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Abstract

Glycols are usually used in the offshore gas industry as hydrate inhibitor in gas pipelines laid deep under the sea. Glycols, in its use, are contaminated by dissolved salts from formation water together with scaling and corrosion products from the pipeline. This results to generation of wastewater containing glycols. Anaerobic treatment may represent an alternative to the aerobic treatment of glycol wastewater. Laboratory-scale studies were carried out to investigate the treatability of glycol wastewater using anaerobic digester reactor. The substrate is a high strength wastewater of about 15000 mg/l COD consisting mostly of monoethylene glycols (MEG) with a pH of 5.4. The microbes used for this study were those from the sewage sludge of IVAR sewage plant and process conditions namely temperature, pH, nutrient requirements, and organic loading rate were optimized to ensure efficient biodegradation. The wastewater treatability and the reactor performance were examined during the study based on the COD removal. Also, the effects of parameters such as pH, SRT, organic loading rate and alkalinity on the COD removal and gas production were monitored. This study was carried out in two experiments. The first experiment was a continuation of an existing working anaerobic reactor while the second was a new system set up. The composition of the nutrients added during the 2nd experiment differs slightly from that of the 1st experiment. From the results in both experiments, there was more gas production in experiment 2 than in experiment. At maximum gas production, COD mass balances of 34.4 % and 83.67 % were obtained in experiment 1 and experiment 2 respectively. The failure of the system may be attributed to the possible presence of toxic substances such as hydrogen sulphide, lack of nutrients and high organic loading rate.

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Abbreviations

ABR	Anaerobic Baffled Reactor
ACP	Anaerobic Contact Process
AD	Anaerobic Digestion
AF	Anaerobic Filters
AMR	Anaerobic membrane reactor
COD	Chemical Oxygen Demand
DEG	Diethylene glycol
EGSB	Expanded granular sludge bed
FB	Fluidized bed
HAc	Acetic acid
HRT	Hydraulic retention time
LCFA	Long chain fatty acids
MEG	Monoethylene glycol
MLSS	Mixed Liquor Suspended Solids
SCFA	Short Chain Fatty Acids
SRT	Solid retention time
TEG	Triethylene glycol
TREG	Tetraethylene glycol
TSS	Total Suspended Solids
UASB	Upflow anaerobic sludge bed
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids

1. INTRODUCTION

In the offshore gas industry, hydrate formation is a well known problem and is often found in gas pipelines deep under the sea which are exposed to extreme low temperature condition. This is highly undesirable as these hydrates might agglomerate and plug the flowline and cause flow assurance failure and damage valves and instrumentation [1]. Also this can cause salt water and corrosion attack on the transport pipelines.

Glycols are usually introduced, in long multiphase pipelines that convey natural gas from remote gas fields to an onshore processing facility, to inhibit the formation of hydrates. This hydration inhibition may be based on removal of water before transport or by reducing the temperature at which hydrates are formed. Glycols typically used in the industry include ethylene glycol (MEG), diethylene glycol (DEG), triethylene glycol (TEG), and tetraethylene glycol (TREG). MEG is the most commonly used glycol in industry.

In the use ethylene glycol, they are contaminated by dissolved salts from formation water together with scaling and corrosion products from the pipeline. This results to the quality deterioration of ethylene glycol and will need to either be regenerated and recovered or replaced. In either of these cases, wastewater is produced which contains large or small amount of glycols and needs to be treated and disposed properly.

Glycol wastewaters are commonly treated by aerobic biological treatment system otherwise known as activated sludge system. A number of studies document the excellent aerobic treatability of ethylene glycol in activated sludge and natural systems [2-4]. This method of treatment is efficient, however it has a high operational and energy cost due to oxygen supply by aeration and large sludge production which also need to be disposed. EG wastewater has low nutrients contents and for the optimum performance of the activated sludge system, there is high biomass yield which also results to high nutrient requirement. There are other forms of treatment methods for glycol wastewater which may include chemical, physical, thermal and other biological methods.

An alternative biological method, which is cost effective, for this type of wastewater is anaerobic digestion system. Anaerobic digestion is one of the oldest means of wastewater treatment and until modern times, the primary application has been the stabilization of primary sewage sludge which then results to substantial solids reduction. This is a biological method such as the activated sludge system but is carried out in the absence of oxygen; hence it has a low energy requirement compared to the activated sludge system. Energy is also generated in form of methane gas as one of the final products in anaerobic treatment of glycol wastewaters. Other advantages include very low sludge production for further handling, low biomass yield which results in low nutrient requirements. The high methane generation, low nutrient requirements and low sludge production make anaerobic treatment an economic viable treatment method. However, anaerobic digestion consists of more complex processes involving different kinds of microbes and is also very sensitive to various factors compared to the aerobic system. A few problems have historically hampered the implementation of anaerobic systems: e.g., slow growth rates resulting in long start-up times, long retention times and poor solid-liquid separation [5]. Due to this complex and sensitive nature of the method, careful attention and monitoring are required for optimal performance. Another common problem encountered in anaerobic treatment of industrial wastewaters is biomass washout due to too low retention time. This problem, for example, can be addressed by incorporating the use of membranes with the anaerobic digester for the purpose of biomass retention [5].

The objective of this thesis is to investigate, in laboratory scale, the effectiveness of anaerobic treatment of high glycol contaminated wastewater for generation of methane gas. An efficient treatment can be related primarily to the COD removal. Glycols in study are mostly monoethylene glycol (MEG) type. This study will also tend to explain in details the various processes involved in anaerobic digestion and the important factors that influence the process. Also examined in the study, were the organic loading rates and effluent qualities achieved, nutrient requirements and operational mixed liquor concentrations. Emphasis will also be made on identifying the critical factors affecting performance of anaerobic reactor so that by maintaining optimal operating conditions, efficiency can be well improved.

2. LITERATURE REVIEW

2.1 Anaerobic Digestion Overview

Anaerobic digestion involves a complex consortium of micro-organisms and this multistep nature of anaerobic operation is depicted in Figure 2.1. Three basic bacteria group (acidogens, acetogens, and methanogens) are recognized in this process, and it is the cumulative actions of these groups of bacteria that ensure process continuity and stability. The process works in such a way that the products from the activity of a particular bacteria group serves as substrate for another bacteria group and in so doing results in production of methane from organic wastes. The actions of these bacteria groups and the biochemical processes could be divided into four basic processes:

1. Hydrolysis: Enzymatic breakdown of complex organics to monomers that can be utilized by microorganisms.
2. Acidogenesis: The end products of hydrolysis are converted to short chain fatty acids, alcohols, and hydrogen in the process also called fermentation.
3. Acetogenesis: The fermentation products not in form of acetic acid and hydrogen are then converted to acetic acid and hydrogen.
4. Methanogenesis: Bacteria conversion of acetic acid and hydrogen to methane.

These four basic processes can also be subdivided into other categories as will be detailed in the subsequent sections. This will also include the stoichiometry and kinetics involved in each stage of anaerobic process.

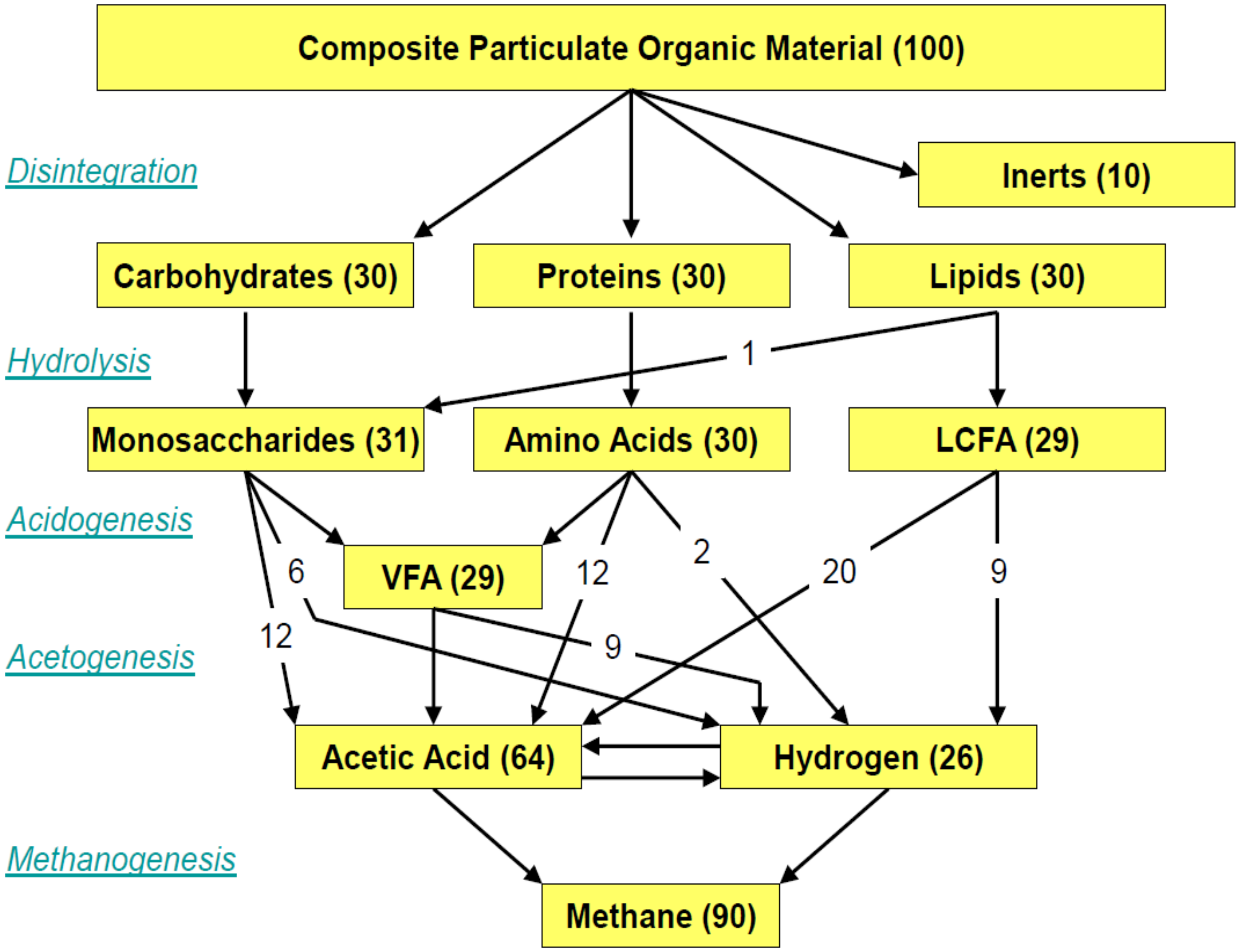

Composite Particulate Organic Material (100)
Disintegration
Inerts (10)
Carbohydrates (30)
Proteins (30)
Lipids (30)
Hydrolysis
1
Monosaccharides (31)
Amino Acids (30)
LCFA (29)
Acidogenesis
6
VFA (29)
12
2
20
9
Acetogenesis
12
9
Acetic Acid (64)
Hydrogen (26)
Methanogenesis
Methane (90)

Figure 2.1 Reactions involved in anaerobic decomposition of organic matter [6]

2.1.1 Disintegration

Disintegration is the first step of degradation for complex organic composite materials and particulates (i.e. colloidal (10 – 1000 nm) and larger particles greater than 1000 nm). These materials are first disintegrated by physical shearing and dissolution. Some extracellular enzymes may also be involved in this process. The end products of disintegration include dissolved polymers of polysaccharides, proteins and lipids. Disintegration is, by default, set to a first order process mainly due to empirical studies and also due to lack of detailed information [6].

$$r_{dis} = k_{dis} \cdot X_{composite} \qquad (2\text{-}1)$$

$k_{dis} = 0.4\ d^{-1}$ for mesophilic bacteria and $1.0\ d^{-1}$ for thermophilic bacteria [6].

2.1.2 Hydrolysis

Large polymeric materials such as carbohydrates, proteins and lipids cannot be directly metabolized by anaerobic bacteria and hence must be reduced to simpler products of a size enough to allow their passage across the cell membrane of the micro-organisms. This is done in the process of hydrolysis and carried out by extracellular enzymes secreted by the fermentative or acidogenic microorganisms that feed on the end products of hydrolysis. Extracellular enzymes are of two types: hydrolytic and lytic extracellular enzymes. Although the process is often referred as hydrolysis, lytic enzymes also depolymerize (in addition to hydrolases) [6, 7]. The main group consists of proteases (acting on proteins), cellulases, amylases, glucanases (all acting on polysaccharides), and lipases (acting on fats and oil; lipids). In this process, carbohydrates, proteins and lipids are converted to monosaccharides, amino acids and long chain fatty acids respectively. These products of hydrolysis serve as substrates for the acidogenic organisms in the next stage. There is an expenditure of energy in hydrolysis reactions. The energy for hydrolysis and synthesis is obtained from the catabolism of the smaller molecules resulting from hydrolysis.

Stoichiometrically, polymers are hydrolyzed to dissolved readily biodegradable substrates of their monomeric composition; however, some lipopolysaccharides are converted to monosaccharides and low chain fatty acids [6].

Hydrolysis of particulates is modeled as a first order reaction with respect to hydrolysable compounds:

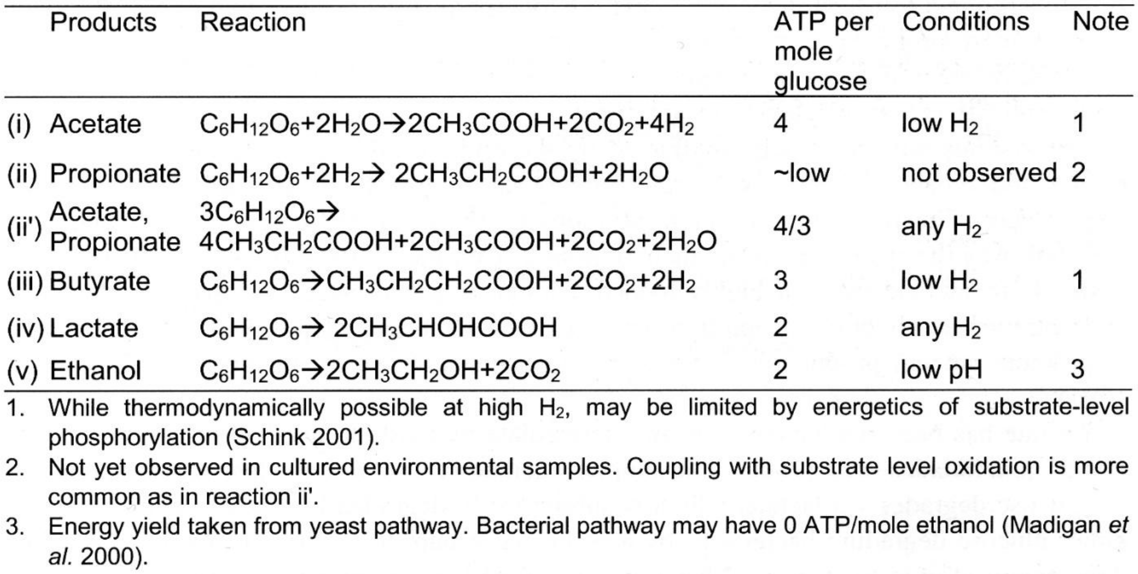
$$r_{hydr} = k_h \cdot X_{polymers} \tag{2.2}$$

$k_h = 0.3 - 0.7\ d^{-1}$ [6]

2.1.3 Acidogenesis/Fermentation

Acidogenesis (or fermentation) is the anaerobic conversion of the hydrolysis products (sugars and amino acids) to volatile fatty acids (VFAs). Fermentation is carried out by acidogens (same organisms that perform hydrolysis reactions) and is relatively fast. The growth rates of acidogenic bacteria is comparable to aerobic rates with $\mu_m \sim 2 - 7\ d^{-1}$. The growth is described according to the Monod equation. The end products from acidogenesis are mainly short chain fatty acids (SCFA) such as acetic, propionic and butyric acids. Alcohols such as ethanol, propanol and butanol may also be produced in addition to lactic acid and formic acid. Due to the lack of 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 fraction of energy associated with the excreted fermentation products cause the remaining energy for growth to be limited and thus the growth yield is low: Y~ 0.1 - 0.2 gVSS/gCOD [8, 9].

Table 2.1 Stoichiometries of product formation using Glucose as model substrate [6]



	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 (Schink 2001).
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 (Madigan *et al.* 2000).

Only certain compounds are fermentable, and a requirement for most fermentations is that an energy-rich organic intermediate be formed that can yield ATP by substrate-level phosphorylation [6].

Lipids are converted by lipase activity to glycerol and fatty acids. The glycerol backbone is fermented to acetate through acidogenesis using H^+ as electron acceptor. Fatty acids are oxidized to Acetyl-CoA by β-oxidation, and electrons are transferred to protons (electron acceptor) to form H_2. Acetyl-CoA is combined with CO_2 to acetate under substrate level phosphorylation.

Soluble substrates (C_s) generated in hydrolysis are converted into various fermentation products which includes volatile fatty acids, alcohols, H_2 etc. The composition of fermentation products depends on various factors such as substrate composition, environmental factors (pH, temperature, etc) and operational factors (loading rate, retention time, etc) in the reactor. The growth yield in acidogenesis is low (Y_{Ac} = 0.1 - 0.2 gVSS/gCOD) 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 with Monod:

$$\mu = \frac{\mu_{maxAc} \cdot C_s}{K_s + C_s} \qquad \frac{dX_{Ac}}{dt} = \mu \cdot X_{Ac} = \frac{\mu_{maxAc} \cdot C_s}{K_s + C_s} \cdot X_{Ac} \tag{2-3}$$

The substrate removal rate is expressed as:

$$\frac{dC_s}{dt} = \frac{\mu \cdot X_{Ac}}{Y_{Ac}} = \frac{\mu_{maxAc} \cdot C_s}{K_s + C_s} \cdot \frac{X_{Ac}}{Y_{Ac}} \tag{2-4}$$

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

$$\frac{dC_A}{dt} = (1 - Y_{Ac})\frac{dC_S}{dt} = (1 - Y_{Ac}) \cdot \frac{\mu \cdot X_{Ac}}{Y_{Ac}} \tag{2-5}$$

Among the fermentation products, only acetic acid and hydrogen are directly utilized by the methanogenic bacteria. The other products must be converted to acetic and hydrogen for them to be utilized by the methanogenic bacteria.

2.1.4 Acetogenesis

Only a part of acetic acid is formed directly during fermentation. Most of it is formed by syntrophic reactions, and until now only a few cultures have been isolated which are capable of this [10, 11]. Other fermentation products must be converted to acetic acid for its utilization by the methanogenic bacteria. Organisms responsible for this conversion are known as the acetogens and utilize, as substrates, the products from acidogenesis while they form acetic acid and hydrogen as the end products. Acetogenesis is also required for the low chain fatty acids being formed during lipase activity on lipids and glycerols. The products (H_2 and formic acid) must be kept at a low concentration in order to favor thermodynamically their formation reaction ($\Delta G^o < 0$). This low concentration is maintained by the hydrogen utilizing methanogens.

The interaction between generation and consumption of hydrogen is called interspecies hydrogen transfer and is illustrated in Figure 2.2 where $\Delta G'$ is related to different hydrogen concentrations for the anaerobic oxidation of propionate, butyrate, and palmitate [6, 7].

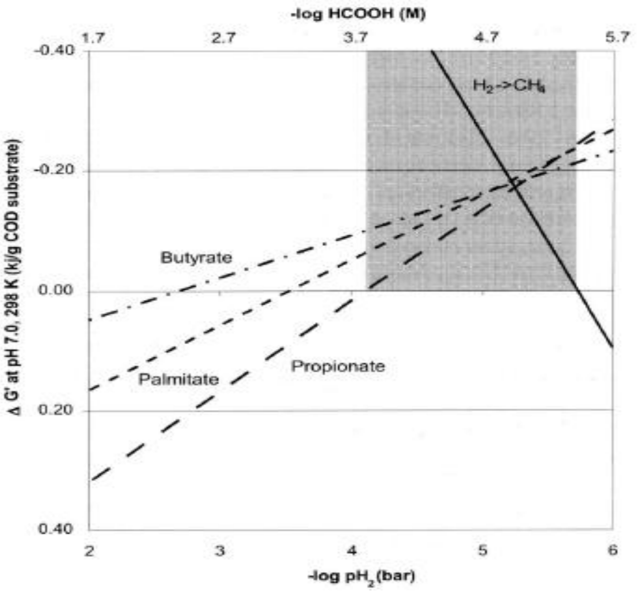


Figure 2.2 Interspecies hydrogen transfer

From Figure 2.2, there is an upper limit set by the acetogens, and a lower limit set by the methanogens of syntrophic thermodynamically transfer of VFAs to methane. The local H_2 concentration must be kept within the so called "hydrogen window", which is in between the partial pressures of 10^{-4} to 10^{-6}, otherwise autotrophic methanogenesis or acetogenesis will be inhibited [6].

Table 2.2 Stoichiometry showing the product formation of the different substrates [12]

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.

Acetic acid and H_2 are used directly by the methanogens while the other fermentation products are converted into acetic acid and H_2 in acetogenesis:

Other products (C_P) → Biomass (X_{Ace}) + Acetic acid (C_{Ac}) + H_2

The growth rate of acetogenic organisms is described with Monod:

$$\mu = \frac{\mu_{maxAce} \cdot C_P}{K_P + C_P} \qquad \frac{dX_{Ace}}{dt} = \mu \cdot X_{Ace} = \frac{\mu_{maxAce} \cdot C_P}{K_P + C_P} \cdot X_{Ace} \tag{2-6}$$

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:

$$\frac{dC_P}{dt} = \frac{\mu \cdot X_{Ace}}{Y_{Ace}} = \frac{\mu_{maxAce} \cdot C_P}{K_P + C_P} \cdot \frac{X_{Ace}}{Y_{Ace}} \tag{2-7}$$

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

$$\frac{dC_{Ac}}{dt} = (1 - Y_{Ace}) \frac{dC_P}{dt} = (1 - Y_{Ace}) \cdot \frac{\mu \cdot X_{Ace}}{Y_{Ace}} \tag{2-8}$$

2.1.5 Methanogenesis

The formation of methane is the ultimate product and last stage of anaerobic digestion. The products of the acetogenesis (i.e., acetic acid, CO_2 and H_2) are utilized as substrates by the methanogenic bacteria to produce methane gas. Although it is also possible that methane-producing bacteria exist which have the ability to use other volatile acids and organic end products from acidogenesis to form methane, none have been isolated yet [13].

This methane formation occurs by two major routes and carried out by two groups of methanogenic bacteria. The primary route is the fermentation of the major product of acetogenesis stage, acetic acid, to methane and CO_2. The methanogenic bacteria that utilize acetic acid as substrate are called acetoclastic methanogens. The overall reaction is:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \Delta G^0 = -31 \text{ kJ mol}^{-1} \tag{2-9}$$

The most common acetoclastic methanogens in reactors treating wastes with high volatile fatty acid content are from the genera *Methanosarcina* and *Methanosaeta*. *Methanosarcina* spp. are coccoid bacteria with doubling times near 1.5 d, and *Methanosaeta* spp. are sheathed rods, sometimes growing as long filaments with doubling times near 4 d [14]. These doubling times occur at optimal conditions for the methane formers. Even though *Methanosaeta* spp. grows more slowly, they are most frequently the dominant genus [13, 14].

In the other route, hydrogenophilic methanogens utilize H_2 as electron donor to reduce carbon dioxide to methane with an overall reaction of:

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^o = -135\ kJ\ mol^{-1}$ [11] (2-10)

Energy generation in methanogens is not driven by substrate level phosphorylation, but reversed electron transport and ATPase [12]. The methane formers are much more fastidious in their environmental requirements than the acid formers. Their rates of metabolism are also lower than the rates of the acid formers and therefore methane production is generally the rate-limiting step in anaerobic digestion [15]. The optimal pH for methane formers 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. The free energies for both acetoclastic and hydrogenophilic methanogens are very low, and these organisms are known to rely on proton or cation motive force energetic through reversed electron flow in the cell membrane [6]. The maximum growth rate of methanogenic bacteria are low, $\mu_{max} \sim 0.3 - 0.5\ d^{-1}$, and long retention is required for methane producing processes [8]. The growth yield is also very low, as the majority of the energy in the substrate is converted into methane gas with typical growth yield of Y ~ 0.05 – 0.1 gVSS/gCOD.

Methanogenesis involves the activity of two groups of methanogens; acetoclastic methanogens using acetic acid as substrate and hydrogenophilic methanogens utilizing H_2 as substrate and the final products are methane gas, carbon dioxide and biomass.

For acetoclastic methanogens, acetic acid is the substrate forming CH_4 and CO_2:

Acetic acid (C_{Ac}) → Biomass (X_{AM}) + CH_4 + CO_2

The growth rate of acetoclastic methanogens (AM) is described with Monod:

$$\mu = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \qquad \frac{dX_{AM}}{dt} = \mu \cdot X_{AM} = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \cdot X_{AM} \tag{2-11}$$

The substrate removal rate of acetoclastic methanogens is expressed as:

$$\frac{dC_{Ac}}{dt} = \frac{\mu \cdot X_{AM}}{Y_{AM}} = \frac{\mu_{maxAM} \cdot C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{AM}}{Y_{AM}} \tag{2-12}$$

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

$$\frac{dC_M}{dt} = (1 - Y_{AM})\frac{dC_{Ac}}{dt} = (1 - Y_{AM}) \cdot \frac{\mu \cdot X_{AM}}{Y_{AM}} \tag{2-13}$$

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

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

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

$$\mu = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \qquad \frac{dX_{HM}}{dt} = \mu \cdot X_{HM} = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \cdot X_{HM} \tag{2-14}$$

The substrate removal rate of hydrogenophilic methanogens is expressed as:

$$\frac{dC_{H_2}}{dt} = \frac{\mu \cdot X_{HM}}{Y_{HM}} = \frac{\mu_{maxHM} \cdot C_{H_2}}{K_{H_2} + C_{H_2}} \cdot \frac{X_{HM}}{Y_{HM}} \tag{2-15}$$

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

$$\frac{dC_M}{dt} = (1 - Y_{AM})\frac{dC_{H_2}}{dt} = (1 - Y_{HM}) \cdot \frac{\mu \cdot X_{HM}}{Y_{HM}} \tag{2-16}$$

2.2 Factors Affecting Performance of Anaerobic Process treatment

The anaerobic treatment process is affected significantly by the operating conditions. As the process involves the formation of volatile acids, it is imperative that the rate of reaction be such that there is no accumulation of acids which will result in the failure of the digester. This in turn, is governed by the loading rate and the influent strength. Temperature and pH are other important factors as the methane producing bacteria are sensitive to these as well [16].

2.2.1 Temperature

As temperature increases, the rate of reaction generally increases. For biological systems, the rate increases are usually not as great as for chemical reactions [15]. Temperature effect is particularly important in anaerobic systems because of the interacting populations. For example, different species of bacteria will respond to changes in temperature in qualitatively similar but quantitatively dissimilar ways [13]. Temperature effect can be grouped under one of the following categories: psychrophylic (0 – 20 °C), mesophilic (20 – 42 °C) and thermophilic (42 – 75 °C). The details of the bacterial processes in all the three temperature ranges are well established though a large section of the reported work deals with mesophilic operation. Changes in temperature are well resisted by anaerobic bacteria, as long as they do not exceed the upper limit as defined by the temperature at which decay rate begins to exceed the growth rate. In mesophilic range, the methanogenic bacterial activity and growth decreases by one half for each drop below 35 °C [16]. Methane has been produced at temperatures down to 10 °C or lower, but for reasonable rates of methane production, temperatures should be maintained above 20°C. Operation in thermophilic range is not generally practical because of the high heating energy requirement and experience at this temperature range has not been satisfactory.

The temperature effect can be expressed as:

$$\mu_{m(20)} = \mu_{m(T)} \cdot \theta^{(T-20)} \tag{2-17}$$

where $\mu_{m(20)}$ = maximum specific growth rate at 20 °C

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

θ = temperature coefficient

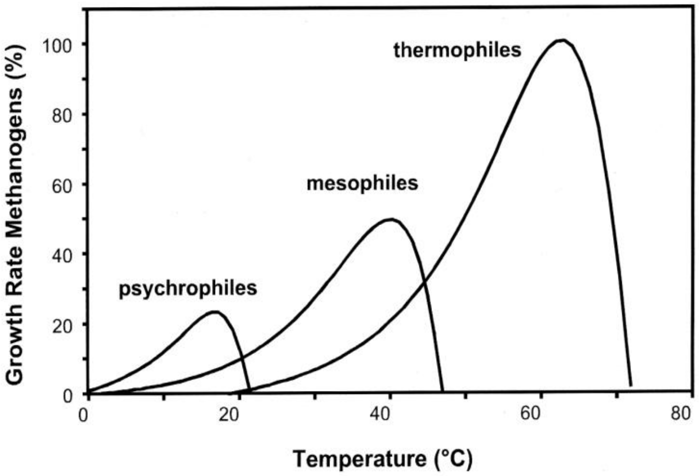


Figure 2.3 Temperature effect on the growth rate of methane forming bacteria

Temperature effect on the hydrolysis and acidogenesis stages of anaerobic process is not very significant as among the mixed population there are always some bacteria which have their optimum within the range concerned. Little information has been reported about the effects of temperature upon the non methanogenic bacteria. It was observed in [13] that there was no significant degradation of the lipid fraction during an operation of a sewage sludge digester at 15°C even at mean cell residence time of 60 days. The performance at 25 and 20°C suggests that the lipid-degrading bacteria were quite sensitive to low temperature and were perhaps lost from the population.

Most anaerobic operations are designed in the mesophilic temperature range. A stable temperature is more conducive to stable operation than any specific temperature [15]. Also, high temperature results to higher specific growth rate which in turn results to lower retention time and smaller volume.

2.2.2 pH

The lower growth rates of the methanogens require that the process be run at pH conditions most favorable to them [15]. Numerous references report that the pH required in anaerobic systems for good performance and stability is in the range of 6.5 – 7.5, although stable operation has been observed outside this range [11, 13, 15].

As far as methanogenic bacteria are concerned, the consensus is that the pH of anaerobic operations should be maintained near 7.0 and that severe problems can result if the pH is allowed to drop below 6.5. The primary effect of pH upon the non-methanogenic population is based on the types of products formed [13]. This changes the substrates available to the acetoclastic and hydrogenophilic methanogenic bacteria, which will in turn influence the rates at which they can operate. It is not yet clear at what pH the best products are formed by the non methanogenic bacteria, but as long as the two populations are grown together, a pH near 7.0 is optimum for the system as a whole.

The system must contain adequate buffering capacity to accommodate the production of volatile fatty acids and carbon dioxide that will dissolve at the operating pressure. Excess alkalinity or ability to control must be present to guard against the accumulation of excess volatile acids. Anaerobic processes can operate over a wide range of volatile acids concentrations (from less than 100 mg/l to over 5000 mg/l) if proper pH control is practiced [15]. A constant pH lends stability to the process. Commonly chemicals used as buffers include lime, sodium carbonate, sodium bicarbonate and sodium hydroxide. Sodium bicarbonate is preferred to others because it gently shifts the equilibrium to the desired value without disturbing the physical and chemical balance of the fragile microbial population [16, 17].

2.2.3 Nutrient Requirements

The chemical composition of anaerobic cells is quite similar to that of aerobic cells ($C_5H_7NO_2$), and consequently the amounts of nitrogen and phosphorus required per unit mass of cells formed are the same. Much of the energy in the original substrate is lost from the liquid as methane, however, so that mass of cells formed per unit mass of COD removed anaerobically is much lower than it is aerobically. Consequently, the amount of nitrogen and phosphorus required per unit mass of COD removed will also be much smaller. When sewage sludge is being digested the possibility of a nutrient deficiency is rare; however it may be necessary to add nutrients to industrial wastes. For a typical activated sludge process, the COD:N:P requirement ratio is 100:5:1 [15] while the required optimum C:N:P ratio for maximum yield of methane has been reported to be 100:2.5:0.5. The phosphorus requirements can be approximated as one-fifth of the nitrogen requirement [16]. There are a number of trace inorganic nutrients required for successful anaerobic treatment especially on industrial wastes. Although these elements are needed in extremely low concentrations, the lack of it has an adverse effect on the microbial growth and anaerobic process performance. Nickel and Cobalt have been shown to promote methanogenesis

[15, 18]. The minimum concentration of macro and micronutrients can be calculated based on the biodegradable COD concentration of the wastewater, cell yield and nutrient concentration in bacterial cells [8, 9]. In general, the nutrient concentration in the influent should be adjusted to a value equal to about twice the minimal nutrient concentration required in order to ensure that there is a small excess in the nutrients added and that the process is not limited by it.

2.2.4 Organic Loading rate

The loading on an anaerobic reactor is defined as the mass of COD added per unit volume per unit time. This plays an important role in the anaerobic process. In the case of non-attached biomass reactors, where the hydraulic retention time is long, hydraulic overload results in biomass washout. This, in turn leads to process failure. For sewage sludge containing high nitrogen, high loading will result to the release of high concentration of ammonia which could eventually lead to toxicity problems. However, there is no established loading limit for soluble wastes which doesn't contain high concentrations of ammonia and other toxic materials. Organic overload can also result to imbalance in the system as more volatile fatty acids will be formed by the acidogens while the methanogens, due to its low growth rate, may not convert as much VFA to methane. Hence this may result to accumulation of volatile fatty acids which reduces the pH and can inhibit the activity of the methane forming bacteria [13, 15]. Fixed film, expanded and fluidized bed reactors can withstand higher organic loading rate. The loading rate can be expressed as:

$$L = \frac{Q \cdot C_i}{V} \qquad (2\text{-}18)$$

Where L= loading rate (mgCOD/l·d); Q= flow rate (l/d); C_i= feed concentration (mgCOD/l); V= reactor volume (l).

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

$$L = \frac{C_i}{HRT} \qquad (2\text{-}19)$$

For a reactor without sludge recycle, the loading is related to the solid retention time (SRT) only because the SRT and HRT is the same [13]. For a reactor with sludge recycle, the SRT is independent of HRT. Low SRT results in high load while long SRT results in low load.

2.2.5 Toxicity

There is considerable effect of the concentration of any material on the specific growth rate of bacteria when all the materials are present in excess. If absolutely none of a needed material is available to a bacterial cell, it can't grow. As the concentration of the material is increased, the specific growth rate will increase until the maximum specific growth rate (μ_m) is reached. This magnitude of range, over which specific growth rate increases with the concentration of the material, will depend upon the particular material under consideration and can be anything from a few attograms per liter to several grams per liter [13]. As the concentration is increased further, there will be a point in which no effect is observed, but eventually a threshold value will be reached at which the specific growth rate starts to decline. At that point, toxicity is said to occur and any concentration in excess of that is said to be toxic. At concentrations above the threshold value, the severity of the toxicity will increase as the concentration increases. A few specific materials are considered:

2.2.5.1 Volatile fatty acids

In anaerobic reactors, accumulation of acids affects the pH of the medium. When the pH is held constant near neutrality, neither acetic nor butyric acids have any significant toxic effects upon hydrogen-utilizing methanogenic bacteria at concentrations up to 10,000 mg/l [19]. Propionic acids, on the other hand, exhibits partial toxicity to methanogenic bacteria at a concentration of 1000 mg/l at neutral pH [13, 19]. Hence it appears that at neutral pH only propionic acid is likely to exhibit toxic effects in anaerobic operations, and then only when the concentration is relatively high. There is no evidence for this with acetic and butyric acids, so that conclusions concerning the generality of this pH-volatile acid interaction must await further study. From this, it can then be said that in anaerobic operations that a little inhibition by volatile acids will occur at neutral pH.

2.2.5.2 Ammonia

Most wastewater sludge contains substantial quantities of protein. Wastes high in protein content will produce significant amounts of ammonia. As the protein is degraded, the nitrogen is released as ammonia but the form (either ammonium ion, NH_4^+, or dissolved free ammonia, NH_3) depends on the pH of the system. Free ammonia can inhibit anaerobic metabolism at high concentrations.

Anaerobes can acclimatize to high ammonia concentrations but large fluctuations can be deleterious the process. Ammonia is a weak base and dissociates in water:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad (2\text{-}20)$$

Both species are inhibitory, but at significantly different concentrations. Free ammonia, which is more toxic than the ammonium ion, is more prevalent at high pH. If the concentration of free ammonia exceeds 150 mg/l, severe toxicity will result whereas ammonium ion concentration must be greater than 3000 mg/l to have the same effect. Both high pH and ammonia levels contribute to process failure but this can be controlled by addition of acid. Also, since one result of ammonia toxicity is a buildup in volatile acids it appears to be more toxic to the methanogenic bacteria than the non-methanogenic bacteria. As noted in Table 2.3, ammonium ion is also an antagonist for inhibition by potassium [13, 15].

2.2.5.3 Light metal cations

pH control usually involves addition of a base to maintain a neutral pH. Care must be taken while doing this; however, because the light metal cations associated with most bases can also exhibit toxic effects, presumably upon the entire microbial community. Sodium, potassium, calcium and magnesium are of particular concern because of their widespread usage and because their toxicity exhibits a complex interaction. They are required for microbial growth and, consequently, affect specific growth rate like any other nutrient. For example, if the concentration of one cation is less than the concentration required to give maximum growth, then the toxicity exhibited by another cation will be more severe than it would be if the first cation were present at its maxima specific growth rate concentration [13]. In addition, if two cations are present at their toxic concentrations the effect will be larger than with either of the cations singly. In spite of these complications some generalities about the effects of various cation concentrations can be made, and these are shown in Table 2.4.

Table 2.4 Stimulatory and Inhibitory concentrations of light metal cations [13]

	Concentrations in mg/l		
Cation	Stimulatory	Moderately Inhibitory	Strongly Inhibitory
Sodium	100-200	3500-5500	8000
Potassium	200-400	2500-4500	12000
Calcium	100-200	2500-4500	8000
Magnesium	75-150	1000-1500	3000

The concentrations which are listed as stimulatory are those which allow maximal reaction rates. These concentrations will ensure optimum metabolic activity of the bacteria under normal condition. The concentrations listed as moderately inhibitory can be tolerated after a period of acclimatization as long as they are applied steadily, however a sudden increase to those concentrations can be expected to retard the reactor significantly for several days. Concentrations listed as strongly inhibitory are those that will inhibit the bacteria growth so severely that extremely long SRT's will be required to prevent process failure. If the toxic effects of a light metal cation cannot be controlled by the addition of stimulatory concentrations of the others, then it will be necessary to dilute the wastes. Table 2.3 summarizes antagonistic responses for the light metal cations and ammonia.

Table 2.3 Antagonistic Responses for Light Metal Cations and Ammonia [13]

Inhibitor	Antagonist
Na^+	K^+
K^+	Na^+, Ca^{2+}, Mg^{2+}, NH_4^+
Ca^{2+}	Na^+, K^+
Mg^{2+}	Na^+, K^+

2.2.5.4 Sulphides

Sulphate can be used as an electron acceptor under anaerobic conditions, resulting in sulphide production. Sulphides are inhibitory to methanogens and sulphate reducing bacteria (SRB) themselves. Wastes high in sulphate can be prone to sulphide toxicity. If the concentration of soluble sulphides exceeds 200 mg/l, then the metabolic activity of the methanogenic population will be strongly inhibited leading to process failure [13] while concentrations up to 100 mg/l can be tolerated with little or no acclimatization. Concentrations between 100 and 200 mg/l may be tolerated after acclimation. Only soluble sulphides exhibit toxicity because only they are available to the bacteria cells. Sulphide reacts with heavy metal cations including iron, to form highly insoluble precipitates. In fact, iron sulphide gives anaerobic processes their characteristic black color. Consequently, iron can be added to eliminate sulphide toxicity when sulphide concentrations are inhibitory [15, 20].

Hydrogen sulphide acts as a weak acid and, consequently, at neutral pH is present in equilibrium with the hydrogen sulphide ion.

$$H_2S \leftrightarrow HS^- + H^+ \qquad HS^- \leftrightarrow S^{2-} + H^+ \tag{2-21}$$

Hydrogen sulfide is sparingly soluble in water, so it will partition between the liquid and gas. Sulfide increases the corrosivity of anaerobic process gas and results in the formation of sulphur oxides when the gas is burned. Consequently, control of the hydrogen sulfide content of the product gas is desirable. This too can be done by adding iron to the bioreactor to precipitate the sulphide anion as iron sulphide. Sulphate itself is not inhibitory to anaerobic bacteria, but it impacts anaerobic processes by providing an electron acceptor that can be used by sulphate reducing bacteria, allowing them to compete with methanogens for the electrons available in the organic compound. This has several effects. First, it produces sulphide, which is inhibitory, as discussed above. Second, it reduces the amount of methane produced because the electrons used to reduce the sulphate are not available for the reduction of carbon dioxide to methane. Third, it reduces the value of the product gas, as discussed above. Fourth, it decreases the removal of COD from the wastewater being treated [13]. The competition between methanogens and sulfate reducing bacteria is very complex and is determined by the growth rates of the bacteria. Faster growing bacteria will dominate.

2.2.5.5 Heavy Metals

Many heavy metals are necessary for the function and structure of enzymes in bacteria but can as well be toxic and inhibitory to reactions at high concentrations. As with other biochernical operations, heavy metals have strong effects on anaerobic processes, as indicated in Table 2.5 by the low concentrations causing 50% inhibition. In spite of this extreme toxicity they need not cause a problem in anaerobic reactors because only soluble metals have an effect and their soluble concentrations can be reduced to nontoxic levels by precipitation with sulphides produced in the process. In situations where inadequate sulphide is produced, sulfur can be added. This must be carefully done since sulphides can also be inhibitory to methane forming bacteria.

Approximately 0.5 mg of sulphide is needed to precipitate one mg of heavy metal. Ferrous sulphide is an ideal chemical to provide supplemental sulphide. Table 2.5 shows that ferrous iron is much less inhibitory than other heavy metals. In addition, the sulphide precipitates of the more inhibitory heavy metals are more insoluble than ferrous sulfide, and consequently the added sulphide will maintain the concentration of those heavy metals at low concentrations. Furthermore, the presence of residual iron will maintain soluble sulphide concentrations at low values. Finally, as long as the pH is 6.4 or above, any excess iron will precipitate as iron carbonate, thereby preventing any inhibition caused by soluble iron [13].

Table 2.5 Concentrations of soluble Heavy metals exhibiting 50% inhibition of Anaerobic Digesters

Cation	Approximate conc. in mg/l
Fe^{2+}	1 – 10
Zn^{2+}	10^{-4}
Cd^{2+}	10^{-7}
Cu^{+}	10^{-12}
Cu^{2+}	10^{-16}

2.2.5.6 Other Organic compounds

As with aerobic processes, a wide variety of organic compounds can cause inhibition in anaerobic process and also these organic compounds can be biodegraded significantly at sufficient acclimatization. Organic compounds that are not very soluble in water or that adsorbed to the biomass can accumulate to high concentrations to cause inhibition to the anaerobic process. Some typical organic compounds reported to be inhibitory to anaerobic process includes Ethyl benzene, Formaldehyde, Ethyl dibromide, chloroform, alkyl benzene sulphonate (ABS) detergent [13]. During acclimation, the activity of a methanogenic bacteria community may almost cease.

2.3 Anaerobic Reactor Systems

Before the advent of improved anaerobic treatment technologies or the high rate anaerobic digesters, anaerobic treatment referred to "anaerobic digestion" of solids generated in aerobic biological wastewater treatment operations [15]. In other words, anaerobic treatment was primarily used for the stabilization or the liquidification of solid components of sewage with the intention of reducing the amount of solids.

Anaerobic reactors have been in use since the 19th century when Mouras and Cameron developed the automatic scavenger and the septic tank to reduce the amounts of sewerage system [21]. The first anaerobic reactor was developed in Germany in 1905 when Karl Imhoff designed the Imhoff tank, in which solids sediments are stabilized in a single tank. In the same decades, Buswell started to adopt the same technology for treating liquid wastes and industrial wastewater [21]. It was not until 1955 that anaerobic contact process was developed to treat soluble organics and dilute wastewaters [22]. A schematic diagram of Imhoff tank is shown in Figure 2.4.

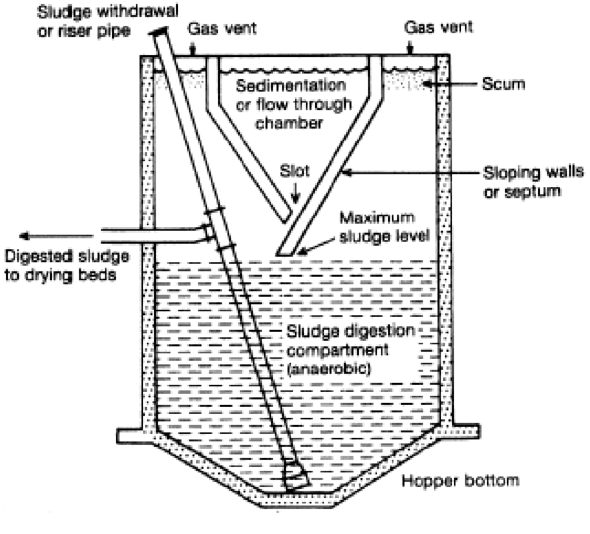

Sludge withdrawal or riser pipe
Gas vent
Gas vent
Sedimentation or flow through chamber
Scum
Slot
Sloping walls or septum
Maximum sludge level
Digested sludge to drying beds
Sludge digestion compartment (anaerobic)
Hopper bottom

Figure 2.4 Imhoff tank [22]

Low rate or conventional anaerobic systems are those which no special features were included on the design to augment the anaerobic catabolic capacity. The process feasibility of these systems was very much dependent on the growth rate of the anaerobic consortia and as a result the bioreactor volume was very large and unstable in operations [11, 13, 21].Conventional treatment consists of a well-mixed reactor without solids recycle. All solids are in suspension. The SRT is equal to the hydraulic retention or detention time (HRT) in a suspended solids reactor without recycle. An SRT of 15 to 20 days at a temperature of about 35°C is typically used; although SRTs as low as 10 days have been used successfully and longer SRTs are employed when greater waste stabilization is required. Other low rate anaerobic systems include the Anaerobic ponds where mixing is typically provided simply by the addition of influent wastewater and by gas evolution. Here a well mixed condition is not generally provided and suspended solids settle and accumulate in the bioreactor [13].

In other to reduce the problems encountered in the low rate anaerobic systems, high rate anaerobic systems were developed. This system utilizes bioreactor configurations that provide significant retention of active biomass, resulting in large differences between the SRT and the HRT [23, 24]. High biomass densities also provide greater resistance to any inhibitory substances in the influent [22]. Three mechanisms are used 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. High biomass concentrations enable the application of high COD loading rates, while maintaining long SRTs at relatively short HRTs [21]. This ability to achieve high organic load allows it to be used relatively in small volume reactors and long SRT provides a stable process [7]. Although the systems are compact and require relatively small area, they achieve a good degree of treatment of biodegradable organic material with a typical BOD_5 removal of 80 to 90% [13, 21, 25].

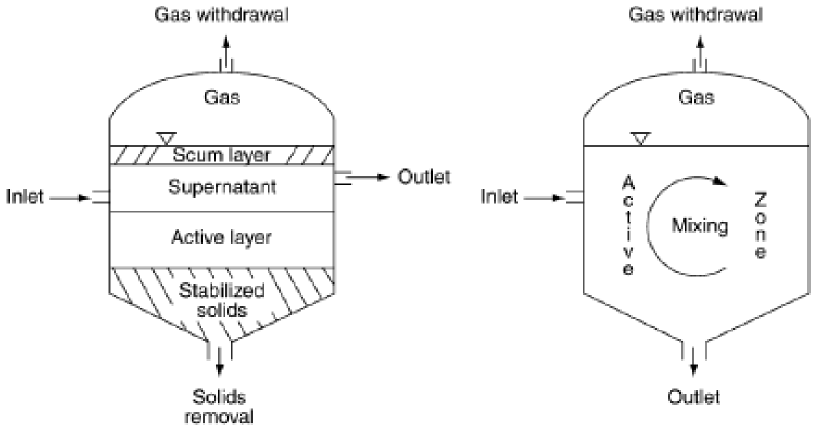


Figure 2.5 Conventional anaerobic digester (a) without mixing and (b) with mixing

Consequently, high-rate anaerobic processes represent a spectrum of bioreactor types ranging from suspended growth to attached growth, with hybrid bioreactors which contain significant quantities of both suspended and attached biomass, in between. These high-rate anaerobic reactors includes 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).

2.3.1 Anaerobic Contact Process (ACP)

Anaerobic contact process, illustrated in Figure 2.6, consists of a completely mixed suspended growth bioreactor, a vacuum degassifier, and a liquid-solid separation device where the bioreactor effluent is separated into a relatively clear process effluent and concentrated slurry of biosolids that is recycled to the bioreactor. Therefore, ACP is essentially an anaerobic activated sludge system [13]. Settling of anaerobic sludge in the clarifier and its return back to the reactor allows further contact between biomass and influent waste. Completely mixed conditions are achieved by mechanical mixing systems similar to those used in conventional anaerobic systems. Conventional clarifiers or plate settlers are often used as the liquid-solids separation device. First generation of ACP had major drawback due to poor sludge settlement which arose from gas formation by anaerobic bacteria in settling tank. If the gas is not removed, bubbles attach to the solids; preventing their settling and subsequent recycle to the bioreactor. This gas formation problem was minimized by employing vacuum degassifier as shown in Figure 2.6. The vacuum degassifier is a device that facilitates removal of carbon dioxide and methane to allow settling of the biosolids in the clarifier.

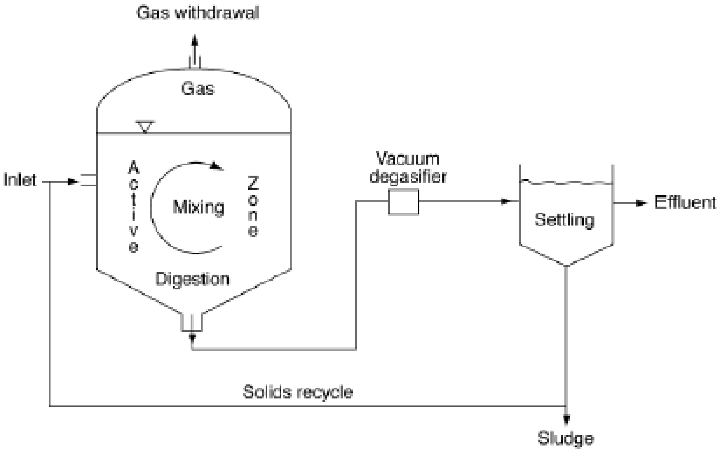


Figure 2.6 Anaerobic contact process, equipped with vacuum degassifier

2.3.2 Anaerobic Filters (AF)

Anaerobic filter or packed bed is a fixed-film biological wastewater treatment process in which a fixed matrix (support medium) provides an attachment surface that supports the anaerobic microorganisms in the form of a biofilm. Treatment occurs as wastewater flows upwards through this bed and dissolved pollutants are absorbed by biofilm, hence was referred to as upflow anaerobic filter (UAF). Anaerobic filters were the first anaerobic systems that eliminated the need for solids separation and recycle while providing a high SRT/HRT ratio [22]. The presence of packing allows for the growth of some attached biomass, but the primary role of the media is to retain suspended growth [26]. The media may be thought of as performing like a set of tube settlers, which provide enhanced liquids-solids separation and retention of suspended biomass within the bioreactor [13]. Various types of support material can be used, such as plastics, granular activated carbon (GAC), sand, reticulated foam polymers, granite, quartz and stone. These materials have exceptionally high surface area to volume ratios ($400m^2/m^3$) and low void volumes (Figure 2.8). Its resistance to inhibitions makes AF suitable for the treatment of both dilute and high strength wastewaters. Figure 2.7 provides a schematic of the overall AF process.

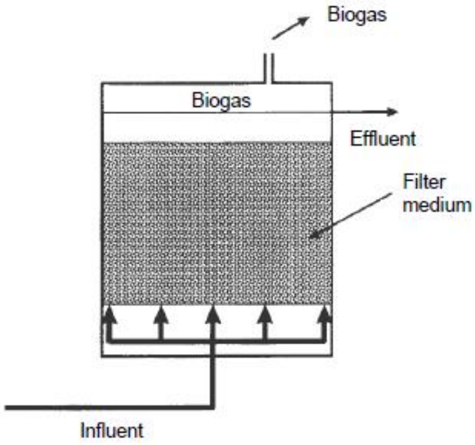


Figure 2.7 Anaerobic Filter (packed bed)

Limitations of anaerobic filter are mostly physical ones related to deterioration of the bed structure through a gradual accumulation of non-biodegradable solids. This leads eventually to channeling and short-circuiting of flow, and anaerobic filters are therefore unsuitable for wastewaters with high solids contents. Additionally, there is a relatively high cost associated with the packing materials.

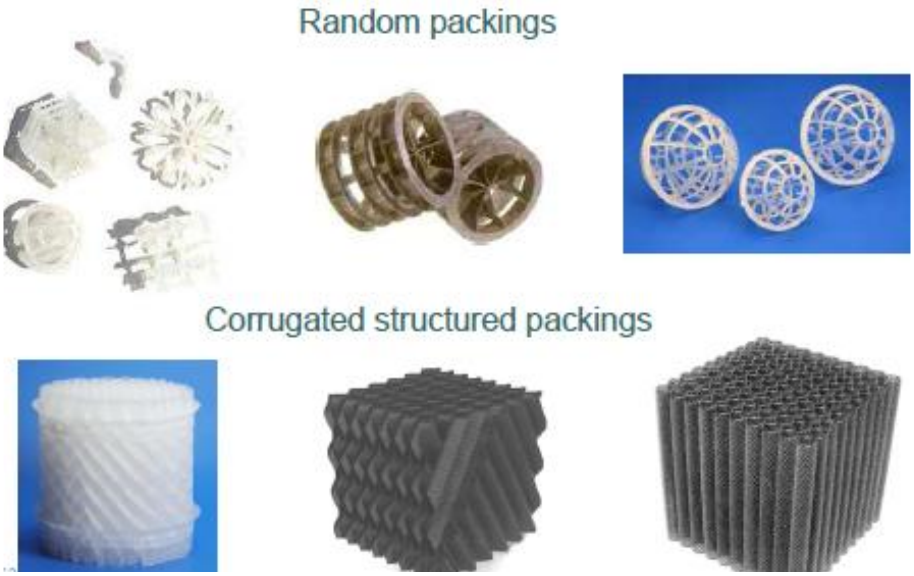


Figure 2.8 Anaerobic filter packings [22]

2.3.3 Upflow Anaerobic Sludge Blanket (UASB)

The problem associated with anaerobic filters and fluidized bed reactors led to development of unpacked reactors that still incorporate an immobilized form of particulate biomass [22]. The upflow anaerobic sludge blanket (UASB) process was developed in the Netherlands [27]. The UASB is by far the most commonly used high rate anaerobic system for domestic and industrial wastewater treatment [15, 22]. The reactor relies on development of a dense, active sludge mass in the lower portion of the reactor and is also integrated with a gas-liquid-solid separation (GLSS) system [13, 15].

The wastewater passes upwards through anaerobic sludge bed where the microorganisms contact with wastewater substrates, as shown in Figure 2.9. The sludge bed is composed of microorganisms that naturally form granules (pellets) of 0.5 to 2 mm in diameter that have a high sedimentation velocity and thus resist wash-out from the system even at high hydraulic loads.

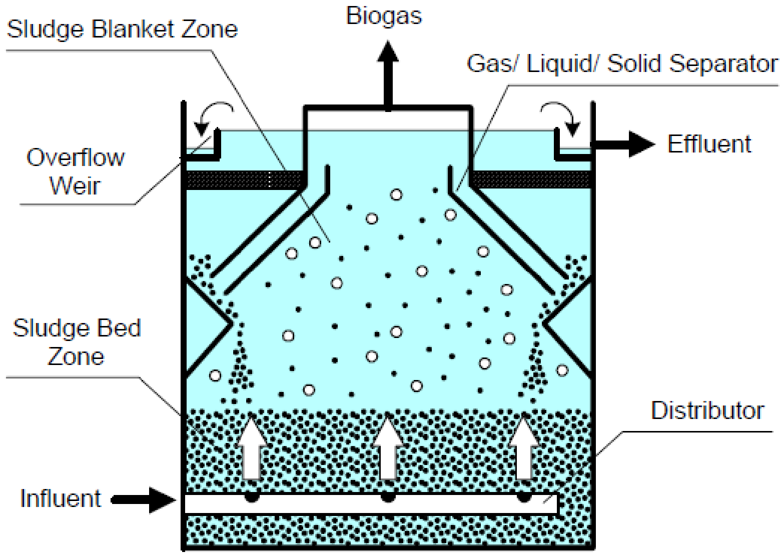


Figure 2.9 Modified upflow anaerobic sludge blanket (UASB) reactor

Upward flow speed is controlled under 10 m/d with recirculated effluent and inflow wastewater. The upward motion of released gas bubbles causes hydraulic turbulence that provides reactor mixing without any mechanical agitation. At the top of the reactor, the gas bubbles are separated from the water in hoods and the rising flocs which show a lower settling rate are carried up by gas/liquid flow. Gas is collected in the hoods and removed from the reactor. Liquid/ solid separation takes place in the settler section. The clarifier effluent overflows the weirs and is discharged while separated solids settle back into the reaction zone [11, 13, 15]. Design of the gas-liquid-solids separation device requires insight into the physical processes occurring there and experience with specific devices in a variety of applications [13].

2.3.4 Hybrid Upflow Anaerobic Sludge Blanket/Anaerobic Filter

Hybrid UASB/AF systems combine aspects of the UASB process with aspects of the AF process [13]. As illustrated in Figure 2.10, influent wastewater are distributed across the bioreactor cross-section and flow upward through the sludge blankets where most organic matter conversion is located whereas the removal of a specific fraction of pollutants is located in the filter area at the top [13, 21]. Specific chemical wastewaters show better treatment efficiencies for all compounds using hybrid systems compared to UASB reactor. The most known example is the treatment of purified therephthalic acid (PTA) wastewater [28, 29]. Results showed that the conversion of therephthalic acid to benzoate is only possible at low concentrations of acetate and benzoate. By applying the hybrid systems, the latter two are converted in the sludge bed area whereas, therephthalic acid is then converted in the hybrid section, where specific flora is retained for degrading the refractory compound. The most known disadvantage of hybrid reactors is the deterioration of the filter section after prolonged periods of operation.

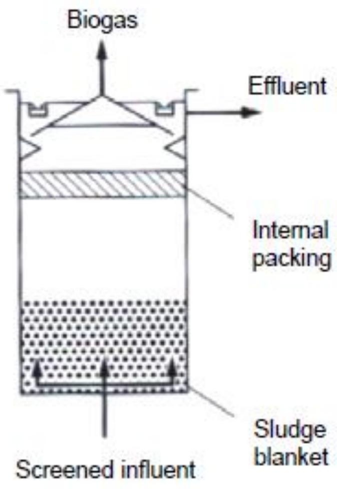


Figure 2.10 Hybrid reactor: UASB with AF process

2.3.5 Expanded Granular Sludge Bed (EGSB) and Fluidized Bed (FB)

EGSB and FB systems are regarded as the second generation of sludge bed reactors achieving extreme organic loading rates exceeding 30 to 40 kgCOD/m^3·d [21]. They differ from those previously considered in that they are essentially attached growth systems with little or no suspended growth [30]. EGSB and FB systems use upflow bioreactors, just like the UASB, AF, and hybrid UASB/AF processes, but the upflow velocities are much higher, resulting in minimal retention of suspended biomass. Instead, the biomass grows attached to granular carrier particles that are fluidized by the upflow of influent wastewater and recirculated effluent [13].

The FB process is based on the occurrence of bacteria attachment to mobile carrier particles, which consist, for example, of fine sand (0.1 – 0.3 mm), basalt, pumice, or plastic. The FB system can be regarded as an advanced anaerobic technology [30], that may reach loading rates of 50 – 60 kgCOD/m^3.d. However, long-term stable operation appears to be problematic. The system relies on the formation of a more or less uniform (in thickness, density, strength) attached biofilm and/or particles. Pre-acidification is necessary and absence of dispersed matter in the feed is required in order to maintain a stable condition with respect to the biofilm development [21, 30]. Despite this, segregation of different types of biofilm still occurs over the height of the reactor and this result to operational problems.

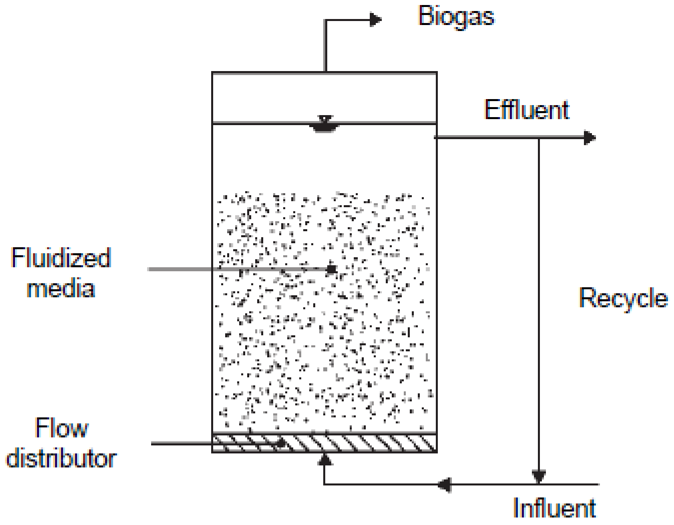


Figure 2.11 Fluidized Bed reactor

Modern FB systems like the Anaflux system [21, 31], rely on bed expansion rather than on bed fluidization. As bed expansion allows a much wider distribution of prevailing biofilms, the system is much easier to operate.

The EGSB system employs granular sludge, which is characterized by good settling property and a high methanogenic activity. And due to this high settling capacity of the granular sludge, a higher flow rate can be applied. This high flow rate together with the lifting action of the generated gas, results to a slight expansion of the sludge bed. This gives a better contact between the sludge and wastewater and eventually leads to significant higher organic loading capacity compared to conventional UASB systems [21].

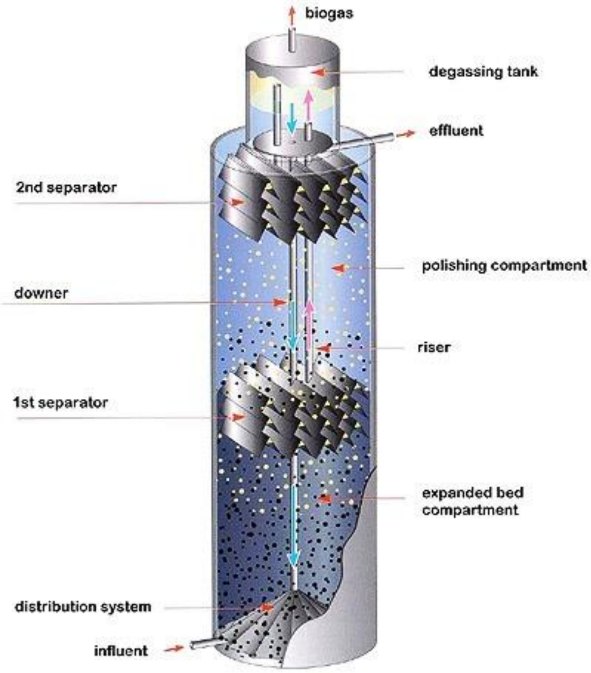

biogas
degassing tank
effluent
2nd separator
polishing compartment
downer
riser
1st separator
expanded bed
compartment
distribution system
influent

Figure 2.12 IC Reactor [21]

A special version of the EGSB is the Internal Circulation (IC) reactor (Figure 2.12). The produced biogas is separated from the liquid halfway the reactor by means of a gas/liquid separator device and conveyed upwards through a pipe to a degasifier unit. The separated gas is removed from the reactor while the sludge-liquid mixture drops back to the bottom of the reactor through a different pipe. This gas lift transport results to an improved contact between the sludge and wastewater [21].

2.3.6 Anaerobic Baffled Reactor (ABR)

Anaerobic baffled reactor (ABR) consists of a number of UASB reactors connected in series. Wastewater passes over and under the staggered vertical baffles as it flows from inlet to outlet. Unique baffled design enables ABR to reduce biomass washout, hence retain high active biomass content, and it can also recover remarkably quickly from hydraulic and organic shock loads. Owing to its compartmentalized configuration, it may function as a two-phase anaerobic treatment system with separation of acidogenic and methanogenic biomass. ABR has a simple design and requires no special gas or sludge separation equipment. It can be used for almost all soluble organic wastewater from low to high strength wastewaters. Considering its simple structure and operation, it could be considered a potential reactor system for treating municipal wastewater in tropical and sub-tropical areas of developing countries [22].

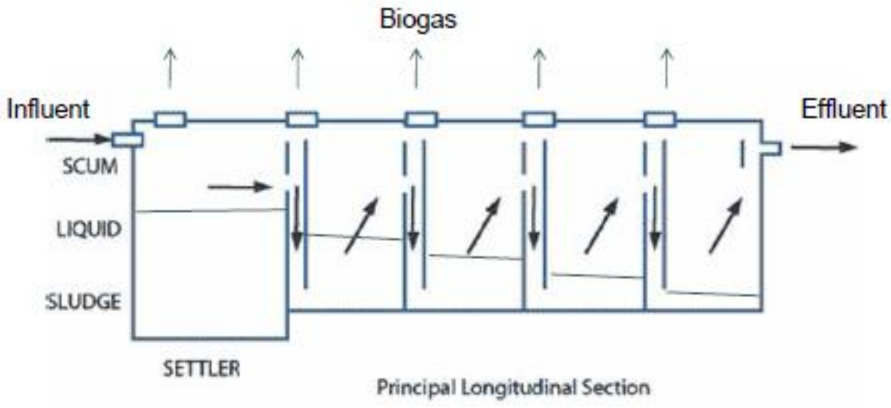


Figure 2.13 Anaerobic baffled reactor (ABR)

2.3.7 Anaerobic Membrane Reactor (AMR)

More recently anaerobic membrane bioreactors are intensively researched. Membrane technology can be considered an interesting option in those areas where established technologies may fail. Higher biomass concentrations in AMR reduce the size of reactor and increase organic loadings. Almost complete capturing of solids (much longer SRT) results in maximum removal of VFAs and degradable soluble organics and provide a higher quality effluent. The big challenge in AMR is the organic fouling which is typically caused by accumulation of colloidal materials and bacteria on the membrane surface. High liquid velocities across the membrane and gas agitation systems might be used to minimize membrane fouling. High pumping flow rates across the membrane may lead to the loss of viable bacteria due to cell lysis. Developments in membrane design and fouling control measures could make AMR a viable technology in future. At present, only a few full scale AMR systems are in operation and considering the sharp drop in membrane prices, an increase in this emerging technology is expected [21, 22].

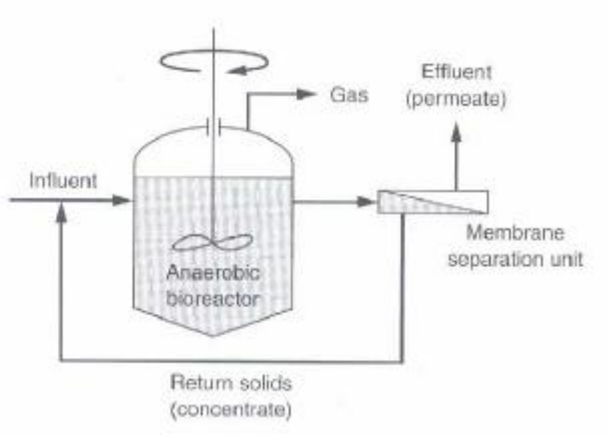

Gas
Effluent (permeate)
Influent
Anaerobic bioreactor
Membrane separation unit
Return solids (concentrate)

Figure 2.14 Anaerobic bioreactor with external membrane separation unit

2.4 Comparison of Different Anaerobic Treatment Processes

The loading rate ranges in terms of COD and HRT for processes discussed are summarized in Table 2.6.

Table 2.6 Characteristics and Energy use of Anaerobic Processes [15]

Parameter	Conventional	Contact	Filter	UASB	Fluidized bed
HRT (d)	15	5	1	1	0.5
Loading rate ($kgCOD/m^3 \cdot d$)	0.5-6.0	2-10	5-30	0.5-40	1-30
Heat energy consumption (MJ/m^3)	105	95	93	93	93
Mixing & pumping energy consumption (MJ/m^3)	88	26	0.1	0.1	1-29
COD for energy self sufficiency (kg/m^3)	26	17	14	14	15-19

3. MATERIALS AND METHODS

This part describes the laboratory-scale experiment of anaerobic digestion of glycol contaminated wastewater for generation of energy in form of methane gas. This also includes the operational, maintenance and control procedures, as well as the analytical methods used in the study.

Two different experiments were conducted in the study, one with an existing anaerobic bioreactor (from 14th February to 12th March) and second with a new system (15th March to 6th April 2011). The procedures were the same in the both experiments unless where stated otherwise.

3.1 Characterization of wastewater (glycol)

The raw wastewater was provided by Nature Technology Solutions AS (NTS) and sourced from Statoil Sleipner field location. The characteristics of the raw wastewater are shown in Appendix A3. It consists primarily of monoethylene glycol (MEG) and propylene glycol, and also has a high COD value of about 15000 mg/l. There were also some trace-nutrients already present in the raw wastewater thereby reducing the total micronutrients requirements for an optimum performance of the anaerobic digester.

3.2 Experimental Apparatus

The anaerobic bioreactor was operated as a batch system and consisted of a continuously stirred flask on a hot plate magnetic stirrer with heat and speed control. The heat control helps in maintaining an average temperature of 37 ˚C. The flask has a liquid volume of 2 liters and this volume was carefully maintained during the feeding of raw wastewater and any other reagents or solutions. The digester flask was fitted with three different holes and rubber stoppers to allow for feeding, withdrawal of digested sample, sampling of the mixed liquor and collection of the gases. These outlets were also air tight to avoid gas exchange or leaks and introduction of air.

At the initial set up, the glycol wastewater was not fed in immediately rather glucose was used. This was done since the biodegradation rate of glucose is known to be very fast. After some weeks this was then gradually replaced with the glycol wastewater. For the generated gas monitoring, a small diameter rubber pipe was connected from one of the holes of the reactor to a graduated rubber cylinder which was filled with water. In order to restrict the gas monitored to just methane, calcium hydroxide was added to the water for carbondioxide absorption. A phenolphthalein indicator was also added for a clear observation of the gas level. The volume of the generated gas was recorded from the graduated cylinder, but however, gas analysis for the determination of its composition was not carried out.

During Experiment 2, the set up was the same with that of the Experiment 1 except that glycol wastewater was fed in to the bioreactor from the first day and there was no addition of glucose.

In both experiments, the microbes used for the laboratory scale investigations were those from the sewage sludge of IVAR sewage treatment plant.

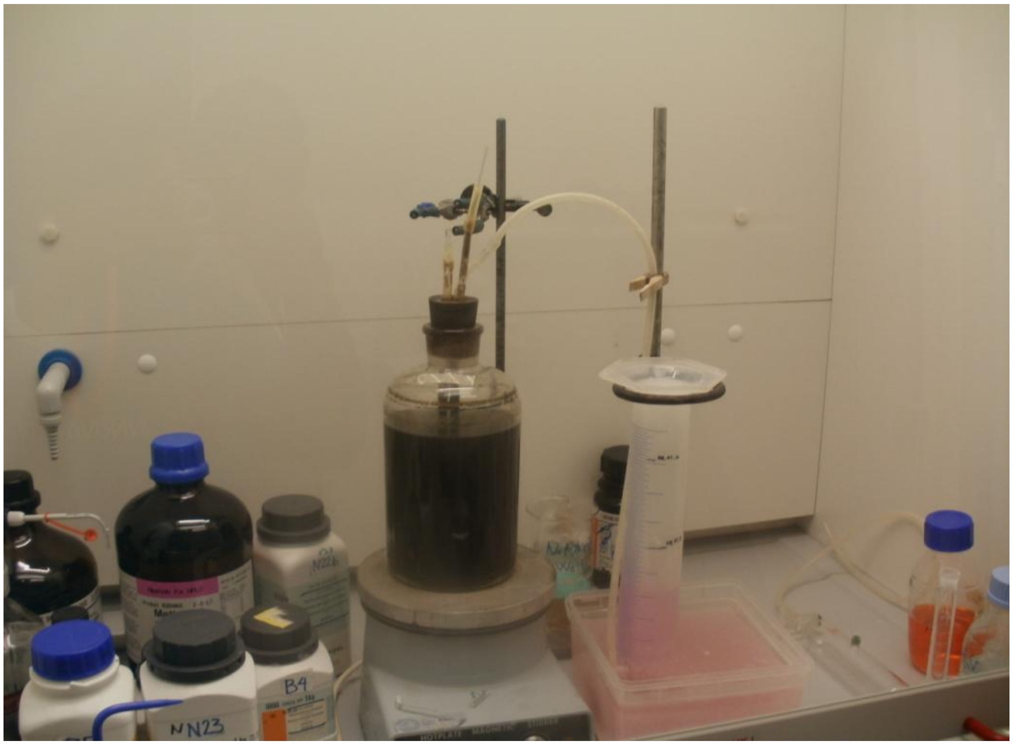


Figure 3.1. The Experimental setup

3.3 Operation

On daily basis the following parameters were measured: temperature, pH, conductivity, and gas level. Monitoring of these parameters especially the temperature and pH were of extreme importance for an optimum performance of the anaerobic reactor. These parameters were controlled and adjusted if needed. Every two days, the Total Suspended Solids(TSS) and Volatile Suspended Solids(VSS) analysis were carried out and also 5-point titration for alkalinity and volatile fatty acids determination. Also in order to check the availability of enough nutrients for microbial growth, phosphorus measurement was done once in a week. COD tests were also carried out periodically after about 10 filtered samples have been collected.

During the first experiment, the domestic wastewater from IVAR plant was used as the nutrients source but towards the end of the first experiment, a prepared solution of nutrient was then used. Usually two stocks of nutrient solutions were used: one for the macronutrients and the other for micronutrients. However due to the availability of the micronutrients already present in the glycol wastewater, only a solution of macronutrient was prepared. The solution was made up with a tap water and stored in darkness at 4 °C. The composition of the macronutrients used both in the first and second experiments are presented in Tables 2.1 and 2.2 respectively.

Table 2.1. Composition of macronutrients in Experiment 1

Compound	Concentration (g/l)	Element	Element concentration (g/l)
NH_4Cl	19.5	N	5.1
Na_2HPO_4	7.78	P	1.7
KCl	1.62	K	0.85
$MgSO_4·7H_2O$	7.6	Mg	0.74
$CaCl_2·xH_2O$	1.2	Ca	0.43

Table 2.2. Composition of macronutrients in Experiment 2

Compound	Concentration (g/l)	Element	Element concentration (g/l)
NH_4Cl	19.5	N	5.1
Na_2HPO_4	7.78	P	1.7
KCl	1.62	K	0.85
$MgCl_2·6H_2O$	6.27	Mg	0.74
$CaCl_2·2H_2O$	1.5	Ca	0.43

The difference in the table being that in the second experiment, $MgCl_2$ was used instead of $MgSO_4.7H_2O$. The $MgCl_2$ change was to remove the sulphur content which was initially suspected to may have been responsible for the abrupt end of the first experiment. The amount of nutrients added was determined based on the calculated requirements for growth according to the COD of glycol wastewater added, and multiplied by a safety factor of about 2-3 in order to avoid nutrients limitation for microbial growth. It was also ensured that excess nutrients were not added as this was the case during the first experiment. Phosphorus content is measured once in a while to ensure the nutrient availability.

3.4 Feeding of glycol wastewater

The digester was fed with the glycol wastewater once per day via one of the holes on top of the lid. The feeding was done in addition with the required amount of nutrients and sometimes with some amount of buffer solution if the pH reduces below the optimum. Withdrawal of digested sample was accomplished once in a day in the same way as the feeding via a different hole on the lid. Feeding and withdrawal were usually done with a syringe.

In ensuring a constant bioreactor volume, the same amount being withdrawn from the system was added back to maintain the 2 liter volume of the reactor. In the first experiment when source of nutrient was mostly the domestic wastewater, it was ensured that its addition to the glycol wastewater sums up to the amount that has been withdrawn. In both experiment, the amount being added and withdrawn increased with time; from 50,100,150 to 200 ml.

3.5 Maintenance and Control

The optimum temperature condition was maintained by ensuring that the thermostat of the magnetic hot plate was working properly and well adjusted to the suitable temperature. A pH of 6.8 -7.0 has to be maintained for a good performance of the bioreactor. The pH control was done by adding buffer solutions of either sodium hydrogen bicarbonate ($NaHCO_3$) for pH increment or acetic acid for pH reduction. The choice of $NaHCO_3$ is due to the fact that it changes the pH slightly and hence be easily controlled and acetic acid because it can also serve as substrate for the methanogenic bacteria. Since $NaHCO_3$ addition has influence on the conductivity, the amount of $NaHCO_3$ added at each instance has always been within the range of 1-2 g in order to maintain consistent values in the conductivity values. Addition of acetic acid was done carefully as this may not necessarily change the pH in the long run since acetic acid will also serve as substrates to the methane producing bacteria.

The walls of the anaerobic reactor were cleaned regularly to prevent biofilm growth.

3.6 SAMPLING AND ANALYTICAL PROCEDURES

3.6.1 Sample Preparation

The objective of sampling was to collect a portion of material small enough in volume to be transported conveniently and yet large enough for analytical purposes while still accurately representing the material being sampled. This implies that the relative proportions or concentrations of all pertinent components will be the same in the samples as in the material being sampled , and that the sample will be handled in such a way that no significant changes in composition occur before the tests are made [32].

In the experiments, samples were collected from the mixed liquor of the bioreactor and then filtered immediately for TSS and VSS analysis. The filtered sample was split into three: one for titration analysis for short chain fatty acids (SCFA) and alkalinity determination, the other preserved frozen for nitrates and total N determination while the third part was then preserved with H_2SO_4. The sample preserved with acid was used for COD tests; acid added is about 1% of sample volume to be preserved. Also the non filtered sample was also frozen.

It was also, as a good laboratory practice, ensured that all sampling equipment and containers were clean and free of contaminants and that the sample itself does not become contaminated or compromised before it was analyzed. The sample containers were clearly labeled for easy identification and retrieval.

3.6.2 Analytical Procedures

pH

A Metrohm 744 pH meter equipped with a Schott Blueline 11 pH electrode was used for the measurement of the pH of the sample as soon as it is taken from the bioreactor and also in the 5-point titration for SCFA and alkalinity determinations. The pH meter also shows in its display the temperature of the sample and hence provides temperature value for the titration process. The electrode was immersed into the buffer solutions of pH 4 and pH 7 for standardization, then rinsed and immersed into the beaker containing the sample. It was stirred and the pH value was recorded from the pH meter after the value had become stable. It was used also during the pH control on the addition of Sodium hydrogen carbonate buffer solution prior to its addition to the reactor. The pH meter also measures the temperature and voltage.

Conductivity

A portable WTW Multi 340i pH/O_2/conductivity meter equipped with a standard conductivity cell TetraCon 325 (WTW) was used for conductivity measurements. Calibration with a standard KCl solution was performed weekly. The unit was also noted correctly as this need to be converted to a unit consistent with the TITRA 5 software.

TSS and VSS analysis

Firstly the samples were filtered with VWR GF/C glass microfibers filter with 1.5 µm particle retention size. The filter was dried at 105 ˚C for at least 15 minutes prior to weighing to ensure complete dryness. The sample to be filtered was measured in a graduated cylinder; this volume depends on the amount of solids present in the sample. For high solids content, small sample volume was used and also for a faster filtration process, samples with high solid contents are allowed to settle for a while before filtration as the clarified liquid is filtered first and then the concentrated sludge added last.

For TSS determination, the filter paper with the solids was dried at 105 ˚C for at least 2 hours after which it is then weighed. The difference between the dried sum and the filter paper gives the TSS.

For VSS determination, the filter paper with the solids was combusted in an oven at 550 ˚C for about 30 minutes, during which all the volatile or organic compounds are burned off leaving the inorganic suspended solids (ISS). The difference between the TSS and the ISS gives the VSS. In each case, the concentration was calculated by dividing by the volume of sample used.

COD measurement

The measurement of COD was based on the "closed reflux, colorimetric method" described in Standard Methods [32]. Digestion solution was first prepared by adding 10.216 g of $K_2Cr_2O_7$, previously dried for 2 hours at 103 °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. 2.5 ml of each sample was placed in Hach COD vial tubes and 1.5 ml of digestion solution was added. Then 3.5 ml of sulphuric acid reagent was carefully run down the inside of the tube so that an acid layer was formed under the sample/digestion solution layer [33]. 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 in place of the sample. The mixtures were then incubated in a Hach COD reflux reactor (Model 45600) at 150 °C for 2 hours. The reactor was turned off and the vials allowed to cool to about 120 °C or less for 20 minutes. Each of the vials was inverted several times while still warm after which they were placed in cooling rack and allowed to cool to room temperature and vent to relieve any pressure generated during digestion [32]. After cooling, the samples were analyzed on a HACH DR 2000 scanning Spectrophotometer at a wavelength of 600 nm. Potassium hydrogen phthalate (KHP) was used to prepare standard solutions in the range 100-1000 mg/l. KHP has a theoretical COD of 1.176 mg COD/mg KHP.

Samples were analyzed in duplicates for accuracy. Also, due to high COD content, preliminary dilutions were made for both the original sample and the effluents in order to reduce the error inherent in measuring small sample volumes.

In Experiment 2, the COD measurement was based on the adapted version of Colorimetric closed reflux method as described by Merck. This version differs from the previously described method in the sense that already prepared digestion reagents, free of Mercury (Hg), were used. In this method, 2 ml of sample were added to the COD vial containing these premixed reagents and then digested in Merck Spectroquant thermoreactor (Model TR620) at 148 °C for 2 hours. The COD analysis and measurements was also done using Spectroquant Pharo 300 (Merck).

620
TR620
MERCK
F1
F2
F3
F4
Spectroquant Pharo 300
MERCK

Fig 3.2. Merck Spectroquant thermoreactor (Model TR620) and Pharo 300.

Volatile Fatty Acids and Alkalinity

Volatile fatty acids concentration and alkalinity were determined using TITRA 5, a 5-point titration procedure [34]. In the procedure, a sample of appropriate volume was filtered and diluted to 50 ml and then put on a magnetic stirrer at low rotation to minimize or avoid CO_2 input or loss. The initial pH was measured and the volume read after which it was then titrated with HCl. Titration were repeated to pH of 6.7, 5.9, 5.2 and 4.3(+/- 0.1) and the volume of acids added were read. The temperature and conductivity measurements were also recorded. These parameter values were then inputted into the computer program TITRA 5 for the calculations of the Volatile fatty acids concentration and alkalinity, which were then presented in units of mg/l as Acetic acid (HAc) and mg/l as $CaCO_3$ respectively. The specific short chain fatty acids were not measured.

Hydrochloric acid (HCl) was used in the titration and the molarity was determined to be 0.1029 M.

Phosphorus

This was occasionally done to determine the phosphorus content and also to ensure that the reactor performance is not limited by nutrients. The phosphorus requirement for the reactor was calculated theoretically based on the daily COD loading.

PhosVer 3 Phosphate Reagent Powder Pillow for 25 ml was used for the measurement of reactive phosphorus (orthophosphate). A sample cell was filled with 25 ml of a filtered sample and then one Phosphate reagent powder pillow was emptied into it and swirled immediately to mix. Also a blank was prepared, for standardization, by filling another sample cell with 25 ml sample without adding the powder pillow. With the selection of appropriate method and wavelength of 890 nm, the determination was done in Hach DR 2000 spectrophotometer. This measurement was necessary for a check on the nutrient availability in the reactor.

4. RESULTS AND DISCUSSIONS

This chapter presents the results and discussions and is presented in first section for the Experiment 1and second for the Experiment 2.

4.1 Experiment 1 (Feb 14 2011-March 12 2011)

Experiment 1 was a continuation of an existing system that was started in September 2010 by a previous researcher. The key parameters for the operating condition of this experiment are presented in Figure 4.1.

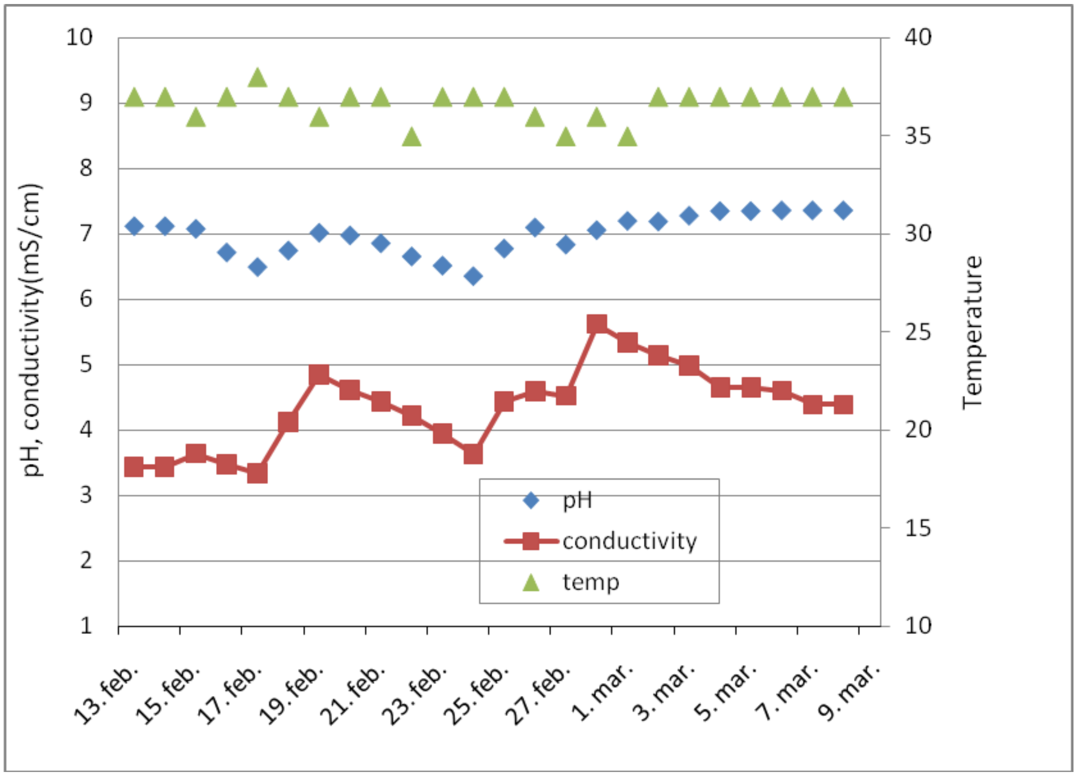


Figure 4.1. Temperature, Conductivity, and pH during Experiment 1

pH

The pH, being a very important parameter in the operation of anaerobic process, was maintained within the range of 6.5 and 7.5. In cases of pH dropping below 6.5, a buffer solution was added. Based on the experience of the previous experimenter, addition of sodium hydroxide for this purpose caused a huge change in the pH and could not be controlled easily, hence $NaHCO_3$ was used. On three occasions during the experiment, the pH was observed to be less or almost less than 6.5. This drop in pH could be attributed to the high accumulation of volatile fatty acids. This may also result to low gas production as the methanogens are known to be very sensitive to pH changes. On 18th February, the pH dropped to 6.5 resulting to a little increase in the VFAs concentration, however there was no negative effect on the gas production. But on the 24th February, the pH dropped from 6.52 to 6.36 on 25th February despite the addition of buffer solution. This pH change affected both the gas production and the VFAs concentration drastically as the value of the former reduced to nothing and the latter increased so much. From this point, there was no more gas production. The pH change with gas production is illustrated in Figure 4.3.

Temperature

The temperature remained almost constant (Figure 4.1) throughout the experiment period and was well maintained within the range of 35-38 °C.

Conductivity

From Figure 4.1, it can be seen that the conductivity reduced with time except on few occasions when the reactor was buffered causing a slight increase on the value. Generally the conductivity value throughout the experiment period ranged from 3.34 - 5.62 mS/cm.

TSS and VSS

From the data on Table 4.1 and information on Figure 4.2, the TSS and VSS were more or less constant during this period of experiment hence the reaction can be said to have reached a steady state condition.

Table 4.1. Average results obtained at different organic loading rate

Period	**Organic loading rate kg COD/m³·d**	**SRT days**	**pH**	**TSS mg/l**	**VSS mg/l**	**VFA mg/l HAc**	**Alkalinity mg/l$CaCO_3$**	**Gas production ml**	**COD removal %**
14 feb-15 feb	0.375	40	7.12	471	268	1066.2	815.7	20	96.9
16 feb-21 feb	0.75	20	6.84	471	323	934.3	588.7	70.8	93.4
22 feb-06 mar*	1.125	13.3	6.97	401	249.3	1264.3	1194.2	10	88.5
7 mar-9 mar	1.5	10	7.36	400	244	906.7	1350.3	13	88.1

***** From 27th march, there was relatively no gas production any longer

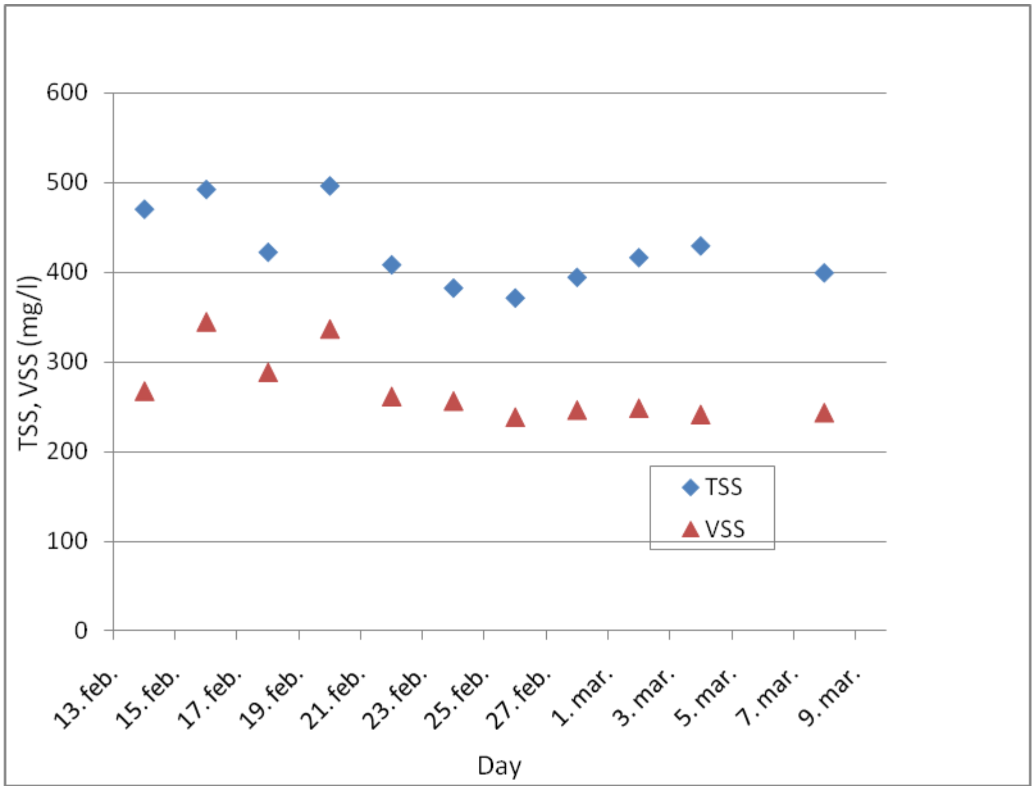


Figure 4.2 TSS and VSS concentrations in experiment 1

Recalling that this experiment was a continuation of one that has been started over 3 months ago, the suspended solids have decreased over time before reaching a steady state condition. Also the low value can be attributed to the fact that there was no means of biomass retention as the treated wastewater was withdrawn along with the biomass on daily basis. This can also be prevented or controlled by incorporation of the anaerobic reactor with a membrane system, which will result to biomass retention and hence an efficient performance of the anaerobic reactor system [5].

Gas production

In the experimental setup, there was no analysis of gas collected and hence couldn't put into consideration the generated gas compositions and concentration in the investigation. Only the gas level in the graduated cylinder was monitored. It was also assumed that the gas being primarily monitored was methane as calcium hydroxide was added for CO_2 capture.

The gas production decreased over time and more gas was produced during the 2nd period (Table 4.1), with peak production observed on the 18th and 19th February. Low pH value is usually believed to account for low optimal performance of methanogens in gas generation; however this was not the case as can be seen in Figure 4.3 when low pH value (6.5) was observed on the peak gas production day (18th February). It is also important to point out here that the pH measurement was usually done after the corresponding gas production had taken place hence a drop in pH may be observed after an optimum pH must have resulted to an optimum gas production.

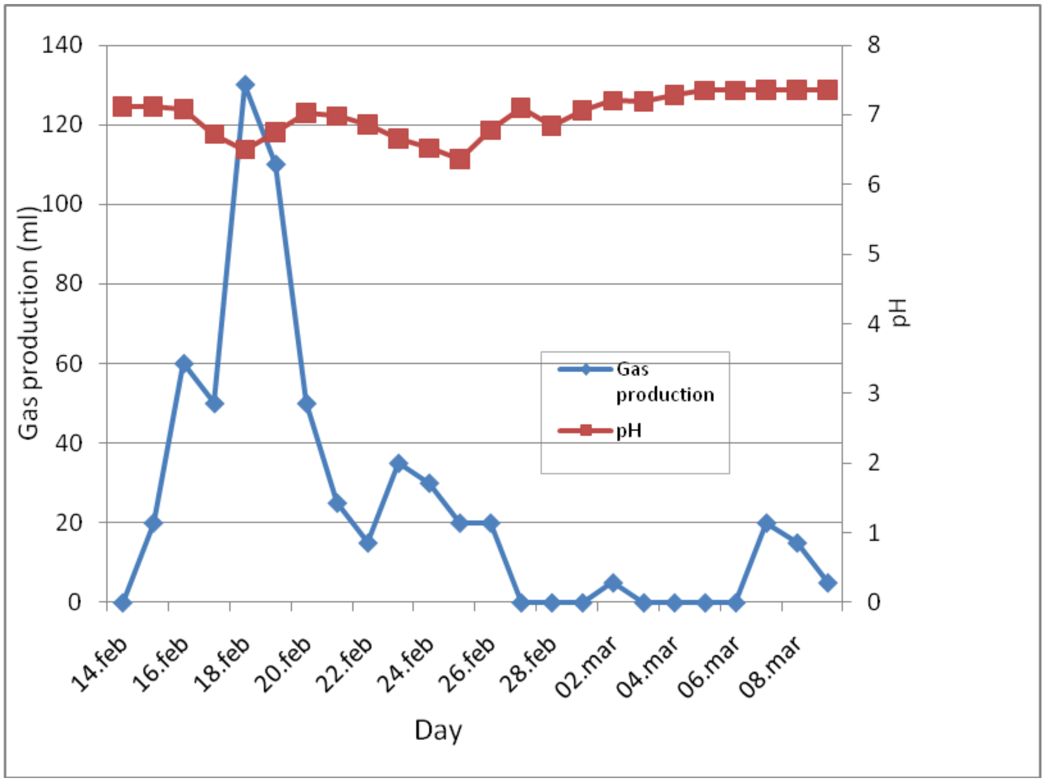


Figure 4.3 pH effect on gas production in experiment 1

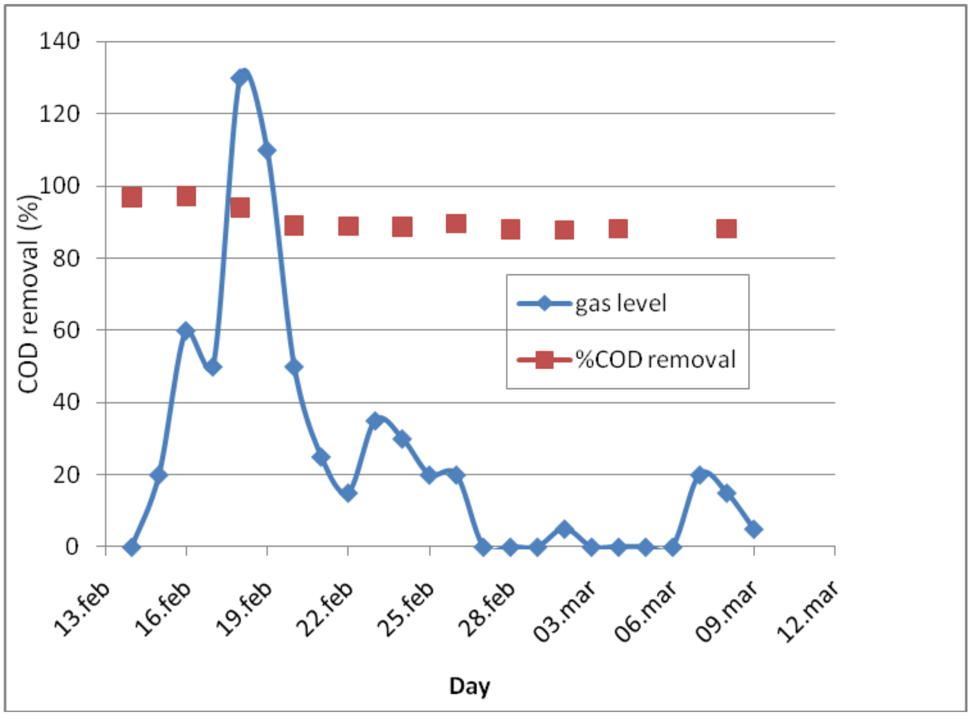


Figure 4.4 COD removal rate and gas production level in experiment 1

These two parameters (% COD removal and gas production) determines the effectiveness of an anaerobic digestion process. It is expected from the viewpoint of any wastewater treatment or biodegradation process that COD be reduced. In experiment 1, it was found out that there was an efficient removal of COD. The COD of the untreated glycol wastewater was measured to be about 15000 mg/l and after undergoing a treatment process,it was observed to have dropped significantly. In Figure 4.4, the COD removal was maximum after the second day of the experiment and gradually declined to almost a constant. This shows the efficiency of anaerobic treatment in terms of COD removal. It was also observed that even at low gas production, the COD removal was still good enough. On the other hand, gas production, which makes anaerobic treatment a unique one from other treatment processes like activated sludge system, was also found to be very significant at the beginning of the experiment. The gas production increased from the very beginning of the experiment until the 5th day when it reached peak production and then started declining until there was no longer activity from the methane producing bacteria. Accumulation of acid usually limits the activity of the methanogens responsible for the methane gas generation and in turn results to low or no gas production. However this stop in gas production could not be attributed to acid accumulation as the pH remained at optimum condition (Figure 4.4) during the decline and eventual stop of gas production.

VFAs and Alkalinity

Fermentation process results to formation of various short chain fatty acids which are then converted to methane in the methanogenesis stage. During the experiment, the concentration of these SCFA were measured by the titration method and monitored to ensure that the concentration does not increase beyond an acceptable level as this will result to accumulation of acids and imbalance in the anaerobic reactor. However, in this experiment, specific measurement and monitoring of various SCFAs was not carried out, hence this limited our knowledge of the specific SCFA. For example, propionic acid may exhibit partial toxicity to the methanogenic bacteria [13].

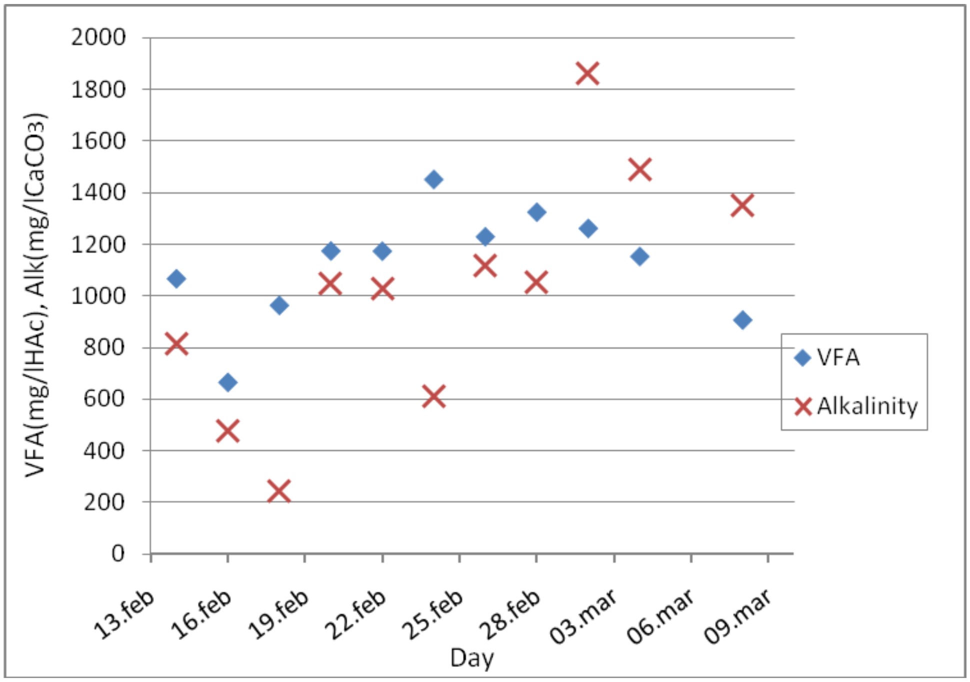


Figure 4.5 VFA concentration and Alkalinity in experiment 1

From Figure 4.5, it can be observed that throughout the experiment period, the VFA concentration was always above the alkalinity except on the last three days of the experiment when it was the reverse, and at this period the reactor had already stopped gas production. This result is actually in contrast with some of literatures that says that the ratio of VFA concentration to bicarbonate alkalinity of more than 0.8 will likely result to a severe drop in pH and imbalance in the reactor.

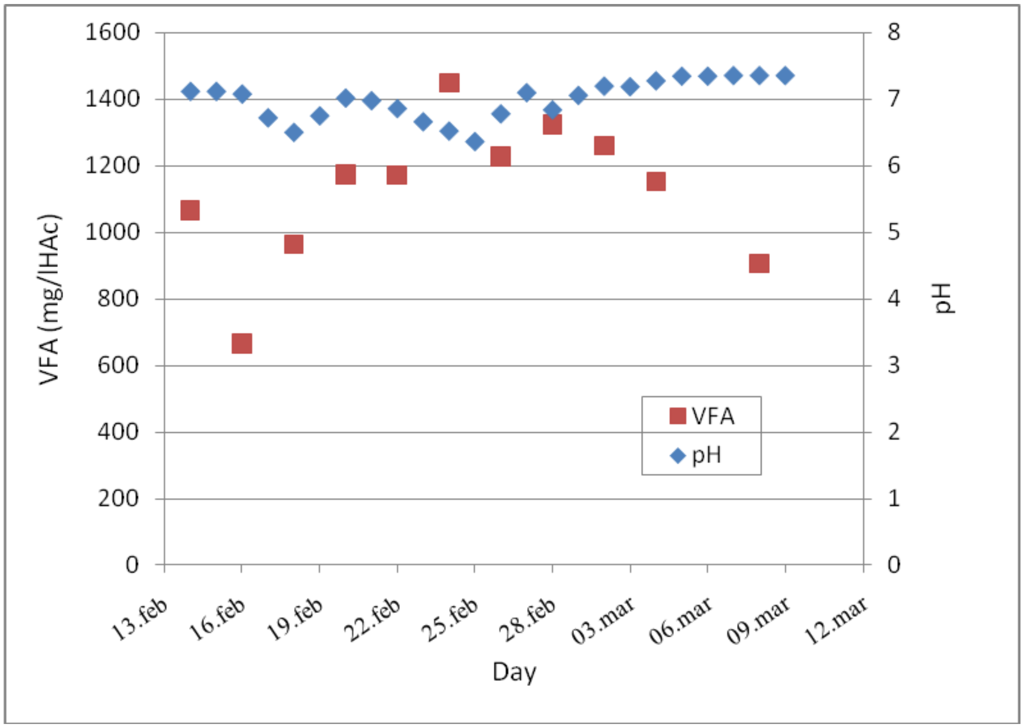


Figure 4.6 Effect of VFA concentration on pH changes in experiment 1

The eventual failure of the reactor system could not be said to have been caused by the accumulation of acid as it can be clearly observed from Figure 4.6 that the VFA concentration had no significant negative effect on the pH. The increase and the deecrease of the VFA concentration did not cause the pH to go out of the optimum condition as there was enough alkalinity to maintain a stable pH condition (Figure 4.5). However, on the 24th february, the VFA concentration of 1449.4 mg/l HAc resulted to the drop in pH to 6.36 the next day. This was controlled by addition of buffer solution which resulted to the VFA concentration decline and pH rise to within optimal range.

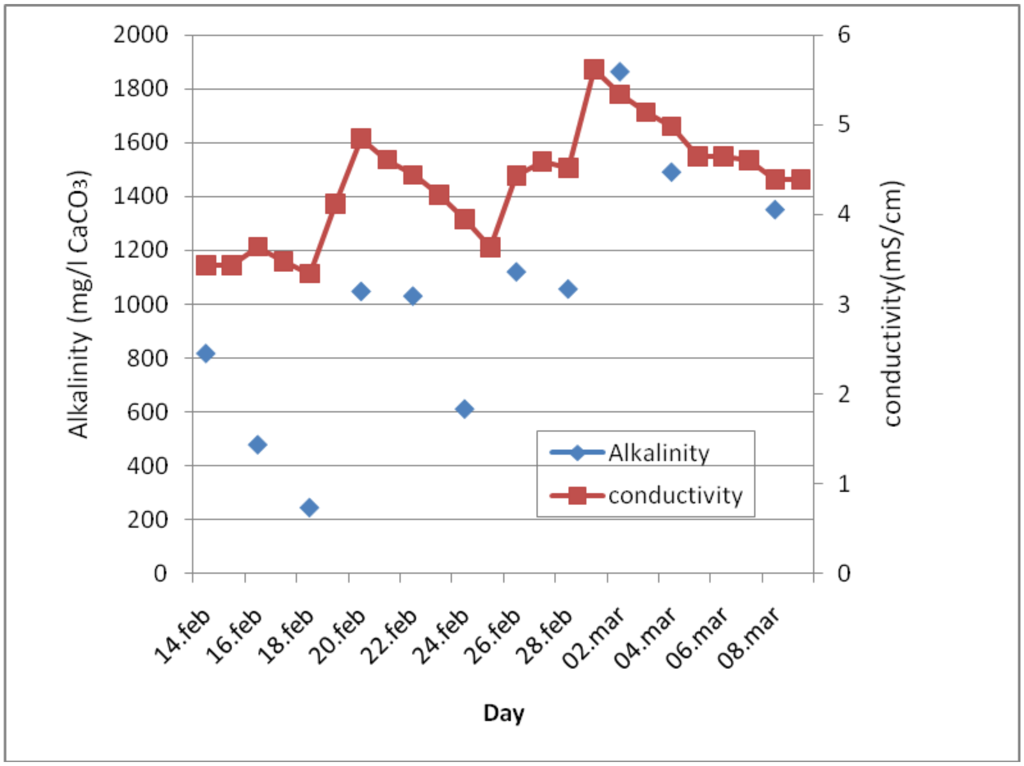


Figure 4.7 Effect of Alkalinity changes on the conductivity in experiment 1

Alkalinity is primarily a function of carbonate, bicarbonate, and hydroxide content and is taken as an indication of the concentration of these constituents [32]. Its increase results to increase in the conductivity and vice versa. From Figure 4.7, both parameters were observed to be uniform in their changes. The two peaks in the figure were due to the addition of buffer solutions which resulted to the alkalinity and conductivity increase.

4.2 Experiment 2 (15th March to 11th April 2011)

Experiment 2 was started from the scratch and like in experiment 1, the same conditions were being monitored and controlled. Being a new system, some of the results obtained were a little bit different from the first experiment.

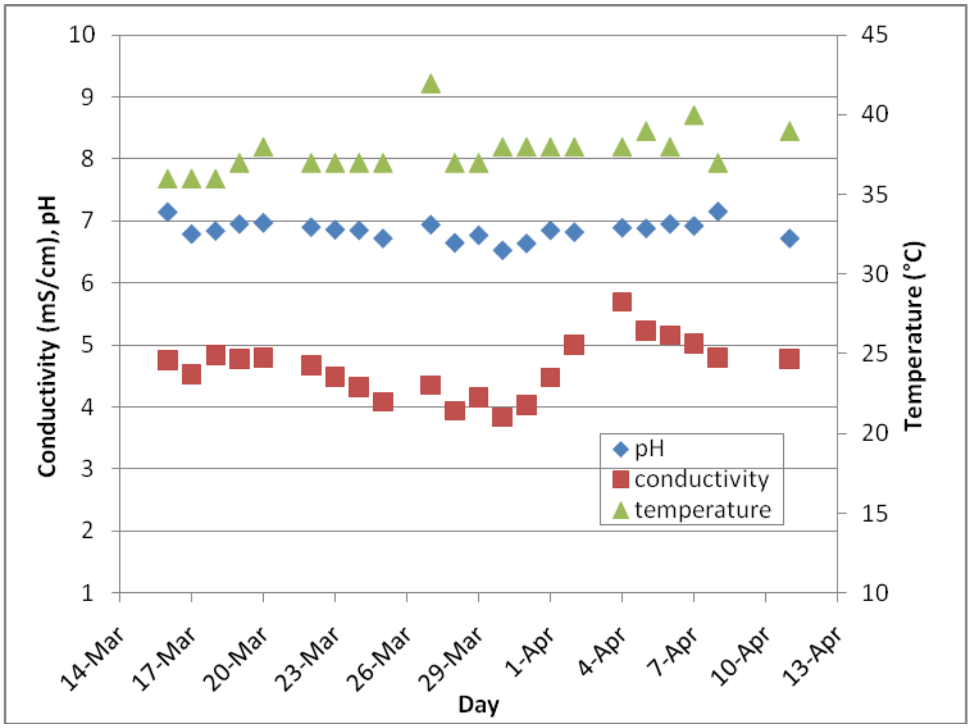


Figure 4.8 Temperature, pH, and Conductivity during Experiment 2

From Figure 4.8, the pH can be seen to be nearly constant throughout the experiment and was more stable than in the first experiment (Fig 4.1). However, between the 28th – 30th march, the pH varied within the range of 6.5- 6.7, hence buffer solution was added to address the problem and to prevent the pH from dropping below the optimum.

Temperature condition was very stable throughout the experiment period and the average daily temperature was 37.72, which is within the optimum temperature range for a good performance of an anaerobic reactor.

The conductivity was also stable and remained almost to a constant level during the first 10 days of the experiment. However it increased steadily from 3.83 on 30th march to 5.69 on 4th April. The increase during this period was due to the buffer solution added at this time to maintain the pH condition. In comparison, the conductivity results in experiment 2 could be said to be more stable than that of experiment 1.

Therefore, in general, the operating condition was well maintained to optimum in experiment 2 than in experiment 1.

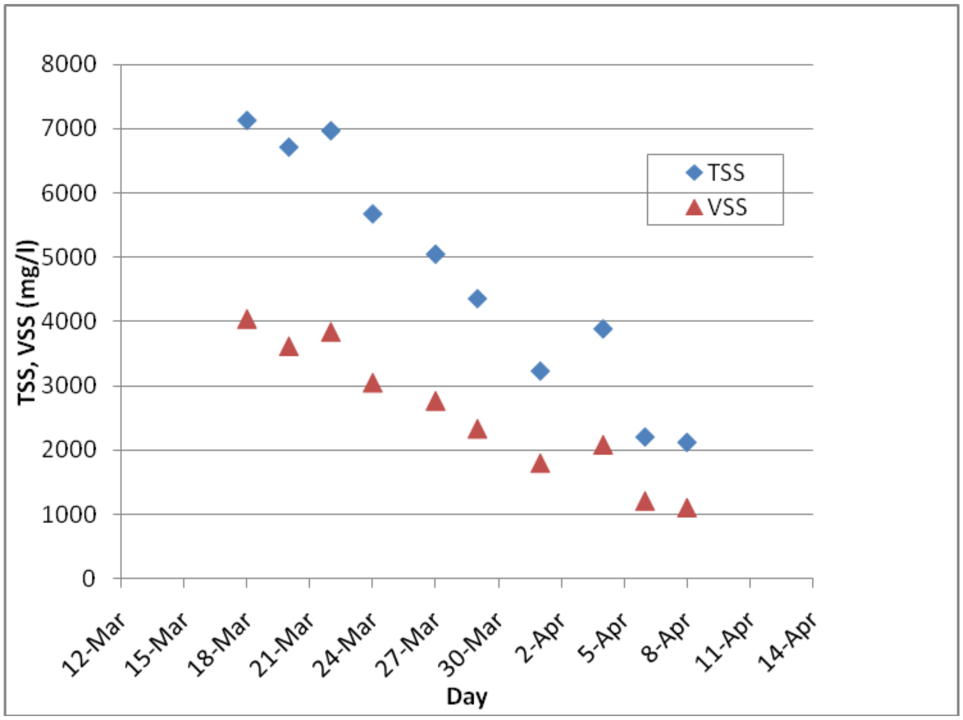


Figure 4.9 TSS and VSS concentrations in experiment 2

During experiment 2, the TSS and VSS concentration were steadily decreasing from the first day of the experiment and never reached a steady state before the failure of the system. This was in contrast to experiment 1 where steady state has already been reached and the TSS and VSS were more or less constant.

Gas production

It was important to note from Figure 4.10 that gas production commenced less than 24 hours after the start-up of the reactor. This was not initially expected recalling that in experiment 1 where the wastewater was not fed immediately with the intention of giving the anaerobes more time to acclimatize to the system. There was an observed increase in gas production after the first 2 days after which the gas production declined steadily until on 22nd March when it increased again. In relation with pH as shown in Figure 4.10, it could be said that pH was almost constant during the period,hence pH change had no remarkable effect on the methanogens responsible for methane gas generation.

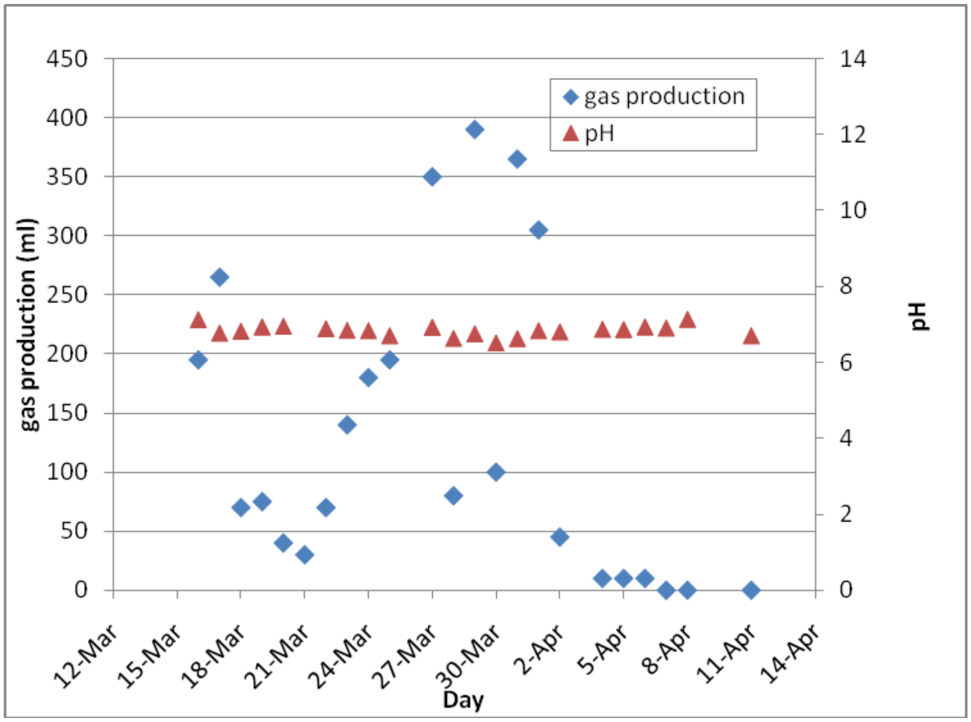


Figure 4.10 Gas production and pH effect in experiment 2

Table 4.2 Average results obtained at different organic loading rate during experiment 2

Period	**Organic loading rate kg COD/m³·d**	**SRT days**	**pH**	**TSS mg/l**	**VSS mg/l**	**VFA mg/l Hac**	**Alkalinity mg/lCaCO₃**	**Gas production ml**	**COD removal %**
16 mar-24 mar	0.75	20	6.91	6613	3645.38	120.85	1085.1	118.3	79.19
25 mar-03 apr	1.5	10	6.74	4205.5	2306.67	707.1	1108.3	228.75	69.31
04 apr-11 apr*	0.75	20	6.92	2732.17	1472.17	1500.95	1246.08	5	52.85

***** From 7th April, gas production stopped

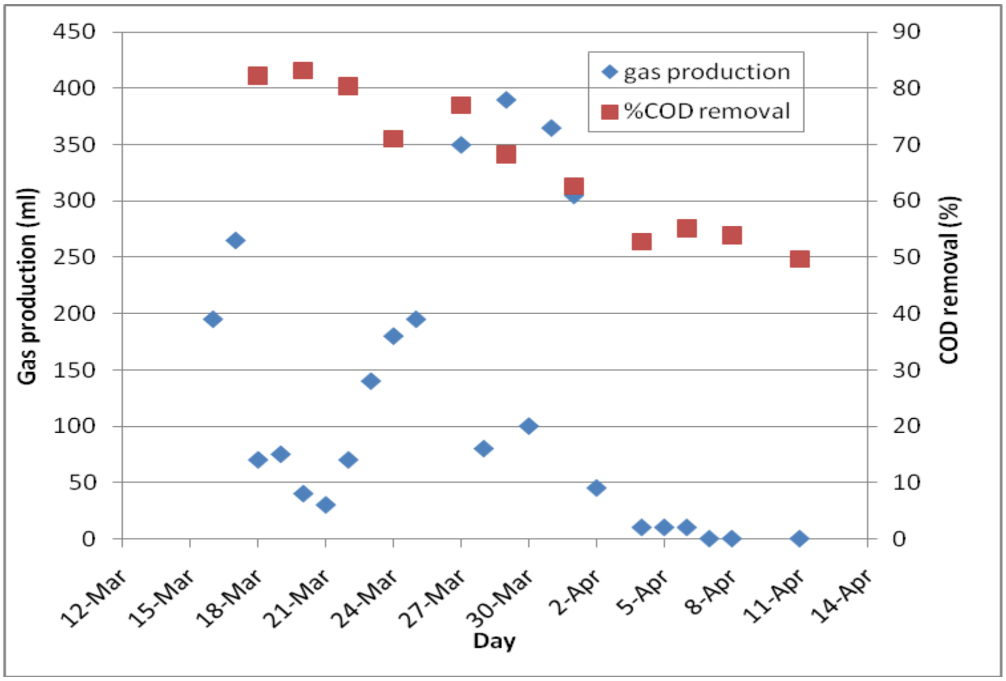


Figure 4.11 Gas production and % COD removal during experiment 2

Like the gas production, the % COD removal was high (78 %) at the beginning as shown in Figure 4.11. This gradually decreased with time to almost 50% when there was no longer gas production. As compared to experiment 1 in Figure 4.4, the COD removal efficiency was better than that of experiment 2.

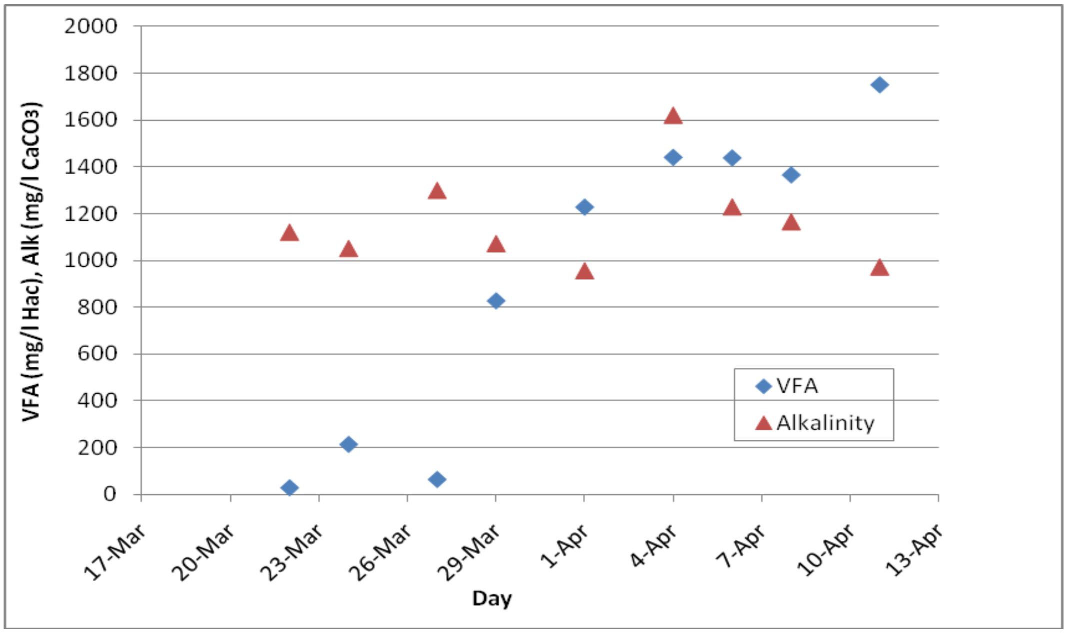


Figure 4.12 VFA concentrations and Alkalinity during experiment 2

At the beginning of the experiment, the concentration of VFA was very low as indicated in Figure 4.12. This can be attributed to the fact that there was a little delay in the acidogenesis process for SCFA formation or that the rate of acetogenesis was very fast. But since there was gas production at this period, it could then be that the VFAs were quickly converted to acetic acid. As time went on, the VFA concentration increased and went on to be higher than the alkalinity from the 6th april.

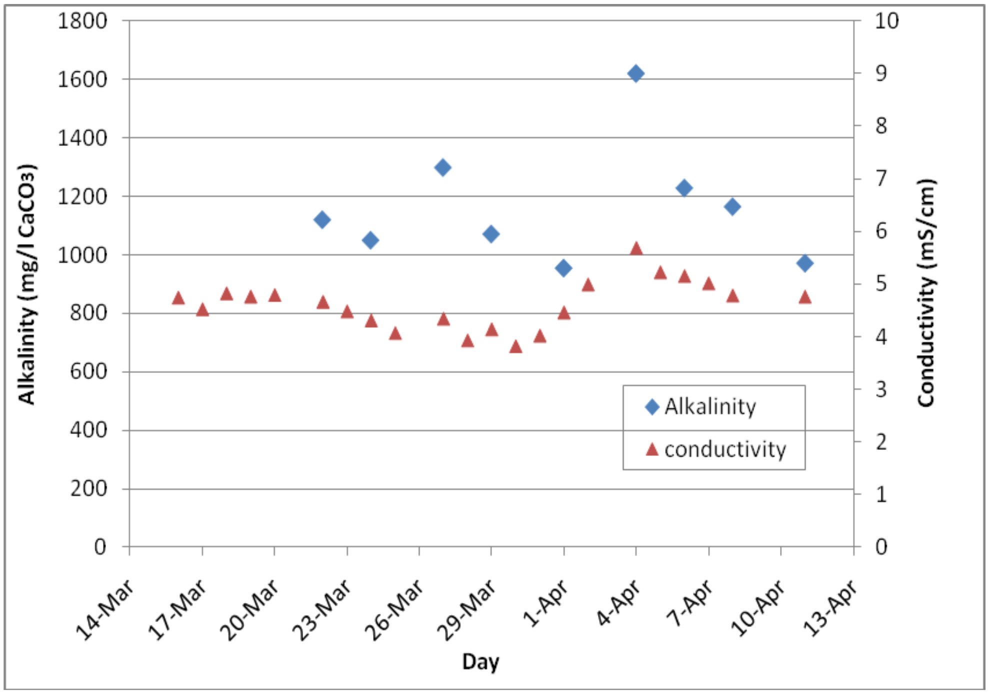


Figure 4.13 Alkalinity changes with conductivity in experiment 2

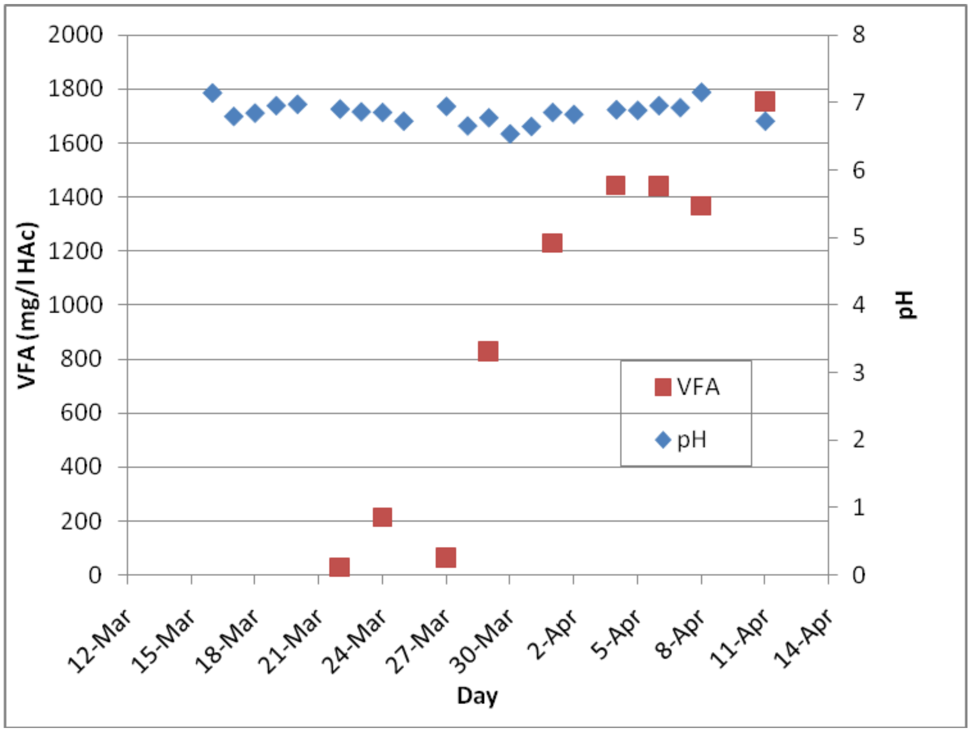


Figure 4.14 VFA concentration effect on pH changes during experiment 2

From Figure 4.14 and similar to experiment 1, it was difficult to say that the reactor failed as a result of VFA accumulation since the large increase in VFA concentration had no significant effect on the pH changes. As the VFA increased, the pH still remained almost constant. This was due to the availability of enough alkalinity to stabilize the pH change.

4.3 COD Mass Balance

COD is generally taken as a control tool to operate an anaerobic system since a perfect mass balance can only be made by using the COD as a parameter [21].

$$COD_{in} = COD_{out} \tag{4.1}$$

For practical purposes, Eq. 4.1 should be expanded to the various outlets of the anaerobic reactor as depicted in Figure 4.15.

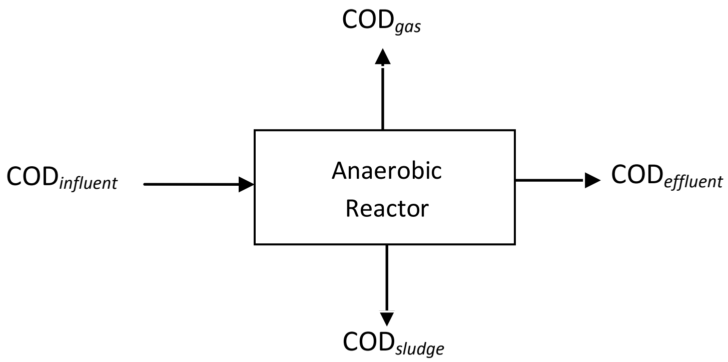


Figure 4.15 COD balance of an anaerobic reactor [21]

$$COD_{influent} = COD_{effluent} + COD_{gas} + COD_{sludge} + \text{Loss} \tag{4.2}$$

Here, COD_{gas} could be referred to as $COD_{methane}$ since a system of CO_2 absorption was incorporated in the experimental set up hence methane was assumed to be the major gas produced. Loss could be due to analytical error from COD measurements or experimental error. When the COD input equals the COD output, it shows that the anaerobic reactor is functioning optimally. Therefore COD mass balance can be used as a tool to monitor reactor performance.

To determine the COD equivalence of methane, the amount of oxygen required to completely oxidize 1 mole of CH_4 at STP is calculated. The balanced reaction is:

$$\underset{16}{CH_4} + \underset{64}{2O_2} \rightarrow \underset{44}{CO_2} + \underset{36}{2H_2O} \tag{4.3}$$

The COD of methane is 64 gCOD/16 gCH_4 or 4 gCOD/gCH_4. The complete metabolism of 1 kg of COD will produce 0.25 kg of CH_4. The number of moles of CH_4 produced will be 250 g/16 g = 15.6 moles. The volume of 1 mole of gas is 22.4 L. The total volume of gas produced per kg COD converted is then 22.4 L/mole × 15.6 moles = 349 L = 0.35 m^3 CH_4/kg COD.

Based on rough calculations, anaerobic reactor in experiment 2 performed better than that of experiment 1. The observed maximum gas production during experiment 1 (at 130 ml) is equivalent to 0.3714 gCOD as CH_4. Using the COD mass balance equation in Eq. 4.2, the COD loss is 0.94 gCOD (mass balance of 34.4 %). While at maximum gas production in experiment 2 (390 ml), which is equivalent to 1.11 gCOD as CH_4, COD loss of about 0.49 gCOD (COD mass balance of 83.67 %) is obtained.

The average COD mass balance in experiment 1 shows that about 2.26 % of the COD fed to the system was converted to biomass and a mass balance of 18.67 % obtained. In experiment 2, 16.4 % of COD load was converted to biomass while a mass balance of 64.6 % obtained.

Based on this COD mass balance, both experiments could be said not to have performed optimally and this can be attributed to inaccurate COD measurements, loss of gas during the experiment, presence of other gases not taken into account in the COD balance and other factors. On the other hand if the COD input and output (apart from COD_{gas}) were accurately determined, then expected amount of gas to be generated from the anaerobic reactor can be calculated theoretically.

4.4 General Discussions

The obtained result indicates, despite the failure of the anaerobic reactor, a significant % removal of COD. In both experiments there were high %COD removal reaching a maximum of 97.1 % and 83.13 % in experiment 1 and 2 respectively. This maximum %COD removal was recorded during the early period of the experiment. Hence the system was working most effectively, as regards to COD removal, during the early stages of the experiments.

Due to the complexity of anaerobic process, much attention is usually given to the operational and environmental conditions for an optimum performance of anaerobic reactor. Based on reported researches and experience, some of these operational parameters and conditions, listed in Chapter 2, significantly affect the system resulting to incomplete methanogenesis, accumulation of VFA, drop in pH value and alkalinity.

Among all the factors, pH inhibition has always been in the forefront as the major influence in performance of anaerobic process. Results show that the pH throughout the experiment 1 & 2 period was within the pH range (6.5 – 7.5) for optimum condition. Hence the failure of the system cannot be attributed to pH inhibition. The results indicate that the minimum pH recorded were 6.36 in experiment 1 and 6.53 in experiment 2. Despite the observed increase in VFA concentration in experiment 2, it was not enough to reduce the pH below the optimum as there was enough alkalinity.

At the failure of the first experiment, it was suspected that maybe there was high sulphur content which may cause sulphide toxicity; hence the composition of macronutrients added was modified in experiment 2. Despite this, there was still a system failure in experiment 2.

This over emphasis on pH inhibition most times causes ignorance on other factors which might be very important for effective anaerobic treatment. For instance, organic shock loading was a factor not well considered during the period of both experiments. In both experiments, the system was observed to be working fine both in COD removal and gas production until there was increase (in this case excess) in the organic loading. Also high withdrawal rate with respect to reactor content or low retention time can result to loss of biomass in the reactor. According to Grady (1999), not more than 5 % of reactor content should be withdrawn one at a time.

Detailed investigation and analysis were required for the wastewater characterization as this would help identify some compounds or substances that may be toxic to the anaerobic process.

TITRA 5 method used in the analysis of alkalinity and VFA content did not give a detailed result on the content of specific fermentation products rather it was measured in mg/l as HAc. Knowledge of these specific fatty acids could have also been important in the entire process. At neutral pH, propionic acid exhibits partial toxicity to methanogenic bacteria at a concentration of 1000 mg/l, and also appears to retard acid forming bacteria in sewage sludge digestion [12].

The failure of the system could have been due to the presence of toxic compounds, for example; hydrogen sulphide and ammonia. The presence and concentration of these gases and other possible toxic gases could have been detected if there was an analysis on the gas generated from reactor system. The system failure may also have been due to lack of nutrients especially when domestic wastewater from IVAR was used as the nutrient source. When prepared nutrients solution was used as the nutrient source, it was possible to determine the required amount based on the calculated requirement for growth according to the COD of glycol wastewater added. But this was not the case when IVAR domestic wastewater was used. It could have also been as a result of high loading rate. Hydraulic overloading could result to biomass washout while organic overload may also result to VFA accumulation. Both conditions, in turn, could lead to process failure.

5. CONCLUSIONS

Based on the information reported and results obtained from this study, anaerobic treatment will present a future viable treatment method for handling of produced water or wastewater contaminated with glycol. An optimal performance of an anaerobic reactor system can only be achieved by maintaining and controlling important factors such as pH, temperature, organic loading, nutrient requirements, and alkalinity. As observed from the experiment, frequent monitoring is very important for a good performance.

Result from these experiments shows that the pH condition throughout the experiment 1 & 2 period was within the pH range (6.5 – 7.5) for optimum condition. Hence, pH inhibition was not a problem and was not the cause of failure.

In both experiments there were high %COD removal reaching a maximum of 97.1 % and 83.13 % in experiment 1 and 2 respectively. This maximum %COD removal was recorded during the early period of the experiment. It was observed that the COD removal efficiency on average were 91.73 % and 67.12 % in experiment 1 and 2 respectively.

Also, on average, COD mass balances of 34.4 % and 83.67 % were obtained during maximum gas production in experiment 1 and experiment 2 respectively. Hence, with respect to the COD mass balances, the reactor in experiment 2 performed better than in experiment 1. This imbalance in COD could be attributed to inaccurate COD measurements, loss of gas during the experiment, presence of other gases not taken into account in the COD balance and other factors.

Despite some of these possible deficiencies and drawbacks during the experiment, significant results were obtained from the COD removal and gas generation. To prevent anaerobic process failure, there is the need for close control of the system and awareness of presence of potential toxic compounds in the wastewater.

On a larger scale, anaerobic treatment technology has the potential for energy savings if used instead of aerobic treatment.

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Appendix

Table A1 Results in Experiment 1

day	temp	gas level	pH	glycol	TSS avg	VSS avg	K	COD	VFAs	Alkalinity	%COD	nutrients
	^{o}C	(ml)		ml	(mg/l)	(mg/l)	mS/cm	(mg/l)	mg/lHAc	mg/lCaCO3	removal	
14.feb	37	0	7.12	50	471	268	3.43	186	1066.2	815.7	96.9	10
15.feb	37	20	7.12				3.43					
16.feb	36	60	7.08	100	493	345	3.64	348	666.1	476.8	97.1	
17.feb	37	50	6.72	100			3.48					
18.feb	38	130	6.50	120	423	289	3.34	740	963.3	242.9	94	
19.feb	37	110	6.75	100			4.12					
20.feb	36	50	7.02	100	497	337	4.85	1352	1173.5	1046.3	89	
21.feb	37	25	6.98				4.61					
22.feb	37	15	6.86	150	409	262	4.44	1377.7	1172.5	1028.9	88.8	
23.feb	35	35	6.66	150			4.22					
24.feb	37	30	6.52		383	257	3.95	1385.2	1449.4	609.4	88.75	
25.feb	37	20	6.36	150			3.63					
26.feb	37	20	6.78	150	372	239	4.43	1288	1228.3	1119.0	89.5	
27.feb	36	0	7.10				4.59					
28.feb	35	0	6.84	150	395	247	4.52	1479.1	1323.2	1055.4	87.99	
01.mar	36	0	7.06	150			5.62					3
02.mar	35	5	7.20	150	417	249	5.34	1510	1260.4	1862.7	87.7	3
03.mar	37	0	7.19	150			5.14					2
04.mar	37	0	7.28		430	242	4.98	1464.4	1152.1	1489.6	88.1	
05.mar	37	0	7.35	150			4.65					2
06.mar	37	0	7.35				4.65					
07.mar	37	20	7.36	200			4.60					4
08.mar	37	15	7.36		400	244	4.39	1461.7	906.7	1350.3	88.1	
09.mar	37	5	7.36				4.39					

Table A2 Results in Experiment 2

day	**glycol**	**temp**	**pH**	**gas level**	**TSS avg**	**VSS avg**	**K**	**COD**	**% COD**	**VFAs**	**Alkalinity**	**nutrients**
	(ml)	**°C**		**(ml)**	**(mg/l)**	**(mg/l)**	**mS/cm**	**(mg/l)**	**removal**	**mg/lHAc**	**mg/lCaCO3**	**(ml)**
initial	200		7.20	0	1625	1070	4.63	3196	78.13			1340
16-Mar	100	36	7.14	195			4.75					
17-Mar	100	36	6.79	265			4.53					
18-Mar	100	36	6.84	70	7120	4050	4.83	2592.5	82.26			
19-Mar	100	37	6.95	75			4.77					
20-Mar	100	38	6.97	40	6705	3625	4.80	2465	83.13			
21-Mar	100			30								
22-Mar	100	37	6.90	70	6960	3850	4.67	2873	80.34	28.1	1119.9	
23-Mar	100	37	6.86	140			4.49					
24-Mar	100	37	6.85	180	5667	3056.5	4.32	4233	71.04	213.6	1050.3	
25-Mar	200	37	6.72	195			4.08					30
26-Mar												
27-Mar	200	42	6.94	350	5041.5	2775	4.35	3357.5	77.03	63.6	1298.5	
28-Mar	200	37	6.65	80			3.94					30
29-Mar	200	37	6.77	390	4350	2340	4.15	4641	68.25	828.3	1071.3	
30-Mar	200	38	6.53	100			3.83					4
31-Mar	200	38	6.64	365			4.03					4
1-Apr	200	38	6.85	305	3225	1805	4.47	5457	62.66	1229.5	955.2	6
2-Apr	200	38	6.82	45			5.00					7
3-Apr												
4-Apr	100	38	6.89	10	3880	2090	5.69	6908	52.74	1442.7	1619.6	2
5-Apr		39	6.88	10			5.23					30
6-Apr	70	38	6.95	10	2200	1216.5	5.16	6556	55.15	1440.4	1228.3	30
7-Apr		40	6.92	0			5.02					
8-Apr	100	37	7.15	0	2116.5	1110	4.79	6743	53.87	1367.5	1164.7	4
11-Apr	100	39	6.72	0			4.77	7359	49.65	1753.2	971.7	

Table A3 Glycol wastewater characterization

Dato		01/08/2008	17/12/2008
Analyse rap.	NOV	028836-08	058407-08
Kasium Ca oppsluttet	mg/L	93	90.9
Kalium K oppsluttet	mg/L	5.5	6.6
Magnesium Mg oppsluttet	mg/L	14	11.5
Natrium Na oppsluttet	mg/L	260	587
Svovel S oppsluttet	µg/L	6700	4000
Jern Fe oppsluttet	mg/L	42	607
Aluminium Al oppsluttet	µg/L	1000	326
Arsen As Oppsluttet	µg/L	2.9	15
Barium Ba oppsluttet	µg/L	280	634
Kadmium Cd oppsluttet	µg/L	<0.4	0.29
Kobolt Co oppsluttet	µg/L	5.4	12
Krom Cr oppsluttet	µg/L	76	234
Kobber Cu oppsluttet	µg/L	5.1	91
Mangan Mn oppsluttet	µg/L	440	5880
Nikkel Ni oppsluttet	µg/L	37	78
Bly Pb oppsluttet	µg/L	4.1	30
AntimonSb oppsluttet	µg/L	<4	2.5
Tinn Sn oppsluttet	µg/L	<2	11
Vanadium V oppsluttet	µg/L	2.2	4.1
Molybden Mo oppsluttet	µg/L	7.7	40
Sink Zn oppsluttet	µg/L	160	146
Strontium Sr oppsluttet	µg/L	2000	3340
Silisium Si oppsluttet	µg/L	1200	1685
Fosfor total	mg P/L	0.23	0.28
Nitrogen total	mg N/L	31.5	39.5
TOC	mg/L	5400	5200
Metanol	mg/L		4500
Monoethylenglycol	mg/L		2600
Diethylenglycol	mg/L		
Triethylenglycol	mg/L		
Propylenglycol	mg/L		17
Sum PAH (16)	µg/L	440	200
Naftalen	µg/L	420	176
Acenaftylen	µg/L	1.1	0.818
Acennaften	µg/L	2.2	2.52
Fluoren	µg/L	10	7.9
Fenantren	µg/L	8.1	7.6
Antracen	µg/L	0.71	0.359
Fluoanten	µg/L	0.56	0.622

Pyren	µg/L	0.51	0.517
Benzo(a)antracen	µg/L	0.08	0.09
Crysen	µg/L	0.1	0.106
Benzo(b)antracen	µg/L	0.03	0.054
Benzo(k)antracen	µg/L	0.03	0.013
Benzo(a)pyren	µg/L	0.19	0.207
Indeno(1, 2, 3, cd)pyren	µg/L	<0.1	0.012
Dibenzo(a, h)antracen	µg/L	<0.1	0.004
Benzo(g, h,i)perylen	µg/L	<0.1	0.021
Fenoler som fenol	µg/L		51000
Kvikksølv, Hg	µg/L	1.23	2.3
Bensen	µg/L	17000	29000
Toulen	µg/L	9900	22000
Etylbensen	µg/L	700	1100
p,m-xylen	µg/L	3800	5500
o-xylen	µg/L	1400	2000
Ftalater:			
Dimetylftalat	ng/L	<100	<50
Dietylftalat	ng/L	<100	4900
Bensylbenzoat	ng/L	<100	<50
Diisobutylftalat	ng/L	165	370
Dibutylftalat	ng/L	335	510
Dimetoksyetylftalat	ng/L	<100	<50
Diisoheksylftalat	ng/L	<100	<50
Di-2-etoksyetylftalat	ng/L	<100	<50
Dipentylftalat	ng/L	<100	<50
Di-n-heksylftalat	ng/L	<100	<50
Bensylbutylftalat	ng/L	<100	<50
Heksyl-2-etylheksylftalat	ng/L	<100	<50
Dibutoksyetylftalat	ng/L	<100	<50
Disykloheksylftalat	ng/L	<50	<50
Di-(2-etylhexyl)ftalat	ng/L	663	2500
Diisononylftalat	ng/L	<100	<50
Di-n-oktylftalat	ng/L	<100	<50
Diisodekylftalat	ng/L	<1000	<1000
Suspendert stoff, SS	mg/L	25	200
pH	pH	4.6	5.4
Sulfid	mg/L	<0.02	0.03
Fenol	ng/L		
2-metylfenol	ng/L		11600000
4-metylfenol	ng/L		6690000
4-etylfenol	ng/L		670000
2,4-dimetylfenol	ng/L		1100000
3,5-dimetylfenol	ng/L		849000
4-n-proplyfenol	ng/L		157000
2,4,6-trimetylfenol	ng/L		55100

2,3,5- trimetylfenol	ng/L	82400
4-n-butylfenol	ng/L	7730
4-tert-butylfenol	ng/L	61200
4-isoproplyl-3-metylfenol	ng/L	3860
4-n-pentylfenol	ng/L	<10
2-tert-butyl-4-metylfenol	ng/L	<10
4-tert-butyl-2-metylfenol	ng/L	<10
4-n-heksylfenol	ng/L	368
2,5-diisopropylfenol	ng/L	<10
2,6-diisopropylfenol	ng/L	<10
2-tert-butyl-4-etylfenol	ng/L	23000
6-tert-butyl-2,4-dimetylfenol	ng/L	1870
4-n-heptylfenol	ng/L	<10
2,6-dimetyl-4-(1,1-dimetylpropyl)fenol	ng/L	<10
4-(1-etyl-1metylpropyl)-2-metylfenol	ng/L	<10
2,6diisopropyl-4-metylfenol	ng/L	<10
4-n-oktylfenol	ng/L	<10
4-tert-oktylfenol	ng/L	172
2,4-di-tert-butylfenol	ng/L	<10
2,6-di-tert-butylfenol	ng/L	<10
4-n-nonylfenol	ng/L	<10
2-metyl-4-tert-oktylfenol	ng/L	<10
2,6-di-tert-butyl-4-metylfenol	ng/L	<10
4,6-di-tert-butyl-2-metylfenol	ng/L	<10

Naftalen	ng/L	541000
C1-Naftalen	ng/L	431000
C2-Naftalen	ng/L	289000
C3-Naftalen	ng/L	10700
Phenantren	ng/L	9860
Antrasen	ng/L	805
C1-Phenantren	ng/L	7430
C2-Phenantren	ng/L	640
C3-Phenantren	ng/L	536
Dibenzotiophen	ng/L	3540
C1-Dibenzotiophen	ng/L	3690
C2-Dibenzotiophen	ng/L	710
C3-Dibenzotiophen	ng/L	506