Ion chromatography differed significantly during these measurements. So, it is more probable that there could be analytical errors, experimental errors or other unidentified errors in the results arising from errors in methods or samples.

4.3.2 Test Reactor B (Yeast extract + Starch + Nutrient Broth + Glycol solutions)

The vertical line on the following figures drawn on 2nd of May indicates when glycol was introduced to the reactor for co-digestion along with yeast extract, starch and nutrient broth solution. The contribution to COD load and volume was 50% from each solution. Before that day, solution comprising of yeast extract, starch and nutrient broth was loaded on the digester of equal COD concentration.

Temperature and pH
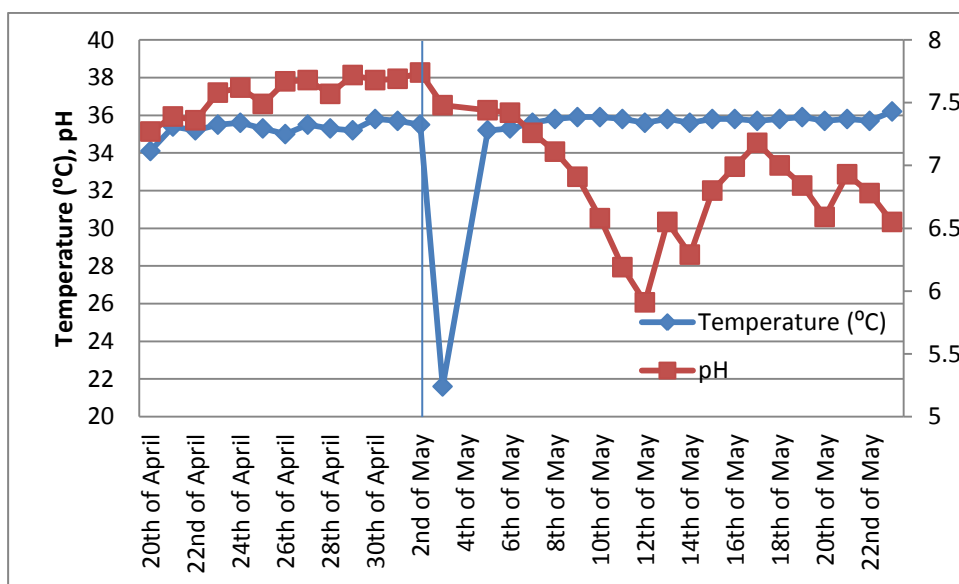
Figure 4.30: Temperature and pH profiles in reactor B during experiment 3

Figure 4.30 shows that the pattern of temperature fluctuations in reactor B was almost similar to the one in reactor A in the experiment 3 (ref. figure 4.25), which is obvious since, both reactors were operated in same water bath.

pH in the reactor varied from 5.91 to 7.74 with an average value of 7.13. pH in reactor B followed almost the same path as in reactor A up to 2nd of May, the day when glycol was introduced in the reactor. After addition of glycol, pH started to descend gradually day by

day. Alkalinity was also decreased at the same time due to accumulation of VFA. As buffering capacity was too low, minor accumulation of VFA would cause reduction in pH. So, to interrupt the pH reduction, 1.6 gm of Na_2CO_3 was added to the reactor once it reached the minimum value of 5.91 on 12th of May. After then glycol solution containing 3.8 gm/l of Na_2CO_3 was loaded as glycol solution. Moreover, several other dosages of Na_2CO_3 were added to restore pH to neutral values. (Refer to appendix A7 for details). Eventually, there were minor variations in pH in the reactor due to accumulation of VFA and efforts to counteract the pH drop i.e. addition of Na_2CO_3.

Gas Production
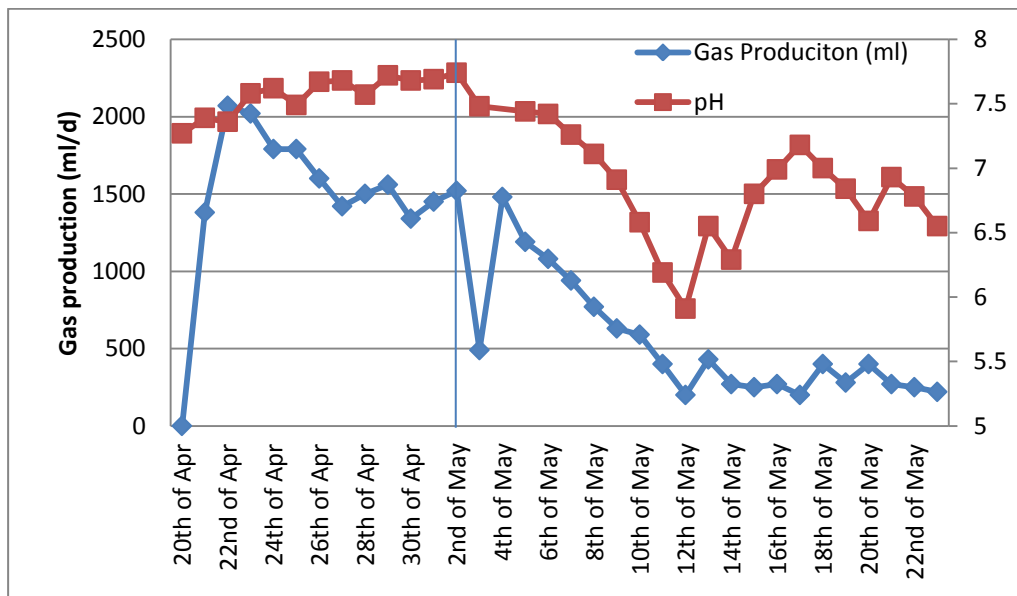
Figure 4.31: pH effects on daily gas production in reactor B during experiment 3

Figure 4.31 shows that the gas production in reactor B was similar to the one in reactor A before addition of glycol (ref. figure 4.36). However, after addition of glycol on 2nd of May, both gas production and pH began to decline slowly. It can be assumed that pH decline might have triggered the reduction of gas production. pH reduction was interrupted after pH dropped to 5.91 by adding Na_2CO_3. The idea was to see if gas production would restore to previous levels after pH restoration. There was only slight rise in daily gas production in addition to prevention from further pH inhibition. Moreover, there was dramatic variation of pH by addition of various dosages of Sodium carbonate such as shift from 5.91 to 6.55, 6.55 to 6.29, 6.29 to 6.8 and so on which is detrimental [7]. Thus, it is most likely that fluctuation in gas production was due to the shifts in pH. The process may be considered to be pH inhibited.
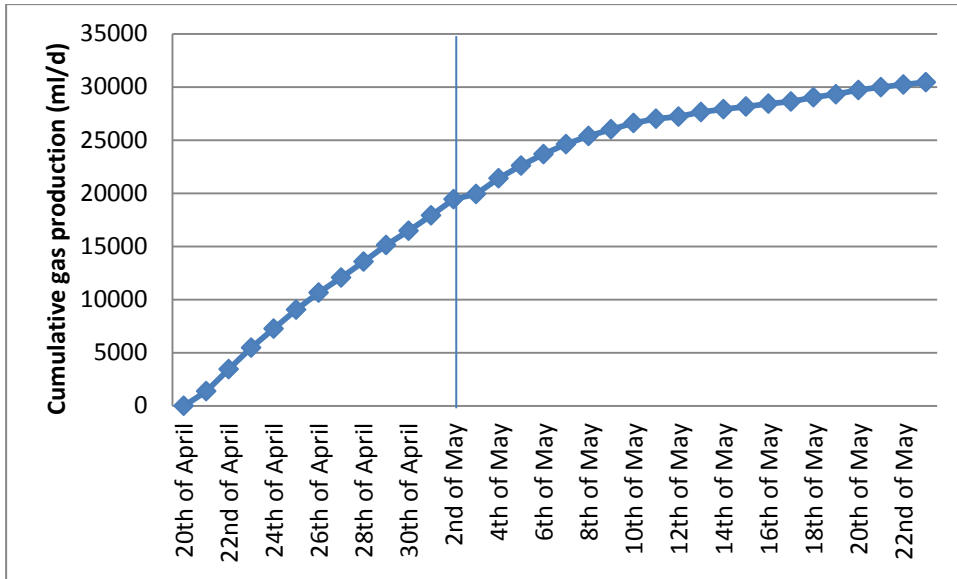
Figure 4.32: Cumulative gas production in reactor B during experiment 3

The total gas production in reactor B during the experiment 3 was 30,450 mL i.e. 30.45 liters which was 7.58 L lower compared to the control reactor A.
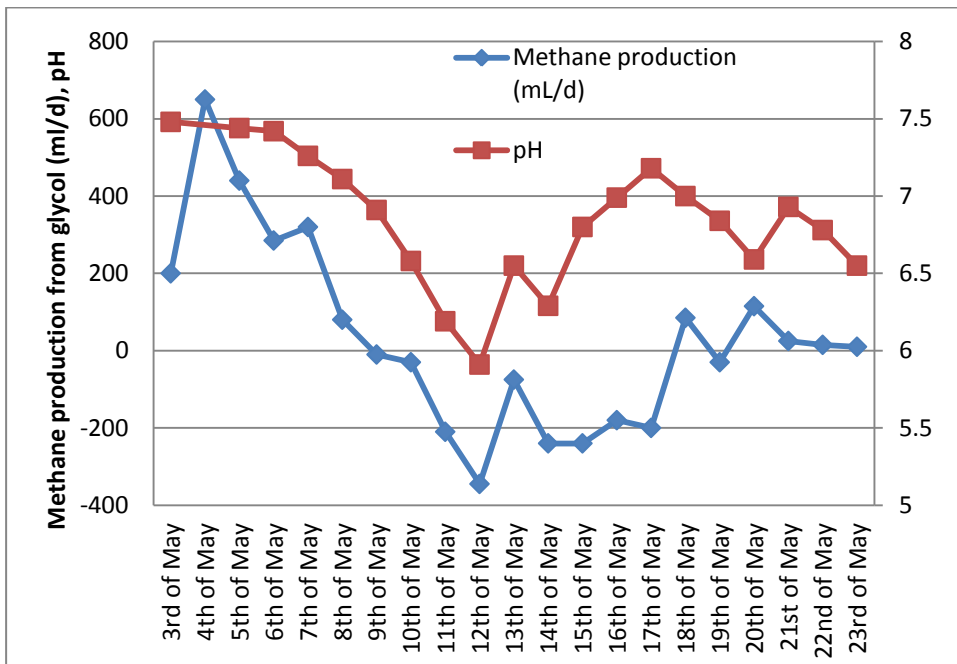
Figure 4.33: Methane production from glycol and pH fluctuations in experiment 3

Figure 4.33 shows that methane production of glycol is highly affected by the pH of the reactor. The fluctuation of methane production and pH follow more or less a parallel trend. With decline in pH, methane production also declined while pH was raised, methane

production also increased. Initially, when pH diminished below 6.91, the value of methane production was negative. As values cannot be negative, it should be taken as zero indicating inhibition. However, when alkalinity was restored back and pH was also raised to almost neutral, then methane production also restored to positive value. Production was positive until pH dropped down to 6.55. From the results obtained, glycol produced peak value of 650 ml/day but the value was higher most likely due to temperature effects. So, if peak value of 440 ml/day is taken, then methane potential of glycol can be determined to be 299.3 mL/g COD at 20°C and 1 atm. During the process, there was high consumption of alkalinity suggesting that inadequate alkalinity was produced during the degradation of glycol.

TS and TVS
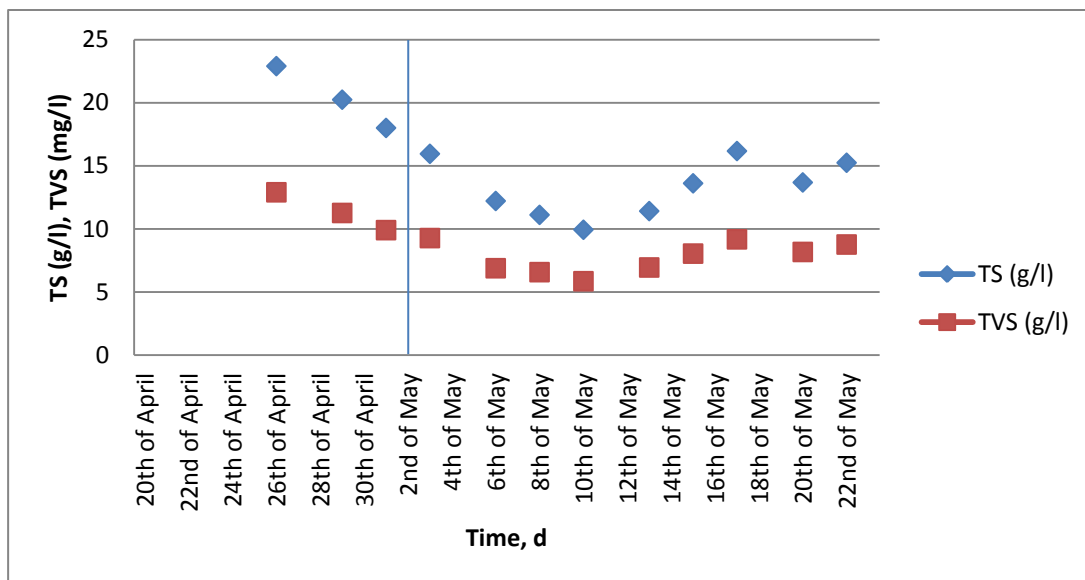
Figure 4.34: TS and TVS profiles in reactor B during experiment 3

Figure 4.34 shows that TS and TVS descended gradually as in other experiments. However, from 13th of May, both TS and TVS slowly rose afterwards. Meanwhile, gas production was decreasing which suggests that the increment is most likely either due to accumulation of undigested TS and TVS from influent substrate or accumulated VFA and monomers.

COD

Total and dissolved CODs of the effluent sludge were determined to be 28.76 g/L and 26.85 g/L respectively on 23rd of May, the final day of the experiment. The values indicate that most of the COD was in the form of soluble COD as the particulate COD was low in the reactor.

Alkalinity and SCFAs
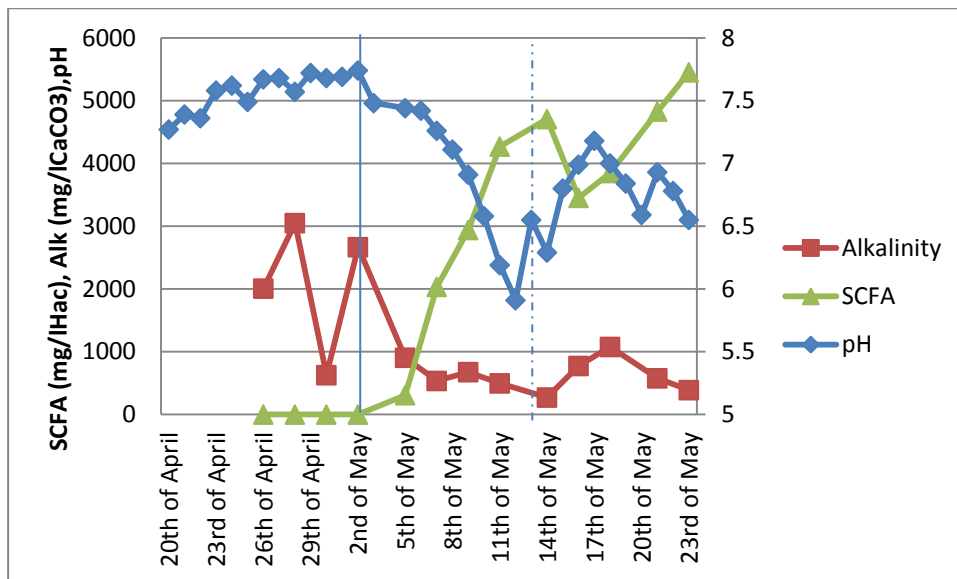
**Figure 4.35**: pH, alkalinity and SCFA fluctuations in reactor B during experiment 3 (*N.B. The additional vertical dotted line drawn on 13th of May indicates that glycol containing 3.8 gm of Na$_2$CO$_3$/l was used as glycol solution afterwards.*)

Figure 4.35 demonstrates the fluctuations of pH, SCFA and alkalinity in the reactor. Initially, alkalinity was higher in the range of approximately 2500 mg/L before addition of glycol except on 30th of April. The value obtained on the date may be taken as an outlier and corresponds to error in measurements. Alkalinity drastically reduced after addition of glycol solution on the reactor. On May 12th, 1.6 gm of Na$_2$CO$_3$ was added in reactor dissolving with glycol solution in order to restore alkalinity in the reactor. Moreover, from 13th of May glycol containing 3.8 gm of Na$_2$CO$_3$/l was used as glycol solution afterwards to maintain alkalinity in the reactor. However, the concentration of Na$_2$CO$_3$ was not able to fully counter alkalinity drop. So, additional amounts of Na$_2$CO$_3$ were added afterwards (refer to appendix A7 for details). pH rose later as the result of adding Na$_2$CO$_3$ in the reactor. When there is sufficient alkalinity in the reactor, pH is less affected by the increased VFA concentration [7].

SCFA began to rise after the addition of glycol in the reactor due to lowering of alkalinity in the reactor. There was also decrease in methane production in the reactor suggesting that methanogens were inhibited due to pH drop caused by SCFA accumulation.

Using Ion chromatography, the concentrations of lactic, propionic, formic, acetic, butyric, and valeric acids were determined to be 5.59 mg/L, 213.13 mg/L, 0.85 mg/L, 5308.75 mg/L, 3354.87 mg/L and 320.55 mg/L respectively on 22nd of May and 1.47 mg/L, 844.94 mg/L, 0.2882 mg/L, 4974.78 mg/L, 3649.06 mg/L and 293.21 mg/L respectively on 23rd of May. The results show that high concentrations of VFAs were accumulated in the reactor causing inhibition. There was accumulation of acids of higher molecular weight, butyric acid which seems to be most likely the prime cause of inhibition to methanogens.

SCFA concentration from TITRA 5 and total VFA concentration from Ion chromatography differed significantly during these measurements. So, it is more probable that there could be analytical errors, experimental errors or other unidentified errors in the results arising from errors in methods or samples.

Ammonium

Table 4.2: Ammonium concentrations in two reactors in experiment 3

Date	Ammonium Concentration (mg/l)	
	Reactor A	Reactor B
22nd of May	614.7901	437.9164
23rd of May	582.4264	354.6957

Table 4.2 shows that ammonium ion was higher in the control reactor compared to the test reactor. The reason behind this may be the lower pH of test reactor compared to other reactor. At low pH, ammonium ion is consumed by acids. Free ammonia is the principal inhibitory species. Ammonium ion (NH_4^+) concentrations as high as 7000 to 9000 mg/L as N have been successfully treated without a toxic response with an acclimated culture, although concentrations as low as 1500 mg/L as N have been reported to be toxic [7]. Ammonia is primarily present as the ionized species at the pH values typically occurring in the anaerobic processes [7], which means that the concentration determined in the experiments most likely may not have been toxic themselves.

Comparison between two reactors

Figure 4.36 presents the comparative performances of two reactors. Reactor containing solution of yeast extract, nutrient broth and starch can be taken as a control reactor which helps to distinguish the effects of co-digestion of glycol with other solution.
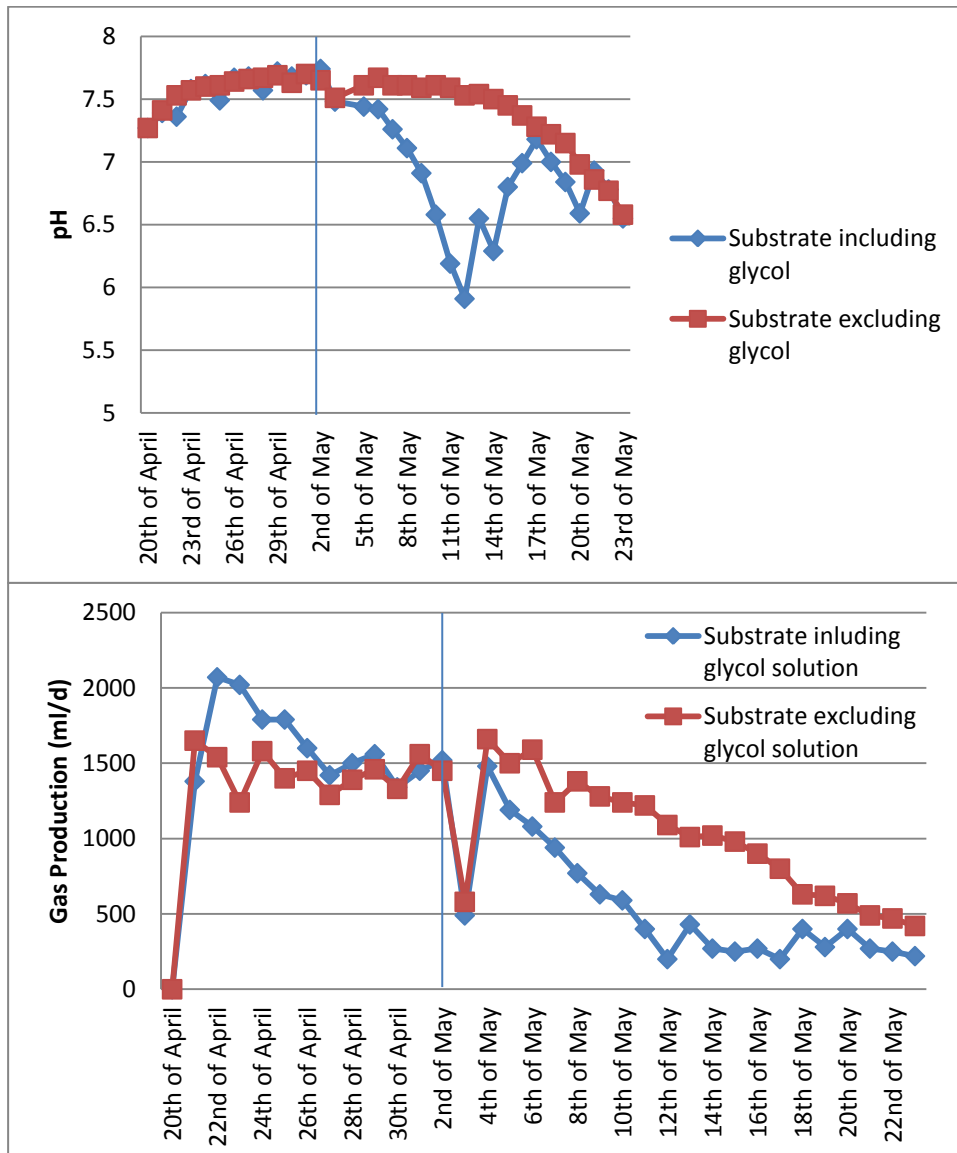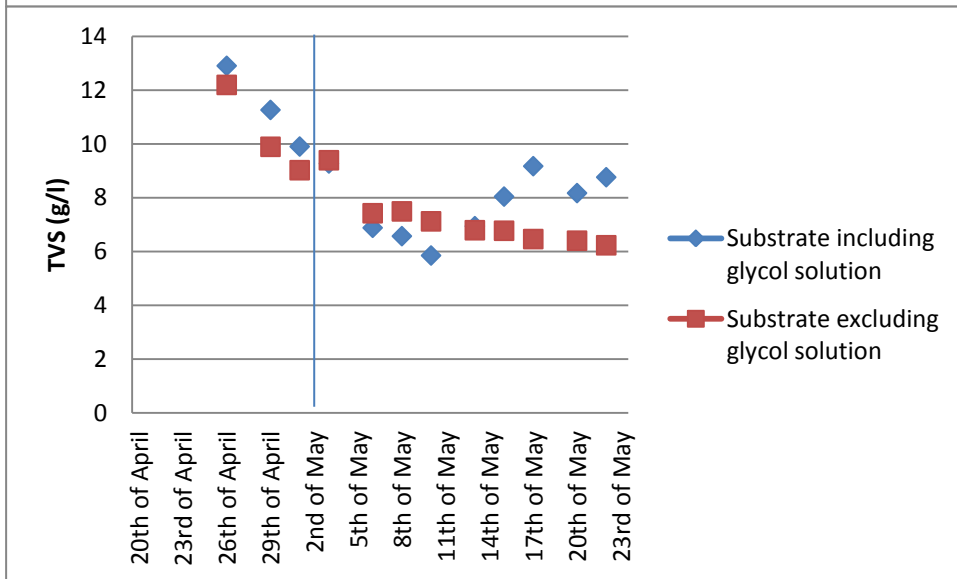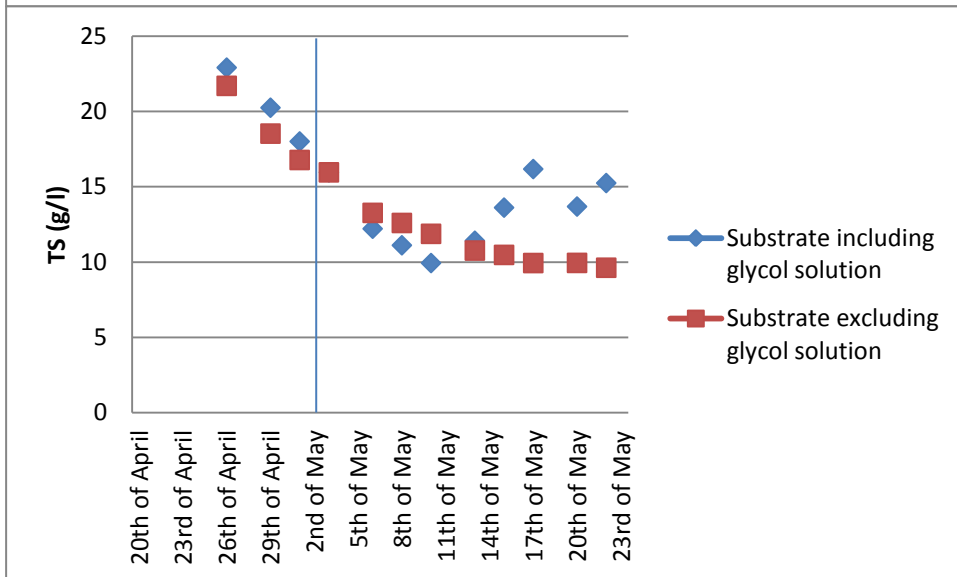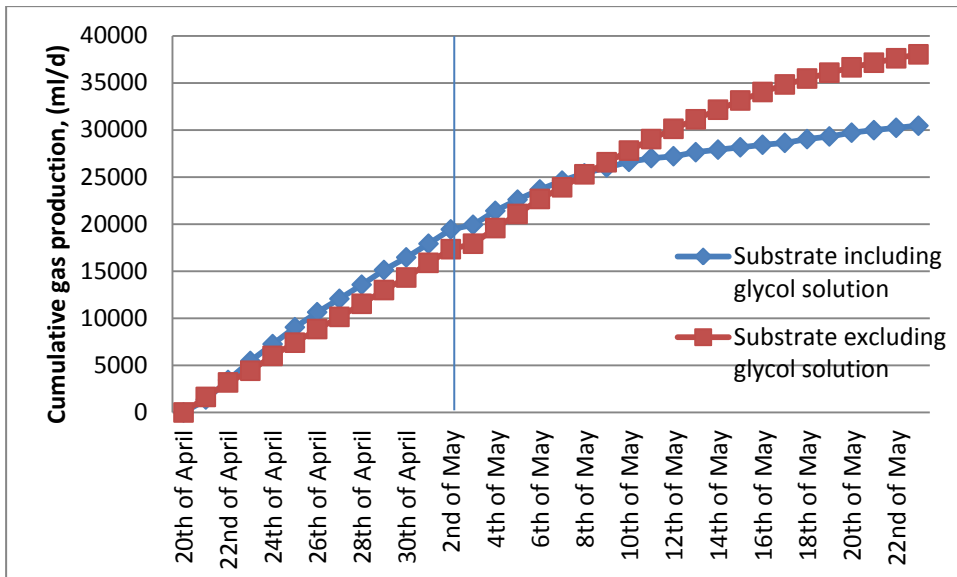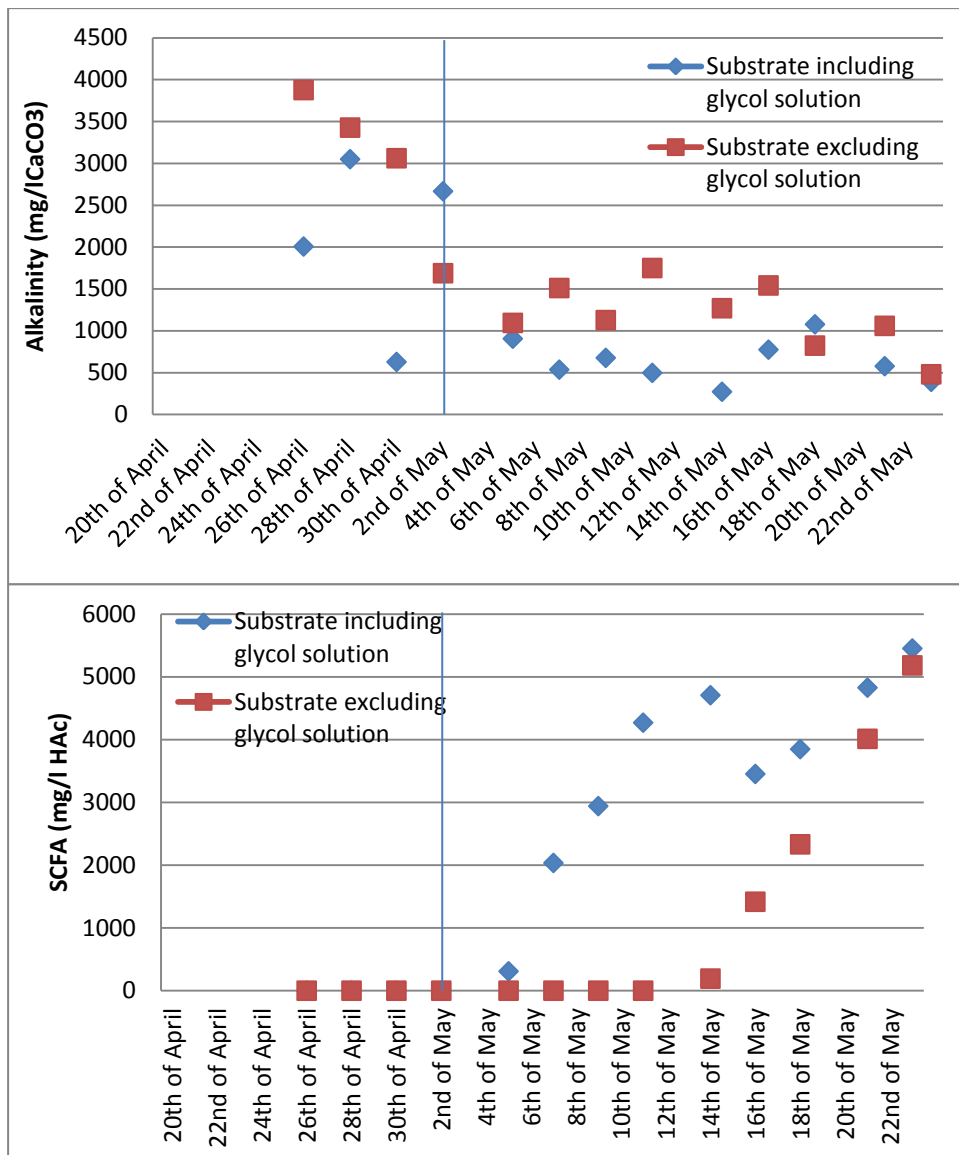
Figure 4.36: Comparison of different parameters between two reactors in experiment 3

Figure 4.36 depicts the impacts of addition of glycol on the reactor performances. There was rapid pH drop in test reactor than the control reactor which is most likely due to inadequate capacity of glycol solutions to generate alkalinity. TS and TVS changes are more or less similar. Some differences should be attributed predominantly to changes in substrate composition and different degrees of inhibition in two reactors causing different amount of VFA accumulation and TVS changes. Slow drop in alkalinity in both reactors is most likely due to the fact that the sludge from IVAR which could generate alkalinity due to presence of proteins was getting replaced with substrate solutions. However, there was rapid decline in alkalinity in test reactor indicating lack of buffering capacity in glycol solutions. At last stages, some alkalinity was added in test reactor which increased alkalinity in the reactor. Rise

in SCFA was tolerated to greater days in control reactor than the test reactor due to availability of alkalinity. However, as the alkalinity diminished in last stages, accumulation of SCFA was almost similar in both reactors.

4.4 Methane Production from Glycol

From the section 2.4.1, 4 moles of glycol produce 5 moles of methane and 3 moles of CO_2. Using ideal gas equation, it can be found that each mole of glycol yields 30.15 L methane at 20°C and 1 atm. pressure. The theoretical value for CH_4 yield at 20°C and 1 atm. pressure is 376 ml/g COD. In experiments 2 and 3, the methane yields of glycol solution was determined to be approx. 211 mL/g COD and 299 mL/g COD respectively during the peak productions of methane. The experimental values may have been lower due to inhibition. The values may be significantly improved if the reactors operate in uninhibited states.

4.5 General Discussions

Anaerobic process is a complex phenomenon where different types of microorganisms work together collectively. The process is highly sensitive to temperature, pH, alkalinity, VFA, heavy metals, organic loading, availability of nutrients etc. There can be multiple factors responsible for the success or failure of the process. So, many factors need to be taken into account during the anaerobic digestion process.

In all three experiments, SRT of 10 days was used. However, analysis of bulk phase samples indicates that high organic loading and short solid retention time were most likely responsible for accumulation of volatile fatty acids causing pH reductions. Besides, pH inhibition caused lower gas productions. Generally, design SRT of 10 days is recommended for use in the design of complete mix anaerobic digesters operating at 35°C for a suspended growth process [16]. However, methanogenic activity is the most crucial factor in the anaerobic process which is slow and requires sufficient contact time to work efficiently. Incorporating this idea, the same reference has suggested using a factor of safety of 5 or more which would give design SRT of 15 days for the experiments. This information is contradictory but it seems that safety factor of 5 or more could possibly enhance the reactor performance. Moreover, for stable performance, not more than 5 % of the total reactor should be withdrawn at a time [7]. In all the reactors, 10% of the solution i.e. 100 ml was withdrawn, which could have been another factor for biomass loss.

Alkalinity concentration in a digester is, to a great extent, proportional to the solids feed concentrations [16]. Glycol solutions do not contain proteins and are not able to produce alkalinity during the anaerobic digestion. The results from the experiments proved that there was insufficient alkalinity in the reactors which were used for co-digestion. Although slight pH drop was observed in experiment 3, it was near neutral all the time in experiment 2, most likely due to difference in substrate composition. Carbon dioxide is the principal consumer of alkalinity in a digester, and not VFA [16]. Carbon dioxide which is generated in anaerobic process solubilizes and forms carbonic acid, which consumes alkalinity [16]. As alkalinity is consumed and there is no further generation in the digester, rise in VFA concentration reduces pH in the reactor. So, pH inhibition due to insufficient alkalinity is most likely the main cause for inhibition in the glycol degradation process. So, pH control by maintaining sufficient alkalinity seems to be indispensable for the anaerobic digestion of glycol. Some other factors also might have triggered the failure of the process. After some days of operation, foul odour was smelled after withdrawing sludge from the reactors indicating possible development of ammonia, hydrogen sulphide etc. which are toxic compounds. However, as glycol solution does not contain protein, it is unlikely that ammonia was produced from glycol solution. Ammonia, H_2S or other unknown toxic gases might have been produced from other co-substrates.

It might be important to analyze the qualitative and quantitative gas production to determine the composition of biogas. Moreover, no analysis was done for nutrients and trace minerals availability in the reactors which would suggest if these compounds were present or not for the growth of microorganisms.

Anaerobic inocula, waste composition, experimental methods and conditions etc. play important role in an anaerobic process [22]. The results may change with the modification of these parameters. The substrate composition is also very significant factor for the anaerobic process. Change in substrate compositions in experiment 3 yielded more gas production than the experiment 2. Moreover, blending ratio between the substrate and co-substrate plays an important role during the co-digestion. In both experiments, the ratio for the glycol solution and other solution was 1:1. It seems that the performance might have been better if higher fraction of other substrate solution was used than the glycol solution to primarily counteract the alkalinity drop. There were some other factors to be taken into accounts during the experimental setup and operation methods. During the anaerobic operation for semi

continuous reactors, flexible silicone tubes were not to be used since oxygen permeates through silicone tubing [41]. Butyl rubber tubes could have been used instead. Although anaerobic cultures adequately cope with very slight addition of oxygen through the feed system, substrate solution or acid, base etc. should have been prepared and maintained in oxygen deficient conditions [41]. The samples preserved for VFA analysis using Ion chromatography were frozen and stored in glass vials. However, some of the glass vials were broken due to the formation of ice whose volume is larger than water. This caused the loss of some samples. Such samples were either to be stored in plastic vials or some space was to be allocated for volume expansion.

UASB reactor might be used for anaerobic conversion of the glycol wastes based on the discussions present in the section 2.5.

5. CONCLUSIONS

Based upon the facts and figures obtained from this study, it can be concluded that it is feasible to convert glycol rich industrial wastewater into biogas in the mesophilic conditions. Anaerobic treatment may represent an alternative to the treatment of glycol wastes in aerobic conditions as a standalone technology or by coupling with other technologies. However, due to lack of alkalinity production during fermentation and potential nutrient limitation, co-digestion with a complex substrate/sludge seems to be required for long term stable anaerobic digester performance. Alternatively, if digested solely, addition of buffering alkalinity along with micronutrients and macronutrients is essential.

pH inhibition due to inadequate alkalinity in the reactor is most likely the prominent cause for failure of the process. With necessary improvements in the overall design, selection of suitable co-substrate and best blending ratio for co-digestion and frequent monitoring and controlling important parameters such as temperature, pH, alkalinity, nutrients, etc. the reactor performance may be enhanced.

Anaerobic treatment of industrial wastewater put forward significant recovery of renewable energy in terms of methane along with waste management opportunity. The study results have shown bright prospects to potential stakeholders who are interested in anaerobic technology.

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Appendix

Table A1: Results from experiment 1

Day	Date	Gas level	Cumulative gas production	Temp	pH	TS	TVS	COD-total	COD filtered
		(ml/day)	(ml)	°C		(g/l)	(g/l)	(g/l)	(g/l)
0	19th of Jan	0	0						
1	20th of Jan	0	0	23					
2	21st of Jan	810	810	30					
3	22nd of Jan	760	1570	27					
4	23rd of Jan	970	2540	27					
5	24th of Jan	1300	3840	32		23.65	12.75		
6	25th of Jan	990	4830	34					
7	26th of Jan	410	5240	34	7.8	22.83	11.22		
8	27th of Jan	620	5860	35	7.8				
9	28th of Jan	550	6410	38.5	7.81	22.71	10.86		
10	29th of Jan	350	6760	35.5	7.95				
11	30th of Jan	510	7270	35.3	7.68				
12	31st of Jan	270	7540	32.9	7.67	21.3	9.5		
13	1st of Feb	360	7900	35.7	7.68				
14	2nd of Feb	350	8250	35.6	7.29	15.26	7.4		
15	3rd of Feb	320	8570	35.5	7.45				
16	4th of Feb	340	8910	36	7.41	15.3	7.5		
17	5th of Feb	440	9350	36.7	7.4				
18	6th of Feb	340	9690	34.5	7.53	13.03	6.3		
19	7th of Feb	300	9990	36.6	7.23				
20	8th of Feb	290	10280	32.7	7.14	12.32	6.18		
21	9th of Feb	320	10600	36.3	7.08				
22	10th of Feb	320	10920	37	7.05	11.82	5.9		
23	11th of Feb	280	11200	35.8	7.01				
24	12th of Feb	280	11480	36.9	7.01	11.46	5.6		
25	13th of Feb	360	11840	40.7	7.02				
26	14th of Feb	200	12040	35.9	7.23	10.99	5.54		
27	15th of Feb	270	12310	34.3	7.12				
28	16th of Feb	280	12590	35.5	7.06	10.79	5.44		
29	17th of Feb	200	12790	34.8	7.23			29.34	24.25
30	18th of Feb	170	12960	35	7.14	10.12	5.2		
31	19th of Feb	170	13130	33	7.12				
32	20th of Feb	260	13390	36.1	7.03	9.69	5.03	27.91	23.83
33	21st of Feb	140	13530	35.2	7.03			27.43	22.77
34	22nd of Feb	210	13740	36.4	7.55	9.36	4.41		
35	23rd of Feb	230	13970	36.9	7.35			27.47	24.08
36	24th of Feb	150	14120	37.1	7.56	9.2	4.57	28.44	25.22
37	25th of Feb	150	14270	37.1					
38	26th of Feb	130	14400	36.9		9.18	4.51		
39	27th of Feb	280	14680	37.8	7.48	9.4	4.95	32.51	27.82

Table A2: Results from reactor A (Yeast extract solution) during experiment 2

Day	Date	Gas level	Cumulative gas	Temp	pH	TS	TVS	COD-total	COD-filtered
		(ml/d)	ml	°C		(g/l)	(g/l)	(g/l)	(g/l)
0	2nd of Mar			37.8	7.12	34.64	18.57		
1	3rd of Mar	1100	1100	33.3	7.23				
2	4th of Mar	1450	2550	33.6	7.28	19.07	10.24		
3	5th of Mar	1380	3930	33.8	7.48			10.1	4.29
4	6th of Mar	1400	5330	34.7	7.51	29.36	14.91		
5	7th of Mar	1240	6570	34.5	7.56			27.75	6.88
6	8th of Mar	940	7510	34.2	7.57	25.02	12.64		
7	9th of mar	1070	8580	35.1	7.59			24.32	6.11
8	10th of Mar	970	9550	35.5	7.6	22.02	11.18		
9	11th of Mar	840	10390	35.5	7.58	20.43	10.8		
10	12th of Mar	670	11060	35.6	7.59			20.58	7.84
11	13th of Mar	640	11700	35.4	7.52	18.07	9.2		
12	14th of Mar	740	12440	35.8	7.46			20.91	13.43
13	15th of Mar	710	13150	35.2	7.52	15.88	8		
14	16th of Mar	640	13790	35.8	7.49			20.51	12.59
15	17th of Mar	660	14450	35.8	7.57				
16	18th of Mar	580	15030	35.9	7.44	14.43	7.52		
17	19th of Mar	580	15610	35.5	7.44			17.7	10.1
18	20th of Mar	570	16180	35.8	7.39	13.41	6.47		
19	21st of Mar	530	16710	35.5	7.39			19.55	12.78
20	22nd of Mar	530	17240	35.8	7.38	12.62	6.05		
21	23rd of Mar	500	17740	35.5	7.37			17.43	11.8
22	24th of Mar	450	18190	35.5	7.4				
23	25th of Mar	430	18620	35.8	7.36	11.79	5.91		
24	26th of Mar	510	19130	36.1	7.26			16.01	12.17
25	27th of Mar	420	19550	35.7	7.33	11.2	5.54		
26	28th of Mar	400	19950	35.9	7.35			22.45	17.36
27	29th of Mar	350	20300	35.8	7.28	10.85	5.48		
28	30th of Mar	330	20630	35.7	7.27			18.87	14.79
29	31st of Mar	310	20940	35.7	7.23				
30	1st of Apr	300	21240	35.7	7.21	10.31	5.31		
31	2nd of Apr	270	21510	35.6	7.27			23.67	20.07
32	3rd of Apr	290	21800	35.6	7.16	10.07	5.25		
33	4th of Apr	240	22040	35.8	7.15			24.13	20.89
34	5th of Apr	250	22290	35.7	7.15	9.87	5.08		
35	6th of Apr	240	22530	35.6	7.14				
36	7th of Apr	280	22810	35.8	7.13				
37	8th of Apr	70	22880	35.9	7.16	9.54	4.84		
38	9th of Apr	230	23110	35.8	7.15				
39	10th of Apr	210	23320	36	7.15	9.42	4.75		
40	11th of Apr	140	23460	35.6	7.16	9.11	4.38	26.32	23.5
41	12th of Apr	230	23690	36.2	7.16	9.12	4.58	26.97	23.49

Table A3: VFA concentrations in control reactor A during experiment 2

Date	Lactic acid	Propionic acid	Formic acid	Acetic acid	Butyric acid	Valeric acid	Total VFA
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
7th of Mar	0.0	15.1	0.8	139.8	3.2	3.1	161.9
9th of Mar	0.8	10.7	1.0	1088.2	4.3	2.4	1107.5
12th of Mar	1.1	6.3	4.9	2372.9	12.2	12.0	2409.4
14th of Mar	0.0	41.1	40.2	3298.5	67.7	66.3	3513.8
16th of Mar	0.2	35.1	4.2	3882.9	18.8	38.6	3979.8
18th of Mar	0.0	697.9	131.2	4780.4	400.2	115.9	6125.6
19th of Mar	0.0	548.3	127.9	4667.0	365.3	129.0	5837.4
21st of Mar	0.0	842.5	133.5	5035.7	570.3	241.9	6823.8
23rd of Mar	0.0	826.1	166.9	4778.2	739.9	296.2	6807.3
26th of Mar	0.0	1044.1	133.7	4956.0	768.4	311.7	7213.9
27th of Mar	0.0	1137.3	179.6	5432.9	1102.7	362.0	8214.4
28th of Mar	0.0	1447.8	197.2	5737.8	1237.4	390.9	9011.1
29th of Mar	0.0	1351.2	182.9	5172.8	1221.6	383.3	8311.7
30th of Mar	0.0	1398.1	185.6	5289.7	1250.3	391.5	8515.3
31st of Mar	0.0	1581.2	204.9	5755.7	1469.8	430.1	9441.6
1st of Apr	0.0	1485.7	198.4	5589.6	1514.9	436.5	9225.1
2nd of Apr	0.0	1948.0	239.2	6132.1	1930.0	487.5	10736.8
3rd of Apr	0.0	1685.1	189.3	5652.1	1619.5	378.7	9524.6
4th of Apr	0.0	1877.9	217.2	5972.5	1984.1	517.7	10569.3
5th of Apr	0.0	1669.2	155.6	5404.3	1524.4	572.0	9325.5
6th of Apr	0.0	1665.4	145.3	5440.7	1702.8	488.8	9443.1
7th of Apr	0.0	1792.3	151.0	5708.3	1939.9	556.7	10148.3
8th of Apr	0.0	1969.6	184.5	5703.2	2135.6	550.3	10543.1
9th of Apr	0.0	1872.7	149.0	5376.6	1919.6	605.9	9923.7
10th of Apr	0.0	1993.6	149.6	5636.3	2103.9	666.0	10549.3
12th of Apr	0.0	2204.9	242.2	4882.3	3129.8	671.1	11130.3

Table A4: Results from reactor B (Yeast extract + glycol solution) during experiment 2

Day	Date	Gas level	Cumulative gas	Methane potential	Temp	pH	TS	TVS	COD-total	COD-filtere
		ml/d	ml	(ml/d)	°C		(g/l)	(g/l)	(g/l)	(g/l)
0	2nd of Mar	0	0		37.8	7.12	34.6	18.5		
1	3rd of Mar	1090	1090		33.3	7.3				
2	4th of Mar	1390	2480		34.2	7.3	13.4	7.36		
3	5th of Mar	1270	3750		34	7.34			15.6	3.85
4	6th of Mar	1290	5040		34.7	7.5	27.3	14.1		
5	7th of Mar	1180	6220		34.5	7.54			26.8	4.87
6	8th of Mar	980	7200		34.1	7.59	23.4	12.0		
7	9th of Mar	1050	8250		35.1	7.58			20.2	5.01
8	10th of Mar	970	9220		35.5	7.63	21.3	11.0		
9	11th of Mar	930	10150		35.5	7.6	20.0	10.2		
10	12th of Mar	690	10840		35.6	7.61			19.0	7.3
11	13th of Mar	690	11530		35.3	7.6	17.9	8.87		
12	14th of Mar	770	12300		35.7	7.57			19.0	11.22
13	15th of Mar	720	13020		35.6	7.58	16.2	8.02		
14	**16-Mar**	**680**	**13700**		**35.7**	**7.5**			**18.5**	**10**
15	17th of Mar	600	14300	270	35.7	7.49				
16	18th of Mar	600	14900	310	35.7	7.35	13.6	7.06		
17	19th of Mar	580	15480	290	35.6	7.26			13.8	6.41
18	20th of Mar	520	16000	235	35.7	7.17	11.5	5.85		
19	21st of Mar	500	16500	235	35.5	7.07			16.4	9.33
20	22- Mar	490	16990	225	35.4	6.96	10.1	5.01		
21	23rd of Mar	440	17430	190	35.6	6.83			14.6	9.6
22	24th of Mar	380	17810	155	35.5	6.71				
23	25th of Mar	300	18110	85	36.1	6.45	8.59	4.25		
24	26th of Mar	200	18310	-55	35.6	6.12			15.4	11.04
25	27th of Mar	90	18400	-120	35.7	5.95	7.92	4.08		
26	28th of Mar	90	18490	-110	35.5	5.85			21.3	19.23
27	29th of Mar	50	18540	-125	35.8	5.78	7.69	4.16		
28	30th of Mar	70	18610	-95	35.5	5.8			19.9	17.1
29	31st of Mar	60	18670	-95	35.5	5.76				
30	1st of Apr	60	18730	-90	35.5	5.69	7.49	4.36		
31	2nd of Apr	40	18770	-95	35.6	5.61			26.6	25.76
32	3rd of Apr	40	18810	-105	35.6	5.69	7.84	4.95		
33	4th of Apr	40	18850	-80	35.5	5.69			26.9	25.63
34	5th of Apr	30	18880	-95	35.7	5.72	8.12	5.39		
35	6th of Apr	40	18920	-80	35.6	5.71				
36	7th of Apr	50	18970	-90	35.7	5.65				
37	8th of Apr	30	19000	-5	35.7	5.48	8.47	5.68		
38	9th of Apr	60	19060	-55	35.5	5.42				
39	10th of Apr	50	19110	-55	35.7	5.3	9.28	6.65		
40	11th of Apr	0	19110	-70	35.6	5.21	9.41	6.55	28.5	27.67
41	12th of Apr	0	19110	-115	35.5	5.17	9.64	7.05	29.1	28.03

From 16th of March, glycol solution was codigested with yeast solution in the reactor

Table A5: VFA concentrations in test reactor B during experiment 2

Date	Lactic acid	Propionic acid	Formic acid	Acetic acid	Butyric acid	Valeric acid	Total VFA
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
5th of Mar	238.04	11.31	0.46	309.02	0.73	6.73	566.29
7th of Mar	214.78	11.74	1.34	278.82	6.29	10.45	523.42
9th of Mar	0.00	28.71	3.80	484.94	21.05	3.48	541.98
14th of Mar	0.00	350.77	39.85	2437.12	187.79	71.04	3086.58
17th of Mar	0.00	712.86	82.53	3745.40	388.90	343.81	5273.49
18th of Mar	8.92	859.21	431.39	4787.16	204.73	302.72	6594.14
19th of Mar	0.00	950.11	396.11	5070.22	260.39	336.85	7013.68
20th of Mar	0.00	1100.17	75.13	4960.60	303.23	334.29	6773.42
21st of Mar	0.00	1104.30	74.98	4645.47	401.97	339.86	6566.58
23rd of Mar	0.00	1592.51	106.36	5985.40	814.09	490.99	8989.35
26th of Mar	0.00	1672.76	111.13	6727.13	1290.84	505.62	10307.48
27th of Mar	0.00	1329.81	88.23	6543.66	1645.37	521.17	10128.25
28th of Mar	0.00	1246.74	91.82	5797.58	2596.68	632.83	10365.66
29th of Mar	11.47	1026.66	86.79	4809.81	2648.71	594.30	9177.74
30th of Mar	27.09	1034.06	98.76	4701.94	2969.60	771.20	9602.64
31st of Mar	39.75	995.78	86.64	5141.27	3117.71	726.16	10107.31
1st of Apr	45.17	827.46	80.73	4747.96	3104.88	699.46	9505.67
3rd of Apr	46.60	591.73	60.81	4088.55	2585.55	516.30	7889.53
4th of Apr	59.75	749.61	65.02	4924.40	3026.23	631.21	9456.22
5th of Apr	60.23	664.36	51.72	4382.58	2638.06	510.96	8307.90
6th of Apr	64.77	574.33	44.42	4038.60	2443.73	443.17	7609.02
7th of Apr	70.03	801.80	57.72	4454.76	2363.87	454.31	8202.50
9th of Apr	86.99	865.52	46.07	4804.84	2079.76	464.76	8347.95
12th of Apr	76.00	544.97	19.82	4138.29	1350.61	240.51	6370.21

Table A6: Results from Reactor A (Yeast extract + Starch + Nutrient Broth solution) during experiment 3

Day	Date	Gas level	Temp	pH	TS	TVS	Alkalinity	SCFA	COD total	COD filtered
		(ml/d)			(g/l)	(g/l)	mg/lCaCO3	mg/lHac	(g/l)	(g/l)
0	Apr-20	0	34.1	7.27						
1	Apr-21	1650	35.9	7.41						
2	Apr-22	1540	35.4	7.53						
3	Apr-23	1240	35.6	7.57						
4	Apr-24	1580	35.5	7.6						
5	Apr-25	1400	35.5	7.61						
6	Apr-26	1450	35.4	7.64	21.69	12.19	3875.1	0		
7	Apr-27	1290	35.3	7.66						
8	Apr-28	1390	35.8	7.67			3426.7	0		
9	Apr-29	1460	35.3	7.69	18.52	9.89				
10	Apr-30	1330	35.6	7.63			3061.1	0		
11	May-1	1560	35.7	7.7	16.77	9.02				
12	May-2	1450	35.6	7.65			1687.3	0		
13	May-	580	21.6	7.51	15.95	9.39				
14	May-4	1660								
15	May-5	1500	35.3	7.61			1093.6	0		
16	May-6	1590	35.4	7.67	13.26	7.42				
17	May-7	1240	35.7	7.61			1510.2	0		
18	May-8	1380	35.5	7.61	12.59	7.49				
19	May-9	1280	35.8	7.59			1126.9	0		
20	May 10	1240	35.9	7.61	11.87	7.12				
21	May 11	1220	35.8	7.59			1749.4	0		
22	May12	1090	35.7	7.53						
23	May13	1010	35.8	7.54	10.76	6.79				
24	May14	1020	36	7.5			1268.9	192.4		
25	May15	980	35.9	7.45	10.47	6.77				
26	May16	900	36	7.37			1541.1	1416		
27	May17	800	35.7	7.28	9.93	6.46				
28	May18	630	35.8	7.22			822.6	2331		
29	May19	620	35.8	7.15						
30	May20	570	35.8	6.98	9.94	6.4				
31	May21	490	35.9	6.86			1058.5	4011		
32	May23	470	35.9	6.77	9.62	6.23				
33	May24	420	36.2	6.58			480	5180	25.3	23.7

The temperature was increased to previous level on 3rd of May after measurement.

Table A7: Results from Reactor B (Yeast extract + Starch + Nutrient Broth + glycol solution) during experiment 3

Date	Gas level	CH4 from glycol	Tem	pH	TS	TVS	Alkalinity	SCFA	COD-total	COD-filter	Na$_2$CO$_3$ dose
	ml/d	ml/d			g/l	g/l	mg/l CaCO$_3$	mg/l Hac	g/l	g/l	g
Apr-20	0		34.1	7.2							
Apr-21	138		35.4	7.3							
Apr-22	207		35.2	7.3							
Apr-23	202		35.5	7.5							
Apr-24	179		35.6	7.6							
Apr-25	179		35.3	7.4							
Apr-26	160		35	7.6	22.9	13	2006	0			
Apr-27	142		35.5	7.6							
Apr-28	150		35.3	7.5			3049	0			
Apr-29	156		35.2	7.7	20.2	11					
Apr-30	134		35.8	7.6			628	0			
May-1	145		35.7	7.6	18	9.9					
May-2	**152**		**35.5**	**7.7**			**2666**	**0**			
May-3	490	200	21.6	7.4	15.9	9.2					
May-4	148	650									
May-5	119	440	35.2	7.4			905	309			
May-6	108	285	35.3	7.4	12.2	6.8					
May-7	940	320	35.6	7.2			536	2035			
May-8	770	80	35.8	7.1	11.1	6.5					
May-9	630	-10	35.9	6.9			676	2940			
May 10	590	-30	35.9	6.5	9.93	5.8					
May 11	400	-210	35.8	6.1			497	4269			
May12	200	-345	35.6	5.9							1.6
May13	430	-75	35.8	6.5	11.4	6.9					
May14	270	-240	35.6	6.2			271	4706			1.6
May15	250	-240	35.8	6.8	13.6	8.0					1.6
May16	270	-180	35.8	6.9			774	3452			1.6
May17	200	-200	35.7	7.1	16.1	9.1					
May18	400	85	35.8	7			1077	3847			
May19	280	-30	35.9	6.8							
May20	400	115	35.7	6.5	13.6	8.1					1.6
May21	270	25	35.8	6.9			577	4827			
May23	250	15	35.7	6.7	15.2	8.7					
May24	220	10	36.2	6.5			390	5451	28.7	26.85	

*Glycol was co-digested along with other solution from 2nd of May and glycol solution containing 3.8gm/l of Na$_2$CO$_3$ was loaded from 13th of May.