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Writer: **Anissa Sukma Safitri**	..... (Writer's signature)

Faculty supervisor:

Roald Kommedal

External supervisor (s):

-

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BIOGAS POTENTIAL OF HIGH STRENGTH MUNICIPAL WASTEWATER TREATMENT IN LABORATORY SCALE UP-FLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTORS

MASTER'S THESIS

ANISSA SUKMA SAFITRI - [227432]

WATER SCIENCE AND TECHNOLOGY
ENVIRONMENTAL TECHNOLOGY STUDY PROGRAM
DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES
UNIVERSITY OF STAVANGER
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Abstract

The main focus of this study is investigating the effectiveness of anaerobic treatment of municipal wastewater for converting organic matter to methane production in anaerobic granular sludge reactors. In-house designed laboratory scale, up-flow anaerobic sludge blanket (UASB) reactor systems were set up for treating of high strength municipal wastewater treatment i.e. below 1200 mg COD/l under mesophilic condition (20 – 25 °C). Three UASB reactors were set up in the study; one reactor (Reactor A) used inactive granular sludge; and two reactors (Reactor I and Reactor II) used active granular sludge. Hydraulic retention time (HRT) applied was between 24 – 1.4 hours in Reactor A and 5.6 – 1.4 hours in Reactor I and Reactor II. Organic loading rate (OLR) was increased gradually from 1.0 g COD/l.d to 15.0 g COD/l.d. The obtained results indicate a significant COD removal efficiency and methane production in UASB reactors. Methane production rate increased with OLR, proportional to the amount of organic matter removed in the UASB reactors. However, in general, COD removal efficiency and methane yield decreased with increasing OLR and decreasing HRT. COD removal efficiency reached a maximum of above 70% in UASB reactors. At the lowest HRT applied (1.4 h) with 15.0 g COD/l.d of OLR, the COD removal efficiency was in the range of 48 – 65%. The optimum biogas potential was occurred in Reactor II; 70% COD removal efficiency was achieved at 3.3 h of HRT with 6.0 g COD/l.d of OLR and 3.0 g COD/l.d of COD removed was converted to methane. Overall methane yields obtained were 0.226 l CH_4/g COD, 0.224 l CH_4/g COD, and 0.286 l CH_4/g COD in Reactor A, Reactor I and Reactor II, respectively, at operating condition. Under these conditions, approximately 22.5 MWh/d of electricity and 121500 MJ/d of heat could be recovered at IVAR Grødaland which has approximately 5000 m^3/d of average hydraulic loading and an OLR of 6.0 g COD/l.d. Anaerobic treatment systems using UASB reactor for treating high strength municipal wastewater represents a feasible and an attractive alternative as pre-treatment for SBR units at IVAR Grødaland by reduction of the SBR inlet total COD to about 300-400 mg/l and conversion organic matter into economically valuable products as methane with 1.3 l CH_4/d of specific volume methane production.

Keywords: *anaerobic treatment, municipal wastewater, UASB reactor, COD removal efficiency, methane production*

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Stavanger, June 2016

Anissa Sukma Safitri

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Abbreviations

ADM1	Anaerobic Digestion Model No.1
AMB	Acetoclastic Methanogenic Bacteria
AMPTS	Automatic Methane Potential Test System
ATP	Adenosine Triphospate
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation
EGSB	Expanded Granular Sludge Blanket
DO	Dissolved Oxygen
F/M	Food Mass Ratio
HAc	Acetic Acid
HMB	Hydrogenotrophic Methanogenic Bacteria
HRT	Hydraulic Retention Time
IVAR	*Interkommunalt Vann Avløp og Renovasjon*
LCFA	Long Chain Fatty Acid
OHPB	Obligate Hydrogen Producing Bacteria
OLR	Organic Loading Rate
SBR	Sequencing Batch Reactor
SRB	Sulphate Reducing Bacteria
SCFA	Short Chain Fatty Acid
SRT	Solid/Sludge Retention Time
STP	Standard Temperature and Pressure
TN	Total Nitrogen
TP	Total Phosphorous
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid
WWTP	Wastewater Treatment Plant

1. Introduction

The continuously increasing production of municipal wastewater with increasing population is one of the main environmental issues of today's society. Municipal wastewater is defined as a combination of the liquid or water-carried wastes removed from residences, institutions, commercial business, industry, together with groundwater, surface water, and storm-water [1]. Many of wastewater treatment methods being developed are designed to deal with decreasing water quality and increasing wastewater quantity. The basic function of the wastewater treatment plant is to accelerate the natural processes by which water purifies itself. Several technological options are available today in the field of wastewater treatment, including aerobic treatment, direct anaerobic treatment, and a combination of anaerobic and aerobic treatment is applied [2, 3].

As the method of a resource preservation and environmental protection technology, the anaerobic treatment combined with other proper methods represents the advanced sustainable technology society needs [4]. Anaerobic treatments are applied initially mainly for food and beverage production. They have been utilized and developed over many centuries, in spite of the fact that the advance application have been achieved in the last few decades with the establishment of various development of high rate anaerobic wastewater treatment processes in which biomass retention and liquid retention are independent [5, 6].

High-rate anaerobic wastewater treatment, however, was developed for high strength industrial wastewater treatment, whereas domestic or municipal wastewaters are characterized as a dilute type of wastewaters. Normal strength municipal wastewater is characterized by the COD concentration of below 1000 mg/l. Besides, low and high strength municipal wastewaters are defined by the COD concentration of below 500 mg/l and over 1000 mg/l, respectively. The concentrated wastewater (high strength) represents cases with low water consumption and/or infiltration [6].

In the mid-seventies of the last century, by Lettinga and co-workers, anaerobic municipal wastewater treatment offers an effective alternative which was already recognized for treating municipal wastewater using e.g. up-flow anaerobic sludge blanket (UASB) reactors [3, 4, 6]. UASB reactors are frequently mentioned as proven pre-treatment systems for treating different industrial wastewaters, including those containing toxic or

inhibitory compounds. This process is also feasible for treatment of municipal wastewater under wide range of temperatures [7].

Low sludge production and high organic loading rates are some of many advantages of anaerobic treatment present over other biological treatments. Nevertheless, the main driver for the increased application of anaerobic processes is the energy and carbon recovery which have a positive net energy production. Furthermore, the biogas (methane) produced can also replace fossil fuel sources and therefore has a direct positive effect on greenhouse gas reduction [5]. It directly results from the ever rising energy prices and the overall concern on global warming [6]. At the same time, it removes the organic fraction from wastewater.

Successful implementation of the anaerobic treatment requires the retention of high levels of active biomass within the system. It allows the application of high organic loading rates, facilitating the use of compact and economical wastewater treatment plants. Currently, the extensive majority of full scale anaerobic waste treatment application are based on the development and maintenance of high sludge granules retention within the systems [8, 9]. Figure 1.1 shows the gradual increase in the number of worldwide installed high rate anaerobic reactors.

Anaerobic processes have been commonly operated under mesophilic condition at optimum methanogenic growth rate of 35 – 37 °C although the temperature of certain wastewater fractions might be either considerably warmer (e.g. pulp and paper industry) or cooler (e.g. landfills) [8, 10]. Treating these wastewaters at initial temperatures would be beneficial because of reduced resources (e.g. no heating or cooling required).

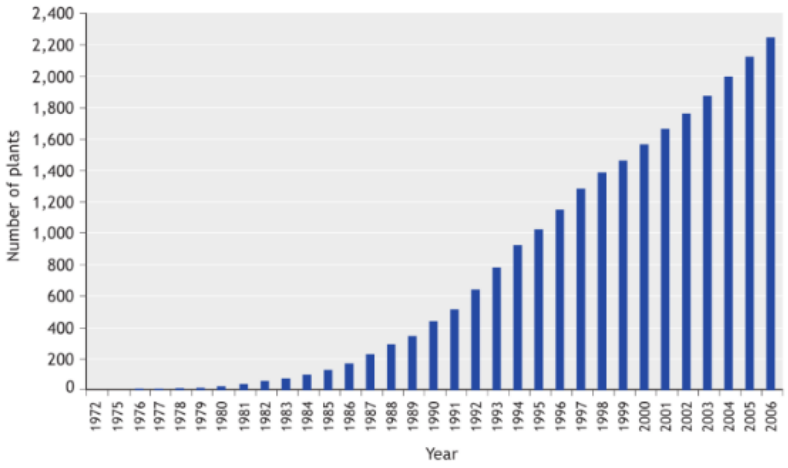


Figure 1.1 *Increase in number of worldwide installed high rate anaerobic reactors [6]*

Some researches have been done under various temperatures including temperature below 25 °C and promising results have already been reported in its studies. Anaerobic treatment of diverse wastewaters at low temperature (below 25 °C) has been proven feasible by laboratory scale trials of granular sludge based reactor systems [2, 7, 11, 12]. The basic advantage of this system is the retention of high levels of active biomass within the system so that the high organic removal can be achieved [11].

1.1. Scope of Work

This study was a part of project in cooperation with IVAR and conducted by Environmental Technology Study Program, University of Stavanger. IVAR *(Interkommunalt Vann Avløp og Renovasjon)* is a Norwegian public company that constructs and operates municipal facilities for water, wastewater and general waste. In this study, laboratory scale tests were conducted using UASB reactors for treating effluent wastewater from dissolved air flotation (DAF) units at Grødaland wastewater treatment plant (WWTP). Figure 1.2 shows the processes configuration at IVAR Grødaland. As reported by IVAR, the plant receives wastewater from several sources that are presented in Table 1.1.

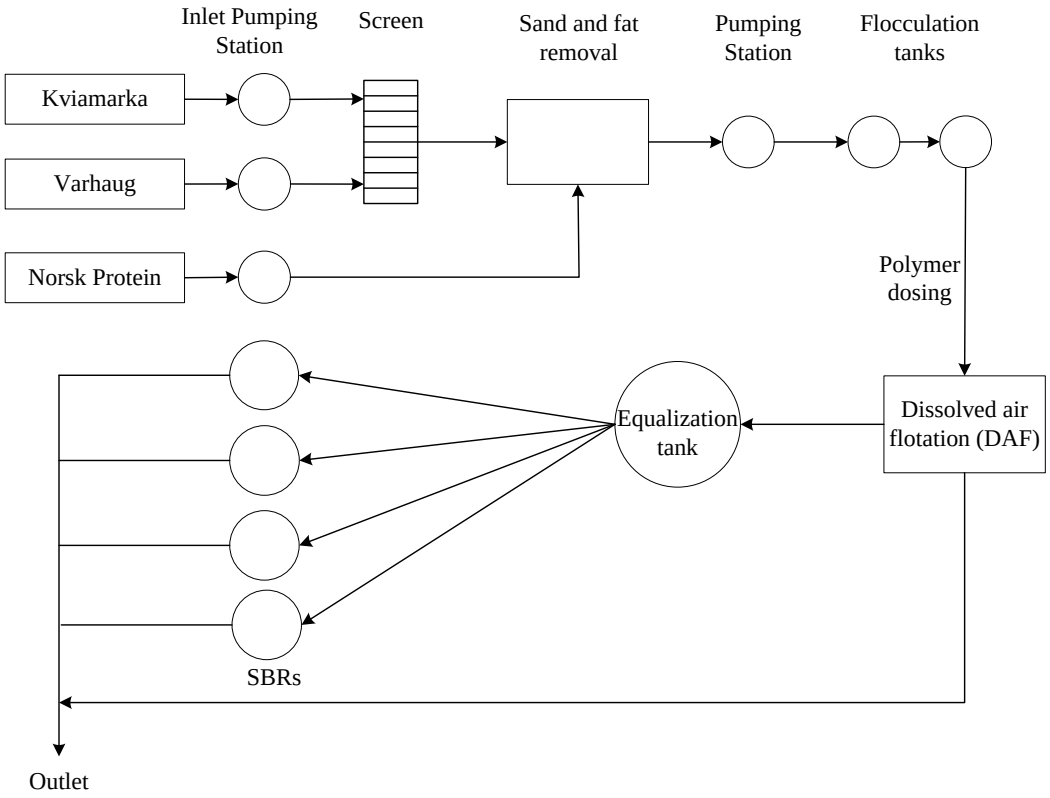


Figure 1.2 *The processes configuration in IVAR Grødaland*

Table 1.1 *Grødaland WWTP wastewater sources*

Wastewater Sources	Average Loading (m^3/d)
a. Animal destruction in Norsk Protein	167
b. Municipal wastewater of 3000 houses in Varhaug and food industry in Fjordland	1680
c. Dairy and chicken slaughtering in Kviamarka	3284
Total Loading	**5131**

The pre-treatments at IVAR Grødaland consist of: bar screen (3 mm opening), sand and grit removal, fat removal, and DAF. Two DAF units with a surface area of 48 m^2 each were designed for 7.5 m/h surface loading with maximum load of 200 l/s. In the DAF units, 30% of the effluent wastewater is recycled as dispersed water with 4 – 6 bar of back-pressure. There are six pressure pumps for each DAF unit and the number of pumps in operation depends on flow rate and temperature. The DAF unit can remove approximately 30 – 40% suspended solid. With polymer addition, the DAF units can achieve approximately 80% of suspended solid removal depending on dosing concentration.

The DAF units cannot remove high fractions of dissolved COD. As reported by IVAR (January – August 2015), the DAF units can remove approximately 20% of total COD with average effluent containing 1304 mg/l COD (of which 849 mg/l is dissolved COD). High concentration of dissolved COD affects the performance of the next stage of treatment, sequencing batch reactor (SBR) unit operations. The SBR process utilizes a fill-and-draw aerobic reactor with complete mixing during the batch reactor step (after filling) and where the subsequent steps of aeration and clarification occur in the same tank [1, 13].

The SBR units at IVAR Grødaland do not work properly due to high fraction of dissolved COD (high organic load) coupled with oxygen deficiency. Such conditions make filamentous microorganisms proliferate. An overabundance of filamentous organisms compared to floc-forming organisms causes the sludge settle poorly, creating what is known as filamentous bulking. A way to resolve this issue is to remove dissolved COD before SBR units through anaerobic granular sludge treatment using UASB reactors as pre-treatment with high solid retention time. Anaerobic granular sludge treatment is expected to give high retention of biomass in granules resulting higher COD removal efficiency and higher COD conversion into biogas (methane).

1.2. Objectives

The main objective of this master thesis was to investigate the effectiveness of anaerobic treatment of municipal wastewater for converting organic matter to methane generation in anaerobic granular sludge reactors. Furthermore, this study was conducted to set-up laboratory scale UASB reactors for treating of high strength municipal wastewater treatment. Several specific objectives are defined in sub-chapter 2.5 after observing the knowledge gaps from literature review and theoretical background in the following chapter.

1.3. Thesis Outline

This master thesis is entitled: **"Biogas Potential of High Strength Municipal Wastewater Treatment in Laboratory Scale of Up-flow Anaerobic Sludge Blanket (UASB) Reactors**" and divided into seven chapters.

1. Introduction;
2. Literature Review and Theoretical Background;
3. Materials and Methods;
4. Results;
5. Discussions;
6. Conclusions; and
7. Recommendations including further research.

Appendixes are included to present supporting materials of the whole study.

2. Literature Review and Theoretical Background

This chapter describes the theoretical explanation of anaerobic processes as well as defines anaerobic stoichiometry. The development of anaerobic treatment treating municipal wastewater in previous studies is also described. Furthermore, the factors affecting anaerobic processes of UASB reactor are also presented. Based on this literature review and theoretical background, in the last of this chapter, the knowledge gaps are well defined as specific objectives of this current study.

2.1. Anaerobic Treatment

Anaerobic treatment is a process by which microorganisms break down biodegradable material in the absence of oxygen (low redox potential) [5, 13]. The metabolic pathways followed in the breakdown of the carbon and energy source are the same for both aerobic and anaerobic process. There are two basic differences between these processes: (a) the terminal fate of electrons produced in the oxidation reactions; and (b) the amount of ATP forms by oxidative phosphorylation. The amount of ATP formed when a pair of electrons is passed through the electron transport system depends on the differences in redox potential between the electron donor and acceptor. Hence, more ATP will usually be released from aerobic respiration [14].

Figure 2.1 presents carbon and energy fate in both aerobic and anaerobic wastewater treatment assuming that the oxidation of 1 kg COD requires 1 kWh of aeration energy. Aerobic treatment is generally characterized by high operational costs (energy); while a large fraction of the influent COD is converted to sludge about 50% (or more) new sludge from the COD converted. The carbon/energy flow principles of aerobic and anaerobic conversion largely affect the set-up of the corresponding wastewater treatment system [6].

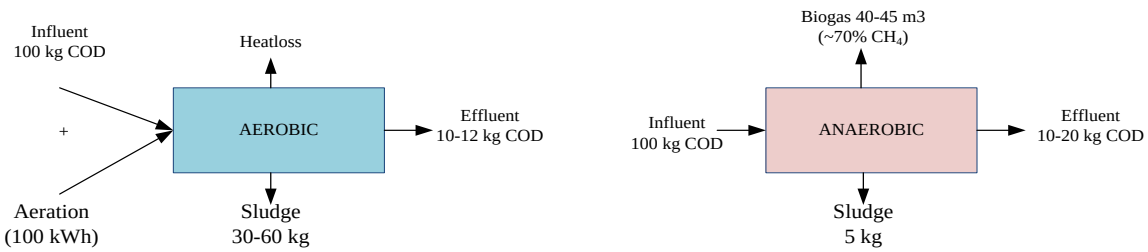


Figure 2.1 *The fate of carbon and energy in aerobic and anaerobic wastewater treatment [6]*

Based on Figure 2.1, the major advantages of anaerobic process compared to aerobic process are: (a) less sludge produced per unit of substrate. Moreover, the amount of excess sludge is well stabilized, even having a market value when the granular anaerobic sludge is produced in the bioreactor; (b) economic value of the methane generated in the treatment process; and (c) higher organic loading potential because the process is not limited by the oxygen transfer capability at high oxygen utilization rates. Disadvantages of the anaerobic process are the elevated temperatures required to maintain microbial activity at a reasonable rate and the incompleteness of organic utilization at economical treatment times [6, 14].

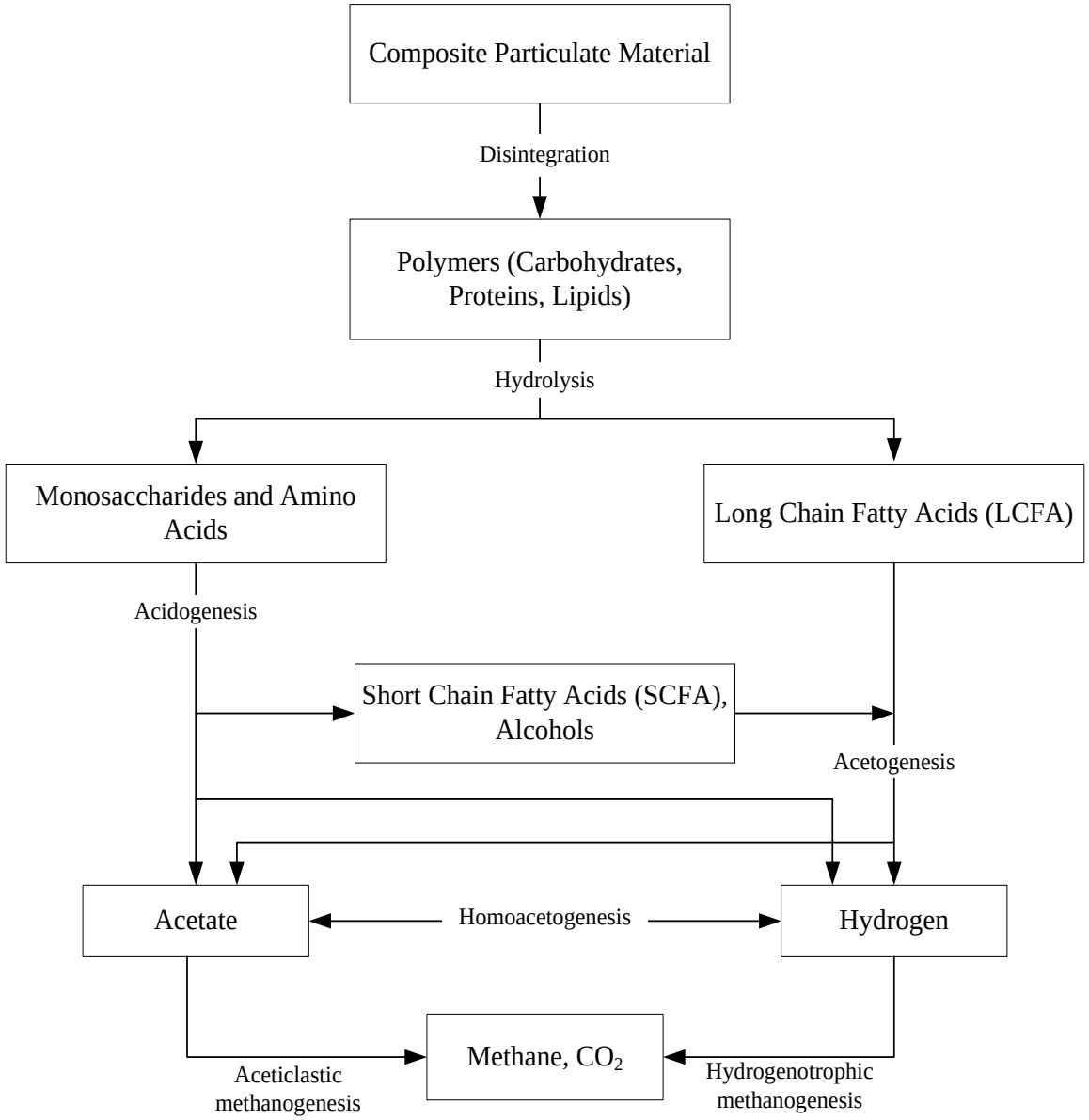


Figure 2.2 *Multistep of anaerobic processes [5, 6, 15]*

Anaerobic process involves a complex consortium of microorganisms and this multistep nature of anaerobic operation is depicted in Figure 2.2. Three basic bacteria group (acidogens, acetogens, and methanogens), as presented in Figure 2.3, are recognized in this process, and it is the cumulative actions of these groups of bacteria that ensure process continuity and stability. The activities of these bacteria groups and the biochemical processes could be divided into four basic processes: (a) disintegration and hydrolysis; (b) acidogenesis; (c) acetogenesis; and (d) methanogenesis. These four basic processes will be detailed in the subsequent sections.

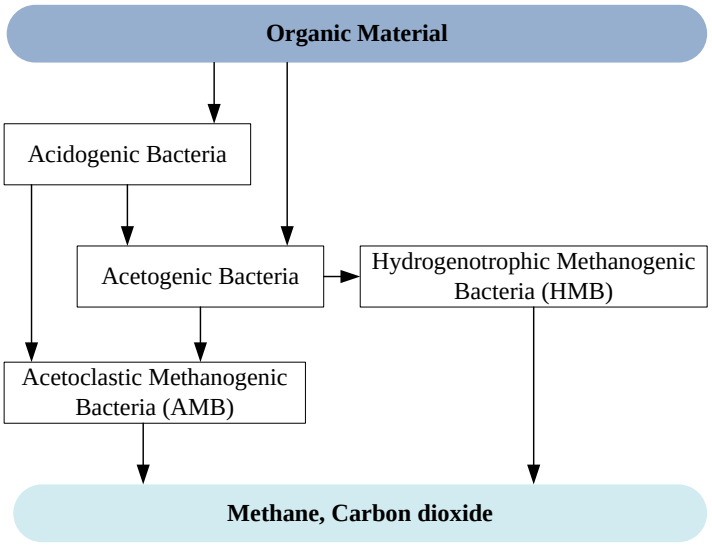


Figure 2.3 *Groups of microorganism in anaerobic processes [5, 15, 16]*

2.1.1. Disintegration and Hydrolysis

Disintegration and hydrolysis are extracellular biological and non-biological processes mediating the breakdown and solubilisation of complex organic material to soluble substrates. The substrates are complex composite particulates and particulate carbohydrates, proteins and lipids. The last three substrates are also products from disintegration of composite particulates [5]. Hydrolysis means the degradation of a defined particulate or macromolecular substrate to its soluble monomers (depolymerisation). Large polymeric materials e.g. carbohydrates, proteins and lipids cannot be directly degraded by microorganisms and therefore must be reduced to smaller products to allow their passage across the cell membrane of the microorganisms. The process is catalyzed by enzymes, which are produced by the microorganism directly benefiting from the soluble products [5]. Although the process is referred as hydrolysis, lytic enzymes also depolymerize (in addition to hydrolases). The main group consists of proteases (acting on proteins),

cellulases, amylases, glucanases (all acting on polysaccharides), and lipases (acting on fats and oil; lipids) [5, 16, 17].

In this process, the products of degradation of carbohydrates, proteins and lipids are monosaccharideserves, amino acids, and long chain fatty acids (LCFA), respectively [5]. These products of hydrolysis are utilized 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 hydrolysed to dissolved readily biodegradable substrates of their monomeric composition; however, some lipopolysaccharides are converted to monosaccharides and LCFA.

2.1.2. Acidogenesis

Acidogenesis (fermentation) is defined as an anaerobic acid-producing microbial process without an additional electron acceptor or donor [5, 13]. This includes the degradation of soluble sugars (monosaccharides) and amino acids to a number of simpler products. Fermentation is carried out by acidogens and is relatively fast. The growth rate of acidogenic bacteria is comparable to aerobic rates with μ_m of 2 - 7 d^{-1} [5]. Because acidogenesis can occur without an additional electron acceptor, and because free energy yields are normally higher, the reactions can occur at high hydrogen or formate concentrations and at higher biomass yields [5, 13].

The end products from acidogenesis are mainly short chain fatty acids (SCFA), called also volatile fatty acids (VFA), 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. 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 [5, 6, 13]. 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 are 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 by 0.1 - 0.2 gVSS/gCOD [8, 9]. Several stoichiometry of product formation using glucose as substrate are shown in Table 2.1. 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 [17, 18].

Table 2.1 *Stoichiometry of product formation using glucose as substrate*

Products	Reaction	ATP per mol glucose	Note
Acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	4	1
Propionate	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$	~low	2
Acetate, Propionate	$3C_6H_{12}O_6 \rightarrow 2CH_3COOH + 4CH_3CH_2COOH + 2CO_2 + 2H_2O$	4/3	
Butyrate	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	3	1
Lactate	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$	2	
Ethanol	$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$	2	3

1. *While thermodynamically possible at high H_2, may be limited by energetic of substrate-level phosphorylation.*
2. *Not yet observed in cultured environmental samples. Coupling with substrate level oxidation is more common as in reaction b.*
3. *Energy yield taken from yeast pathway. Bacterial pathway may have 0 ATP/mol ethanol [16]*

2.1.3. Acetogenesis

The VFAs, other than acetate, which are produced in acidogenesis step are further converted to acetate, hydrogen and carbon dioxide by the acetogenic bacteria [13]. Table 2.2 is shown the stochiometries of acetogenesis product formation. The most important acetogenic substrates are propionate and butyrate, key-intermediates in anaerobic processes [19]. 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. Acetogenesis is also required for VFAs 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 ($\Delta G^0 < 0$). This low concentration is maintained by the hydrogen utilizing methanogens [5, 13, 19].

Table 2.2 *Stoichiometry showing the product formation of the different substrates*

Substrate	Reaction	$\Delta G°$ (kJ/gCOD)	$\Delta G'$ (kJ/gCOD)
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 x 10^{-5} bar, pCH_4 0.7 bar, HCO_3^- 0.1 M, and organic acids 1 mM

The interaction between generation and consumption of hydrogen is called interspecies hydrogen transfer and is illustrated in Figure 2.4 where ΔG' is related to different hydrogen concentrations for the anaerobic oxidation of propionate, butyrate, and palmitate. From Figure 2.4, there is an upper limit set by the acetogens, and a lower limit set by the methanogens of syntrophic thermodynamically transfer of VFA 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 [5, 6, 13].

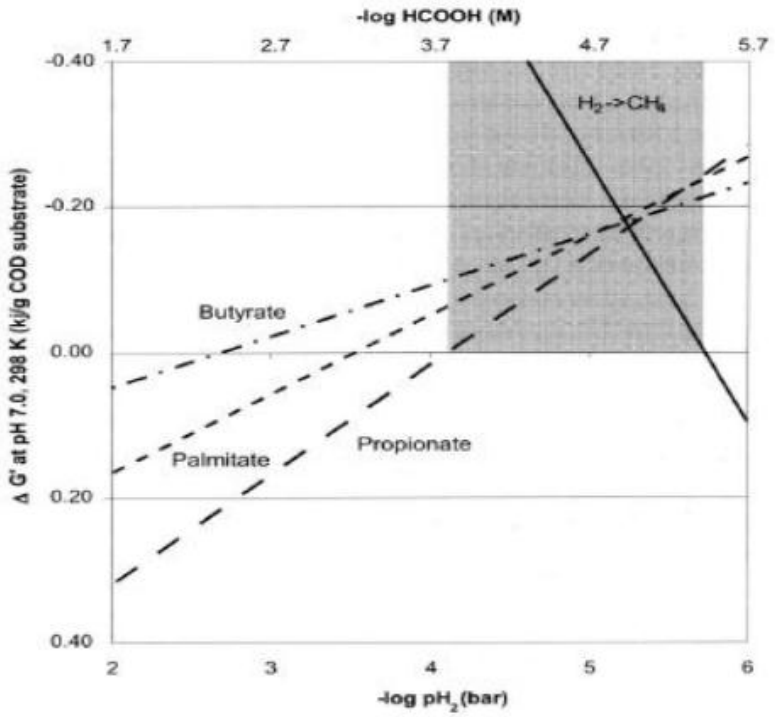


Figure 2.4 *Interspecies hydrogen transfer [5]*

2.1.4. Methanogenesis

Methane generation is the ultimate product and last stage of anaerobic processes. The products of the acetogenesis are utilized as substrates by the methanogenic bacteria to produce methane. This methane generation is carried out in two major routes by two groups of methanogenic bacteria, as presented in Figure 2.3. 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 methanogenic bacteria (AMB). The overall reaction is shown in Equation 2-1.

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \Delta G° = -31\ kJmol^{-1} \qquad 2\text{-}1$$

In the other route, hydrogenotrophic methanogenic bacteria (HMB) utilize H_2 as electron donor to reduce carbon dioxide to methane with an overall reaction in Equation 2-2 [19].

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G° = -135\ kJmol^{-1} \qquad 2\text{-}2$$

Energy generation in methanogens is not driven by substrate level phosphorylation, but reversed electron transport and ATPase [16]. The methanogens are much more sensitives in their environmental requirements than the acidogens. Their rates of metabolism are also lower than the rates of the acidogens and therefore methane production is generally the rate-limiting step in anaerobic processes [18]. The maximum growth rate (μ_{max}) of methanogenic bacteria is low by 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 0.05 – 0.1 gVSS/gCOD [8, 9].

2.2. Anaerobic Stoichiometry

Organic material in wastewater is usually quantified and qualified by the oxygen consumption of organic material oxidation. The following sub-chapters will describe the stoichiometry of anaerobic processes.

2.2.1. Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD), a common parameter of pollutant strength, is a measure of the electrons available in an organic compound, expressed in terms of the amount of oxygen required to accept them when the compound is completely oxidized [13]. The

COD determination involves oxidation of organic compounds in the presence of strong oxidant at a certain temperature and time frame. The number of electrons donated by strong oxidant is expressed as oxygen equivalent in gO_2/m^3 (or mgO_2/l). The electron equivalents of oxygen can be determined by noting that 1 mol of O_2 weight 32 g and contains 4 electron equivalents. Therefore, 1 electron equivalent (eeq) corresponds to 8 of COD, as shown in Equation 2-3 [6].

$$\tfrac{1}{2}H_2O \rightarrow H^+ + \tfrac{1}{4}O_2 + e^- \Rightarrow \tfrac{1}{4}mol\ O_2 \cdot 32\tfrac{g}{mol} = 8\ gram\ O \qquad 2\text{-}3$$

$$1eeq = 8\ g\ COD$$

The theoretical COD of an organic compound $C_nH_aO_b$ can easily be calculated on the basis of the chemical oxidation reaction, assuming a complete oxidation that is illustrated in Equation 2-4 [6].

$$C_nH_aO_b + \frac{1}{4}(4n + a - 2b)O_2 \rightarrow nCO_2 + \frac{a}{2}H_2O \qquad 2\text{-}4$$

Equation 2-4 shows that 1 mol of organic material demands *¼(4n+a-2b)* mol of O_2 or *8(4n+1-2b)* gO_2. For organic material containing nitrogen compounds, Equation 2-4 needs to be corrected for the number of electrons that will stay with N and the total weight of N in the compound, as shown in Equation 2-5 [6].

$$C_nH_aO_bN_d + \tfrac{1}{2}(2n + 0.5a - 1.5d - b)O_2 \rightarrow nCO_2 + dNH_3 + \tfrac{a-3}{2}dH_2O \qquad 2\text{-}5$$

The theoretical COD can be calculated by the oxidation stochiometry of glucose or the mineralization of glucose, as expressed in Equation 2-6. Equation 2-6 shows glucose oxidation requires 6 mol of oxygen per mol glucose. Therefore, 1 gram glucose represents 1.067 gCOD (192/180).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad 2\text{-}6$$

$$180\ g \quad 192\ g$$

The theoretical COD per unit mas may be very different for different chemical compounds. In case of strongly reduced compounds, for example methane, using Equation 2-4, the theoretical COD of methane is high as shown in Equation 2-7.

$$COD_{CH_4} = 4gCOD/gCH_4 \qquad 2\text{-}7$$

The carbon oxidation state of C in methane is -4, the most reduced state of carbon. The lower the average oxidation state of the carbon in the compound, the more oxygen can be bound by the compound, and consequently the higher is its COD value. In case the compound ($C_nH_aO_bN_d$) is completely biodegradable and would be entirely converted by the anaerobic organisms (no sludge yield) into CH_4, CO_2 and NH_3, the theoretical amount of methane gas (and CO_2) produced can be calculated using the Buswell Equation (Equation 2-8) [6].

$$C_nH_aO_bN_d + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3d}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3d}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3d}{8}\right)CO_2 + dNH_3 \quad 2\text{-}8$$

2.2.2. COD Fraction

The total amount of COD in wastewater can be divided into various fractions based on biodegradability, as shown in Figure 2.5. Furthermore, the proportion of biodegradable COD can be divided into particulate and dissolved COD. Dissolved biodegradable COD is readily degraded by microorganisms while particulate biodegradable COD must be hydrolysed into smaller molecules so that can be used for the growth of microorganisms. The proportion of non-biodegradable COD can be also divided into particulate and dissolved COD. Particulate non-biodegradable COD will adsorb to biomass, since it cannot be used by microorganisms, it will be accumulated to sludge. Meanwhile, dissolved non-biodegradable COD will not also be degraded by microorganisms. However, it will not be accumulated to sludge thus will pass through the effluent [5, 6, 13].

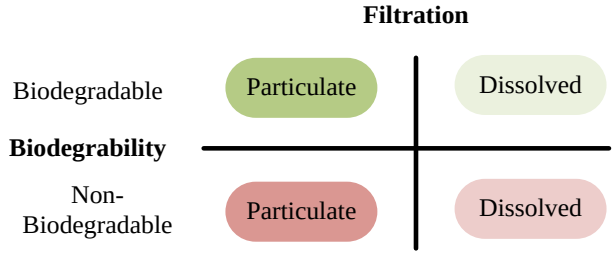


Figure 2.5 *COD fraction in wastewater*

Distinguishing between available degradable (substrate) and total input COD is important, as a considerable fraction of the input COD could be anaerobically not biodegradable. The influent with 100% of biodegradable COD is seldom found. The COD flowchart used in anaerobic processes is presented in Figure 2.6 which shows the COD flow through intermediates and end products of methane. Other products of disintegration are inert particulate and inert soluble material which are 10% of total organic material conversion

as COD [5]. The typical non-biodegradable fractions of total influent COD for raw and settled (primary effluent) wastewaters are shown in **Error! Reference source not found.**.

Table 2.3 *Typical non-biodegradable fraction of total COD for raw and settled (primary effluent) wastewater [6]*

Parameter	Fraction of Total COD	
	Raw Wastewater	Settled Wastewater
Non-biodegradable Soluble/Dissolved	0.03 – 0.08	0.05 – 0.10
Non-biodegradable Particulate	0.13	0.08

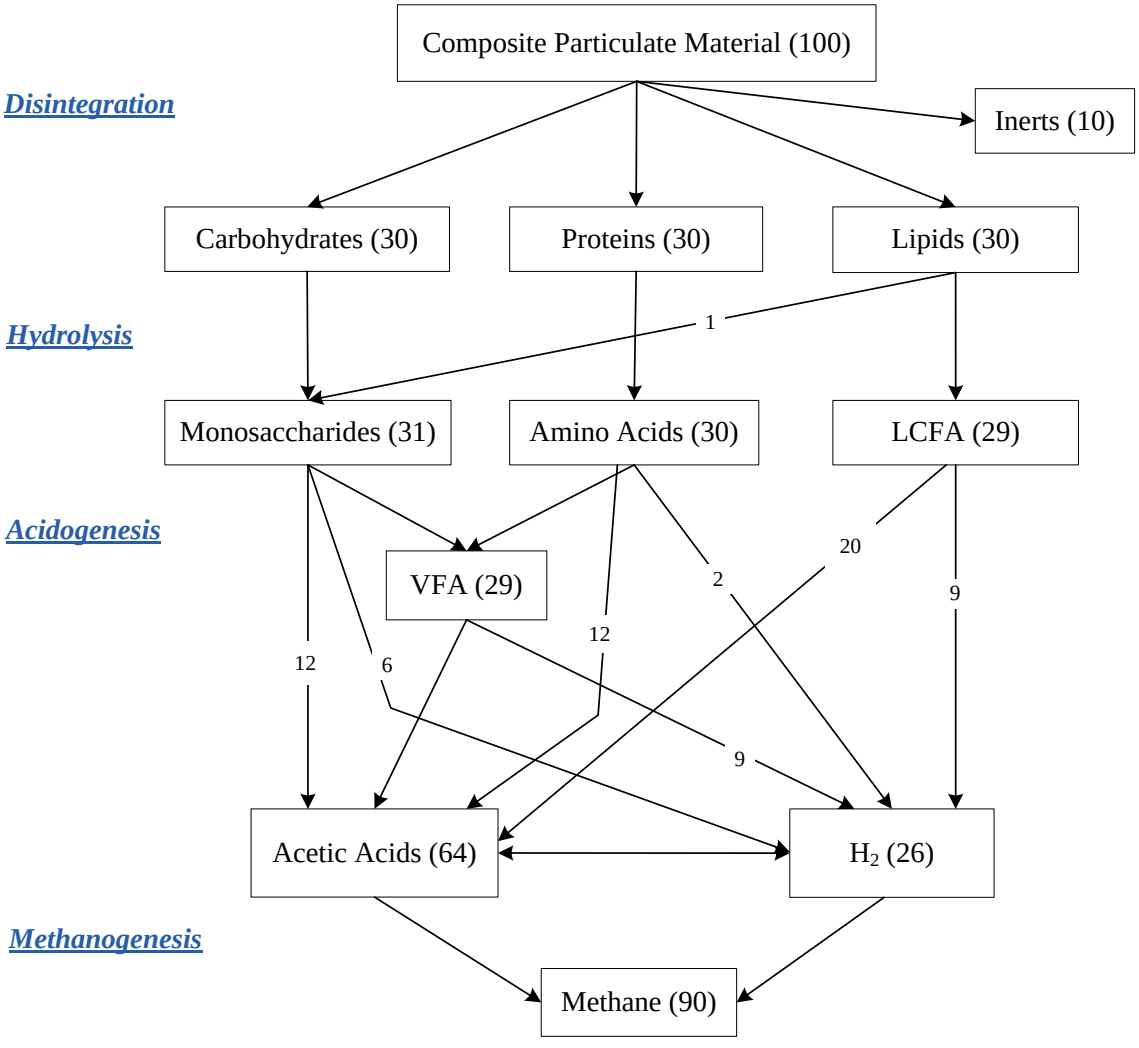


Figure 2.6 *The COD flow in anaerobic processes (percent unit) [5]*

2.2.3. Growth and Substrate Consumption Rate

Bacterial growth is divided into four phases: (a) lag phase which is the period after inoculation of bacteria before growth. The lag phase is thought to be due the physiological adaption of the cells to the new medium; (b) logarithmic phase that is regular constant cell division (the period of exponential growth) and the most sensitive period to inhibitory constituents; (c) stationary phase is when the death rate is equal to division rate and nutrients becoming limiting factor or waste products becoming toxic; and (d) death phase is when the death rate exceeds division rate. Bacteria grow via binary division and will increase exponentially during logarithmic growth phase. The growth rate of bacteria will be inversely with bacterial doubling time (generating time) [16]. It can be expressed as a first order reaction based on biomass concentration as shown in Equation 2-9, where $\frac{dX_B}{dt}$ is biomass growth rate; $t_g$is generation time; μ is specific growth rate (gVSS/gVSS.d); and X_B is biomass (gVSS/l).

$$\frac{dX_B}{dt} = \frac{\ln 2}{t_g} X_B \qquad \frac{dX_B}{dt} = \mu \, . X_B \tag{2-9}$$

The specific growth rate (μ) is dependent on the concentration of the limiting factors such as carbon source, electron donor, electron acceptor, nitrogen, phosphorous, or other factors which are necessary for growth. The relationship can be expressed with different mathematical formulas and the most widely used is Monod Equation (Equation 2-10) with Monod kinetics is illustrated in Figure 2.7, where K_s is half saturation constant which is defined as the substrate concentration where μ is half of μ_{max}; Cs is growth limiting substrate concentration (g/l); and μ_{max} is maximum specific growth rate (gVSS/gVSS.d) [1]. Based on Monod Equation, it can be seen than when Cs is much larger than Ks, μ will be approximately equal to μ_{max}. The biomass will then grow at maximum speed (μ_{max}) and the growth is independent of substrate concentration.

$$\mu = \frac{\mu_{max} . C_s}{K_s + C_s} \qquad \frac{dX_B}{dt} = \mu \, . X_B = \frac{\mu_{max} . C_s}{K_s + C_s} \, . X_B \tag{2-10}$$

Substrate consumption and biomass growth is related to each other by growth yield factor (Y) and substrate consumption rate can be written as Equation 2-11 and Equation 2-12 where Y is proportional coefficient from Equation 2-9.

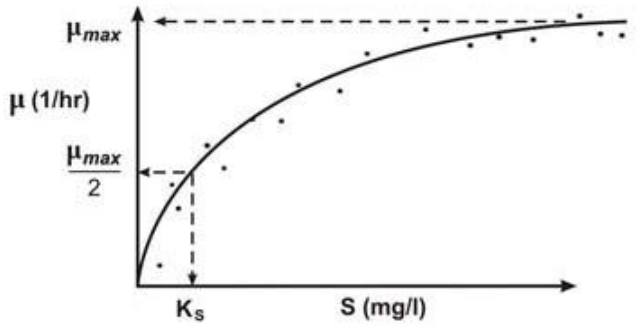


Figure 2.7 *Monod kinetic [20]*

$$\frac{dX}{dt} = Y\frac{dC}{dt} \qquad \frac{dC}{dt} = \frac{\mu . X_B}{Y} \qquad \frac{dC}{dt} = k_m . X_B \tag{2-11}$$

Where $\frac{dC}{dt}$ is substrate consumption rate (gCOD/l.d) and μ/Y (k_m) is specific substrate consumption rate (gCOD/gVSS.d).

The specific substrate consumption rate (k) corresponds to substrate removal in the reactor and is affected by substrate concentration in the same way as μ. Maximum specific substrate consumption (k_{max}) thus corresponds to maximum bacterial growth (μ_{max}). Methane production can be calculated by the percentage of the substrate that is not used for growth and will be converted to methane (1 – Y), as shown in Equation 2-12 [1].

$$\frac{dM}{dt} = (1 - Y)\frac{dC}{dt} = (1 - Y).\frac{\mu . X_B}{Y} = (1 - Y)k_m . X_B \tag{2-12}$$

2.2.4. Methane Production

The total amount of CH_4 produced in the anaerobic process is related to the amount of organic matter present in the sample as CH_4 is equivalent to a certain amount of COD. Generally, not all organic matter are biodegradable and also part of the organic substrate will be used for cell synthesis [6]. Based on the basic influent characteristics, i.e. flow rate, COD concentrations, and the information on the biodegradability of the COD, the expected CH_4 production rate can be estimated followed Equation 2-13.

$$CH_4 + 2O_2 \rightarrow CO_2 + H_2O \tag{2-13}$$

Two moles of oxygen are required to oxidize one mole of methane to carbon dioxide and water. Thus, the COD equivalent of methane is 4 kgCOD/kg CH_4. At STP (standard temperature and pressure) of 0 °C and one atmosphere, this corresponds to 0.35 m^3 of

methane produced per kg of COD (22.41 m^3/64 kgCOD) converted to methane. At temperature 35 °C, this corresponds to 0.40 m^3 of methane produced per kg of COD (25.29 m^3/64 kgCOD) converted to methane.

Table 2.4 shows methane production of 100% COD conversion in function of some temperature variables. The total biogas production can be further determined by the proportion of methane in the biogas. In order to find how much COD inlet is converted into biogas, the COD effluent and the COD in sludge production should be known. The carbon dioxide content of the gas produced in anaerobic process ranges between about 30 and 50% and varies depending on the nature of the substrate [6, 13].

Table 2.4 *Methane production of 100% COD conversion in function of temperature*

Temperature (°C)	**Methane Yield (l CH_4/ g COD)**
0 (273 K)	0.35
20 (293 K)	0.37
25 (298 K)	0.38
35 (308 K)	0.40

2.2.5. COD Balance

Like any biological system, an anaerobic process must be monitored for relevant parameters and the measurements must be evaluated for adequate operation and control. All COD that entered the system end up in the end product of CH_4, minus the COD that is incorporated in the biomass, as shown in Figure 2.8. Since a mass balance can be made by only using the COD as a parameter (Equation 2-14), the COD therefore generally taken as a control tool to operate an anaerobic system.

$$COD_{in} = COD_{out} \qquad 2\text{-}14$$

$$COD_{influent} = COD_{effluent} + COD_{gas} + COD_{sludge}$$

For identifying the fate of COD in an anaerobic reactor detailed analysis of the gaseous, liquid and solid outlets should be performed [6]. Based on the basic influent characteristics, the theoretical COD equivalent for 1 kg bacterial VSS, with an estimated composition of $C_5H_7O_2N$ can be calculated as 1.42 kgCOD/kgVSS. Having both the final products CH_4 and newly grown bacteria expressed as COD, the balance can be made if the influent and effluent are properly measured [6, 13].

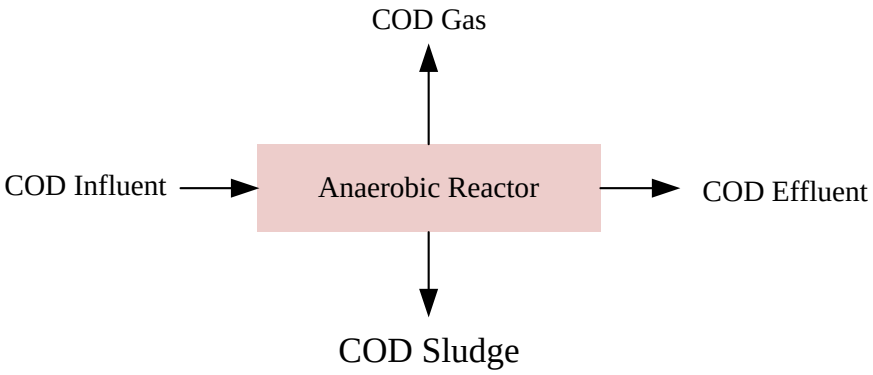


Figure 2.8 *COD balance [6]*

2.3. Factors Affecting The Anaerobic Processes

To effectively design and operate any biological wastewater treatment process, it is necessary to have a basic understanding of factors affecting the processes, such as the nutritional requirement of microorganisms, operating condition and the environmental factors that affect microbial growth. In this sub-chapter, factors affecting the anaerobic processes will be described.

2.3.1. Sludge Retention Time (SRT)

Sludge retention time (SRT) exerts dominant effect on the capabilities and performance of a biochemical operation. It affects the type of microorganisms that can grow in a bioreactor, as well as their activity, thereby determining effluent quality. The selected SRT must always exceed the minimum SRT associated with the microorganisms responsible for a particular required biochemical transformation. The minimum SRT is the value below which a particular group of microorganisms is unable to grow in a suspended growth reactor. Figure 2.9 shows typical SRT values for various anaerobic conversion processes at 35 °C. Longer SRT values will generally be required for lower temperatures [13].

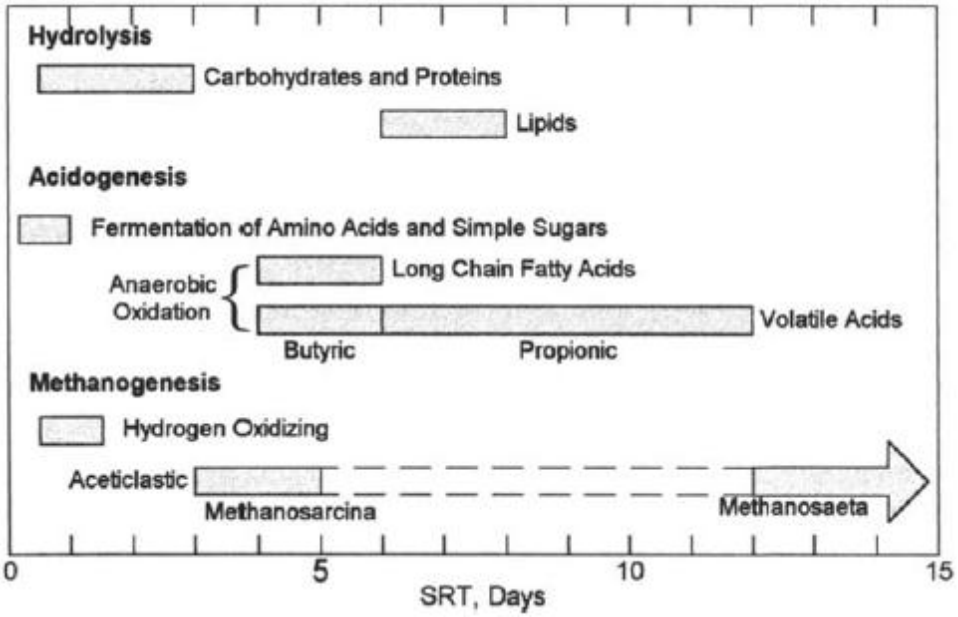


Figure 2.9 *Typical SRT ranges for various biochemical conversions in anaerobic bioreactors at 35 °C [13]*

Hydrolysis of particulate carbohydrates and proteins to produce monosaccharides and amino acids is relatively rapid reaction in about three days. In contrast, the hydrolysis of lipids to form LCFA and others soluble reaction products is a much slower reaction that does not generally occur for SRT values less than about six days. Figure 2.9 illustrates the relative effects of SRT on the growth of the various types of microorganisms found in anaerobic bioreactors and the resulting impact on the types of biochemical conversion that will occur [13].

Anaerobic treatment of wastewater containing carbohydrate and protein with production of methane can be accomplished at SRT values of about 8 days. In fact, significant methane formation will occur at SRT values as low as 5 to 6 days, but significant quantities of propionic acid will accumulate because thus SRT is too short to allow the growth of bacteria which anaerobically oxidize propionic acid to acetic acid and hydrogen. SRT values in excess of 8 days will be required to stabilize wastewaters containing significant quantities of lipids, such as primary sludge from domestic treatment systems. Generally, a minimum SRT of about 10 days is specified to ensure complete and reliable degradation of lipids in anaerobic bioreactors [13, 17].

2.3.2. Organic Loading Rate (OLR)

Organic loading rate (OLR) is related to SRT as mentioned in sub-chapter above through the active biomass concentration in bioreactor. It has been used to characterize the loading on anaerobic treatment systems that can be achieved for a particular process quantifies

how effectively the bioreactor volume is utilized [13]. 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. Organic overload can also result to imbalance in the system as more VFA 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 VFA which reduces the pH and can inhibit the activity of the methane forming bacteria [13, 18]. Fixed film, expanded and fluidized bed reactors can withstand higher organic loading rate [6].

The OLR can be calculated in unit kgCOD/m^3.d or gCOD/l.d as shown in Equation 2-15.

$$OLR = \frac{Q.Cin}{V} \quad \text{2-15}$$

Where Q is flow rate (l/d); C_{in} is feed concentration (gCOD/l); and V is reactor volume (l).

The OLR can also be related to hydraulic retention time (HRT) and the feed concentration (C_{in}), as shown in Equation 2-16.

$$OLR = \frac{Cin}{HRT} \quad \text{2-16}$$

For a reactor without sludge recycle, the loading is related to SRT only because the SRT and HRT is the same. For a reactor with sludge recycle, the SRT is independent of HRT. SRT and OLR are inversely proportional to each other [1, 13]. Equation 2-17 shows that SRT is inversely proportional with the volume (V), and that volume is related to SRT through biomass concentration (*X*) in the bioreactor. The OLR is increased as the biomass concentration is made larger, thereby allowing the bioreactor to be made smaller [13].

$$SRT = \frac{X.V}{Y.Q.Cin} = \frac{X}{Y.OLR} \quad \text{2-17}$$

2.3.3. Temperature

All the processes of growth are dependent on chemical reactions, and the rates of these reactions are influenced by temperature. The rate of microbial growth as well as the total amount of growth can be affected by temperature. As the temperature is increased, a point will be reached where the rate of growth is a maximum. With the further increase in

temperature, the heat-sensitive cell components such as enzymes are denatured and the growth rate drops rapidly [14]. The temperature effect can expressed as Equation 2-18.

$$\mu_{m(20)} = \mu_{m(T)}.\theta^{(T-20)} \qquad 2\text{-}18$$

Where $\mu_{m(20)}$ is maximum specific growth rate at 20 ºC; $\mu_{m(T)}$ is maximum specific growth rate at temperature, T ºC; and θ is temperature coefficient.

Microorganisms are classified into temperature classes on the basis of the optimum temperature and the temperature range in which the species are able to grow and metabolize, as shown in Figure 2.10. The overlapping growth temperature ranges in Figure 2.10 indicate that there is not a clear boundary between these classic groups of psychrophilic, mesophilic and thermophilic microorganisms. The bacterial growth rates of methanogenic thermophiles and mesophiles from anaerobic reactors are well determined [7].

Under psychrophilic conditions, chemical and biological reactions proceed much slower than under mesophilic conditions. Most reactions in the biodegradation of organic matter require more energy to proceed at low temperatures than at a temperature optimum [10]. A strong temperature effect on the maximum substrate utilization rates of microorganisms has been observed by many researchers [8, 12, 21]. In general, lowering the operational temperature leads to a decrease in the maximum specific growth and substrate utilization rates but it might also lead to an increased net biomass yield (g biomass per g substrate converted) of methanogenic population or acidogenic sludge [4, 12].

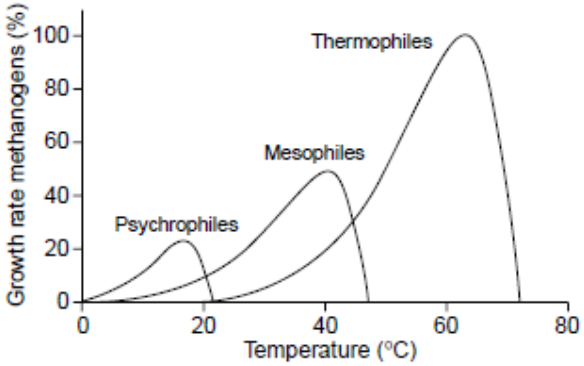


Figure 2.10 *Relative growth rates of methanogens with different temperature [10]*

Practically most of full-scale applications of anaerobic wastewater treatment are restricted to wastewater with temperatures exceeding 18 °C [10]. Nevertheless, it is possible to grow methanogens at lower temperatures, provided that longer SRTs are used to compensate for the lower maximum specific growth rates. Moreover, under moderate climate conditions, many dilute wastewaters, including domestic and industrial wastewaters, are discharged at low ambient temperatures. Besides low temperature, these wastewater usually contain concentrations of organic matter, typically below 1500 mgCOD/l [12, 22]. Most studies with the effect of temperature on anaerobic process show a strong negative effect on the metabolic activity of mesophilic anaerobic methanogenic bacteria at decreasing temperature, as presented in Figure 2.11. This indicates that the capacity of an anaerobic reactor seeded with mesophilic biomass will drop sharply during start-up under low temperature.

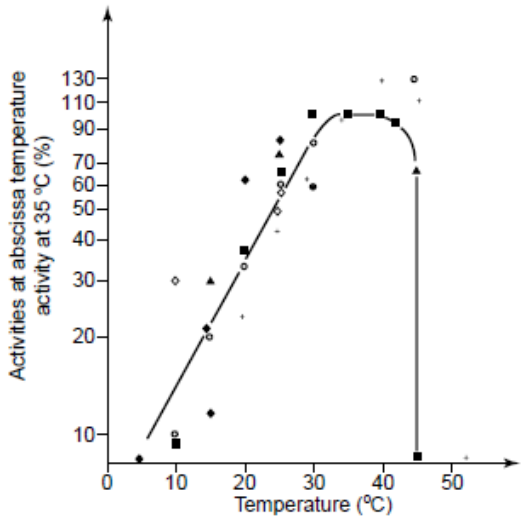


Figure 2.11 *Temperature dependency of the methane production rate of mesophilic anaerobic processes from different researchers, white diamond, black circle, white circle, blank square and cross are research conducted by different researchers [10]*

2.3.4. pH

For most bacteria, and for most wastewater treatment processes, the extremes of the pH range for growth are between 4 and 9. The optimal pH for methane microorganisms 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 AMB and HMB 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 hydrogen-ion concentration is considered to be one of the most important factors that influence enzyme activity [14].

The system must contain adequate buffering capacity to accommodate the production of VFA 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 VFA. Anaerobic processes can operate over a wide range of VFA concentrations (from less than 100 mg/l to over 5000 mg/l) if proper pH control is practiced [18]. A constant pH lends stability to the process. Commonly chemicals used as buffers include lime, sodium carbonate, sodium bicarbonate and sodium hydroxide. Sodium bicarbonate ($NaHCO_3$) 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. The addition of sodium bicarbonate, especially during starting up, is imperative for maintenance of pH around 7 and for keeping the stability of the system [19, 23].

2.3.5. Nutrients

For microorganisms, nutrients (a) provide the material required for synthesis of cytoplasmic material, (b) serve as an energy source for cell growth and biosynthetic reactions, and (c) serve as acceptors for the electrons released in the energy-yielding reactions [14]. 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. For a typical activated sludge process, the COD:N:P requirement ratio is 100:5:1 while the required optimum C:N:P ratio for maximum yield of methane has been reported to be 100:2.5:0.5 [18].

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 [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. 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 [18, 24].

2.3.6. Inhibitory Substances

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. As the concentration of the material is increased, the specific growth rate will increase until the maximum specific growth rate (μ_{max}) is reached [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, as described in several sub-chapters below.

2.3.6.1. Volatile Fatty Acids (VFAs)

In anaerobic reactors, accumulation of acids affects the pH of the medium. When the pH is held constant near neutral pH, neither acetic nor butyric acids have any significant toxic effects upon hydrogen-utilizing methanogenic bacteria at concentrations up to 10000 mg/l [25]. Propionic acids, on the other hand, exhibits partial toxicity to methanogenic bacteria at a concentration of 1000 mg/l at neutral pH [13, 25]. 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 [6]. From this, it can then be said that in anaerobic operations that have a little inhibition by VFA will occur at neutral pH.

The methanogenic bacteria are sensitive to pH changes and a decreased methanogen activity will influence the entire anaerobic treatment processes. When hydrogen consumption by HMB is reduced resulting decreased pH will further affect AMB activity in that way they are inhibited by a higher hydrogen concentration. This will reduce the acetic acid production and the acidic fermentation products or VFA will be accumulated. The acidogenic bacteria are least sensitive to pH. As mentioned in previous chapter, they also have much higher growth rate and yield than acetogenic and methanogenic bacteria. This means that a high concentration of VFA will be produced. If the production of VFA

exceeds the maximum capacity of methanogenic consuming acetic acid and hydrogen, it will lead to further VFA accumulation and decreased pH. This process is called acidification of anaerobic process and illustrated in Figure 2.12.

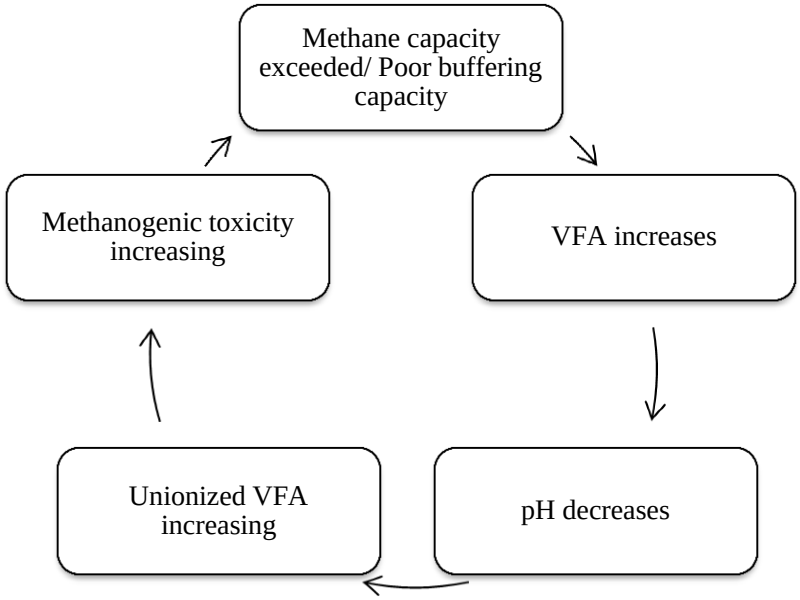


Figure 2.12 *Reactor pH drop as a result of methanogenic overloading and accumulating VFAs [6]*

2.3.6.2. Ammonia

Most wastewater contains substantial quantities of protein. Wastewater 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 [6, 18]. Ammonia is a weak base and dissociates in water, as shown in Equation 2-19.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad 2\text{-}19$$

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 build-up in VFA, 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].

2.3.6.3. Sulphides

Anaerobic process contains mixed microbial communities. Besides methanogenic, other bacteria are present which can compete with the methanogens for substrates. Different microbial can use different electron acceptors such as sulphate or sulphide by sulphate reducing bacteria (SRB). These bacteria convert sulphate into hydrogen sulphide (H_2S). Hence, the main intermediary products of anaerobic degradation process e.g. hydrogen and acetate can be converted by both SRB, methanogens, and/or obligate hydrogen producing bacteria (OHPB) [6]. Because these three groups of bacteria operate in the same environmental condition, they will compete for the same substrates. The competition between methanogens and SRB is very complex and is determined by the growth rates of the bacteria. Faster growing bacteria will dominate [6].

Wastewater 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. Concentrations between 100 and 200 mg/l may be tolerated after acclimatization [13]. Hydrogen sulphide acts as a weak acid and, consequently, at neutral pH is present in equilibrium with the hydrogen sulphide ion, as presented in Equation 2-20.

$$H_2S \leftrightarrow HS^- + H^+ \qquad HS^- \leftrightarrow S^{2-} + H^+ \qquad \text{2-20}$$

Hydrogen sulphide is sparingly soluble in water, so it will partition between the liquid and gas. This result in a lower methane yield per unit of degraded organic waste and, therefore, negatively affects the overall energy balance of the process. The quality of the biogas is reduced since a part of the produced sulphide ends up as H_2S in the biogas [6]. The produced sulphide has a bad smell. Moreover, sulphide increases the corrosivity of anaerobic process gas and results in the formation of sulphur oxides when the gas is burned [13].

2.3.6.4. 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. Like other

biochemical 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 sulphide, and consequently the added sulphide will maintain the concentration of those heavy metals at low concentrations. 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 *Concentration of soluble heavy metals exhibiting 50% inhibition of anaerobic processes*

Cations	Approximate Concentration (mg/l)
Fe^{2+}	1-10
Zn^{2+}	10^{-4}
Cd^{2+}	10^{-7}
Cu^{+}	10^{-12}
$Cu2^{+}$	10^{-16}

2.3.6.5. 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. 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 maximum 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 2.6.

Table 2.6 *Stimulatory and inhibitory concentration of light metal cations in anaerobic processes*

Cations	Concentration (mg/l)		
	Stimulatory	**Moderate 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 SRTs 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 2.7 summarises antagonistic responses for the light metal cations and ammonia.

Table 2.7 *Antagonistic responses for light metal cations and ammonia*

Inhibitors	Antagonists
Na^+	K^+
K^+	Na^+, Ca^{2+}, Mg^{2+}, NH_4^+
Ca^{2+}	Na^+, K^+
Mg^{2+}	Na^+, K^+

2.3.6.6. Other organic compounds

As 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 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

detergent [13]. During acclimatization, the activity of a methanogenic bacteria community may almost cease.

2.4. Up-flow Anaerobic Sludge Blanket (UASB) Reactor

This sub-chapter is divided into two sections. The first section provides information about general concept of UASB reactor including typical operating condition used for successful application, and the second one explains the application of UASB reactor for treating municipal wastewater.

2.4.1. General Concept

The up-flow anaerobic sludge blanket (UASB) reactor is distinguished by the absence of an external sedimentation chamber. Instead, the wastewater is introduced at the bottom of the reactor and flows upward at a velocity that matches the settling velocity of the biomass. In this way a sludge blanket is formed and maintained. A special zone is required to allow the gas formed to escape without carrying sludge particles with it. The biomass in these reactors is in the form of compact granules that contain mixed cultures of bacteria. Because of the good retention of biomass in UASB reactors, they are suitable for treating wastewater with relatively low substrate concentrations. In fact, they have been demonstrated to be capable of effective treatment of municipal wastewater [13].

Successful implementation of the UASB technology is caused by the retention of high levels of active biomass within the system. The retention of a high biomass concentration within the system allows the application of very high organic loading rates, facilitating the use of compact and economical wastewater treatment plants [9]. The basic UASB reactor is illustrated on Figure 2.13. The influent wastewater is distributed at the bottom by a system of tubes which provides a flow through a blanket of granular sludge. Inside these porous particles, VFA and biogas are formed. Ascending biogas bubbles keep the particles partially fluidized. At the top of both reactors, the gas bubbles are separated from the water and the rising flocs which show a lower settling rate are carried up by the gas/liquid flow. Gas is collected and removed [13, 19].

Tracer studies demonstrated that internal mixing was not optimal in a pilot-scale UASB reactor treating municipal wastewater at low temperatures [26]. This produced dead space in the reactor, leading to a reduction in the treatment efficiency. In order to improve the

sludge-wastewater contact and use the entire reactor volume efficiently a better influent distribution was required. Different feed inlet devices, more feed inlet points per square meter or higher superficial velocities have been proposed as solutions. The use of effluent recirculation combined with taller reactors (or a high height/diameter ratio), as shown in Figure 2.13, where a high superficial velocity is applied [12]. This reactor is called expanded granular sludge blanket (ESGB), as shown in Figure 2.13.

In this ESGB reactor concept, the up-flow liquid velocity (>4 m/h) causes the granular sludge blanket to expand, eliminating dead zones and resulting in better sludge-wastewater contact [21, 27]. Accumulation of flocculent excess sludge between the sludge granules is also prevented [12]. Recirculation of the effluent dilutes the influent concentration, but it was extensively proven that low strength wastewater can efficiently be treated in reactors with recirculation [11, 28]. Furthermore, higher organic loading rates can be also accommodated in those systems. Consequently, the gas production is also higher, improving even more the mixing inside the reactor. In tall reactors, the gas loading (in $m^3m^{-2}h^{-1}$) and the hydrostatic pressure at the bottom can be higher than in short reactors and the effect of these parameters on the performance of the process also have to be considered.

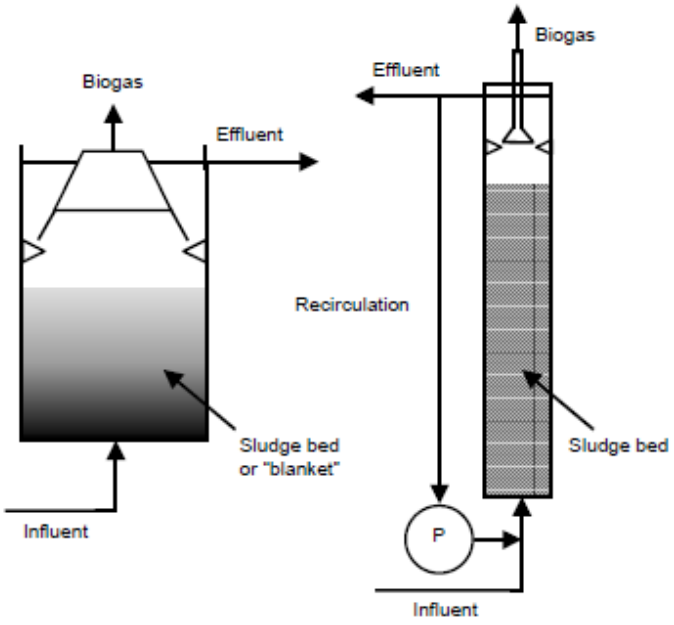


Figure 2.13 *Schematic diagram UASB (left) and ESGB (right) bioreactor [2, 7]*

Rebac [21] research showed the potential of the EGSB reactor as a high rate treatment system for low strength wastewater under psychrophilic conditions (below 12 °C). COD removal efficiencies over 90% were achieved at organic loading rates up to 12 gCOD/l.d and at HRT as low as 1.6 h using a VFA-mixture as feed. VFA measurement could help to decide the cause of increase or decrease in removal efficiency [2, 29]. Higher up-flow velocities, like those applied in EGSB reactors, induce a better sludge wastewater contact and the removal efficiency of soluble substrates is likely to increase. The up-flow velocities (v_{up}) that can be applied in the EGSB system range from 4 to 10 m/h.

2.4.2. Municipal Wastewater Treatment Application

Anaerobic processes is widely used in the treatment of sludge because it provides a significant reduction in the mass of the input material [30]. It is also an effective method for the treatment of high organic wastewater e.g. industrial wastewater [7, 11]. For the past three decades, it has become popular methods for the treatment of low and high strength concentration (below 1000 mgCOD/l) wastewaters like municipal wastewater, providing good treatment efficiencies at moderate temperatures and low HRT [31]. It is considered to become a good alternative as pre-treatment before aerobic treatment that will result in high efficiency removing organic contaminant.

Municipal wastewater temperatures are frequently lower than industrial wastewaters. Only under tropical climate conditions can municipal wastewater reach temperature ideal for anaerobic treatment. The first experiences with compact/high-rate anaerobic treatment using UASB reactors for municipal wastewater treatment started during the early eighties in Cali, Colombia. The results obtained from the operation of the 64 m^3 UASB pilot scale reactor showed the feasibility of the system under the prevailing environmental and municipal wastewater characteristics. The initial trials were rapidly followed by full scale reactors in Colombia, Brazil and India [6].

The application of UASB reactors to high strength municipal wastewater treatment under psychrophilic and low mesophilic condition has been studied since 1976 [2, 3, 7, 26]. Seghezzo [2] reported that a 6 m^3 UASB reactor seeded with digested sewage sludge was operated at hydraulic retention times (HRT) of 14 – 17 h. COD reduction reached 85 – 65% and 70 – 55% at 20 °C and 13 – 17 °C, respectively. He concluded that the UASB concept was a simple, compact, and inexpensive technology for sewage treatment, even at relatively low temperatures.

Based on research carried out on different UASB reactors (0.120, 0.240, 6 and 20 m^3), de Man [26] concluded that anaerobic treatment of low to high strength domestic sewage (500 – 1500 mgCOD/l) can be accomplished at 12 – 18 °C applying HRT of 7 – 12 h with total COD removal efficiencies of 40 – 60%. In order to comply with local regulations for discharge, the UASB system is generally accompanied by a proper post-treatment system, such as: facultative ponds, sand filtration, constructed wetlands, trickling filters, physic-chemical treatment, and activated sludge treatment. The UASB reactor and the post-treatment step can be implemented in more integrated set-up [6].

2.5. Knowledge Gaps (Specific Objectives)

This study aimed to contribute to the development methane potential of anaerobic treatment systems. Furthermore, the main focus was to investigate the effectiveness of UASB reactors for converting organic matter to methane as pre-treatment process for SBR units at IVAR Grødaland. To achieve this objective, in-house designed laboratory scale, UASB reactor systems were set up for treating of high strength municipal wastewater treatment i.e. below 1200 mgCOD/l under mesophilic condition (20 – 25 °C). Based on literature review and theory background, the process performance and reliability of anaerobic treatment process in UASB reactors were needed to be observed. Emphasis was also made on identifying the critical factors affecting performance of UASB reactors so that by maintaining optimal operating conditions, efficiency could be well improved. Therefore, the OLR capacity, up-flow velocity, pH variability, VFA production, alkalinity and nutrient availability, granular sludge behaviours, and the need for post-treatment were also investigated as specific objectives of this study. The productivity of this anaerobic granular sludge system was studied by determining its COD removal efficiency, measuring its methane production rate as well as methane yield and biogas quality (methane fraction in the produced biogas). The COD balance analysis was conducted as well to evaluate reactor performance by investigating COD recovery and COD loss during operation.

3. Materials and Methods

This chapter explains the laboratory-scale experiment of anaerobic treatment of municipal wastewater from DAF effluent, IVAR Grødaland, for generation of energy in form of methane using UASB reactor. Three UASB reactors were used in the study for investigating UASB reactor performance and analysing biogas potential; one reactor (Reactor A) used inactive granular sludge; and two reactors (Reactor I and Reactor II) used active granular sludge. Reactivation of granular sludge was also part of the study using inactive granules as well as to determine the generation of methane after reactivation compared to the last two reactors using active granules. This also includes the operational, maintenance and control procedures, and the analytical methods used in the study. All laboratory works for this master's thesis project were conducted at the University of Stavanger.

3.1. Anaerobic Granular Sludge Reactor Configuration

Setting-up of the laboratory scale UASB reactors required some essential devices, instruments and other materials that were installed together with the reactor body. All of these elements worked together and assured the anaerobic treatment process. This section describes the configuration of all three reactors used in this study.

3.1.1. The Configuration of UASB Reactor A

Figure 3.1 shows the UASB Reactor A that was used for investigating UASB reactor performance and analysing biogas potential using inactive granular sludge. The granules were obtained from Telemark University College and have been kept in the fridge since May 2015. The configuration of Reactor A is shown in Figure 3.2. The reactor was made from polyethylene and fabricated by Ytre Vanntank (ID 350x8) by 5 l of volume. Feed wastewater was pumped from the container into the reactor by a diaphragm pump with adjustable flow rate. The gas went out through the gas trap to gas counter due to liquid height, pressure and suction force given by the recirculation pump. Then, the biogas produced went through CO_2 absorber using NaOH 3M solution (Product No. 106498, Merck) and methane was measured by the next gas counter. Table 3.1 describes the important equipment used in setting-up of the UASB Reactor A.

Figure 3.1 *Photo of the laboratory scale UASB Reactor A*

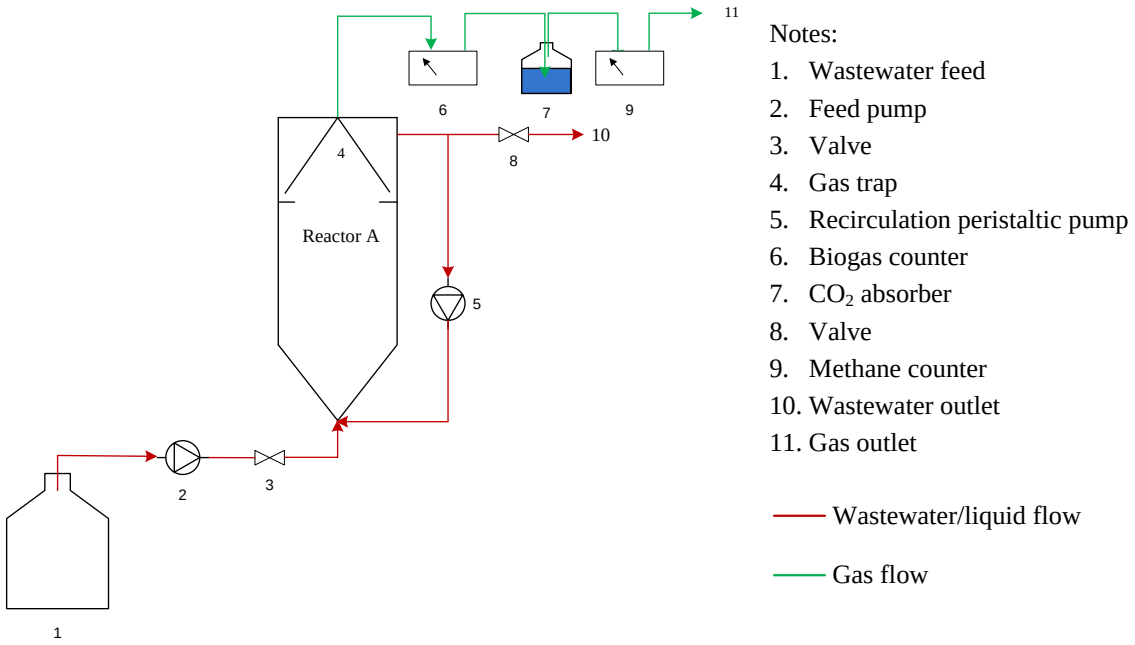


Figure 3.2 *The Flow diagram of the laboratory scale UASB Reactor A*

Table 3.1 *The characteristics of equipment used for the laboratory scale UASB Reactor A*

Equipment	**Manufacturer**	**Specification**	
Feed pump	Brundfos®	Type	: diaphragm pump
		Model	: DDA 7.5
		Flowrate	: 0 – 7.5 l/h
		Max. pressure	: 16 bar
Recirculation pump	Heidolph	Type	: peristaltic pump
		Model	: pumpdrive PD-5201
		Flowrate	: 5-120 RPM/min
Gas counter	Ritter	Model	: MGC-1 V3.3 PMMA

Equipment	Manufacturer	Specification	
		Gas flowrate	: 1 ml/h- 1 l/h
		Max. pressure	: 100 mbar
		Min. pressure	: 5 mbar
		Vol. measurement	: 3.1 ml (biogas); 3.3 ml (CH_4)
		Measuring accuracy	: less than approx. ±1%

3.1.2. The Configuration of UASB Reactor I and Reactor II

As shown in Figure 3.3, Reactor I and Reactor II were designed more highly developed to assured the anaerobic treatment process compared to Reactor A. The granules were made from mixed sources: (a) pulp and paper company treating cellulose and lignin containing (Norske Skog, Moss); (b) agriculture pilot plant treating swine and cow manure supernatant (farm in Skien, Norway); and (c) hydrocarbon oil containing wastewater at Bamble Industrial Park, Telemark. Figure 3.4 shows the flow diagram of laboratory scale UASB reactor. As shown in Figure 3.4, this laboratory scale UASB reactor had a fridge for storing wastewater in a 30 l plastic container to assured the composition of wastewater in feed would be stable. Feed wastewater was pumped from the container into the reactor by a peristaltic pump with adjustable flow rate. Reactor effluent and gas went out through the filter to gas collector/trap due to liquid height, pressure and suction force given by the recirculation pump. This filter was installed to avoid the biomass/granules to be washed out and clogged the tubes or pumps.

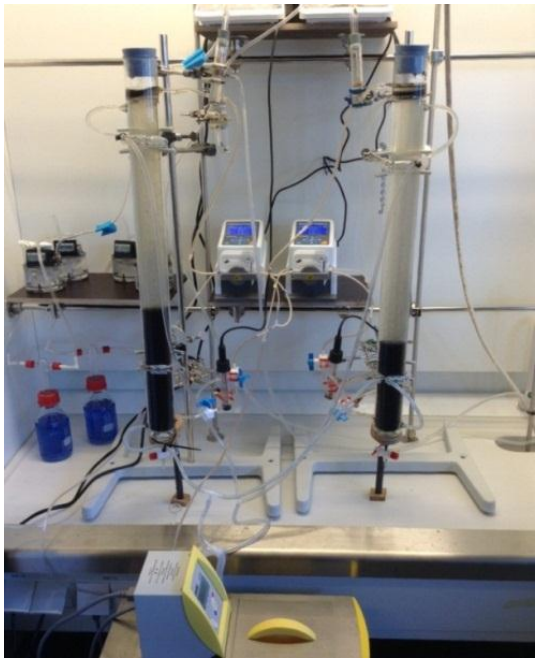


Figure 3.3 *Photo of the laboratory scale UASB Reactor I and Reactor II*

The reactor had a heating system (heater bath and circulation tubes) that allowed the liquid to be warm enough for bacteria inside the reactor. The heater bath generated warm water and circulated it inside the external layer of the reactor body. The heat control helped in maintaining mesophilic condition by average temperature of 25 ˚C. A pH meter, with a pH probe, installed in line with the wastewater flow, allowing an instant measurement of pH of the mixed liquor. Gas coolers (humidity traps) and gas volume meters (gas counters) for countering the produced biogas were also installed. Cold water was pumped from the tap water and circulated through the humidity traps; this allowed produced biogas not to evaporate before it came into the gas counter. Gas counter was used to measure the volume of produced biogas. Table 3.2 describes the important equipment used in setting-up of the UASB Reactor I and Reactor II.

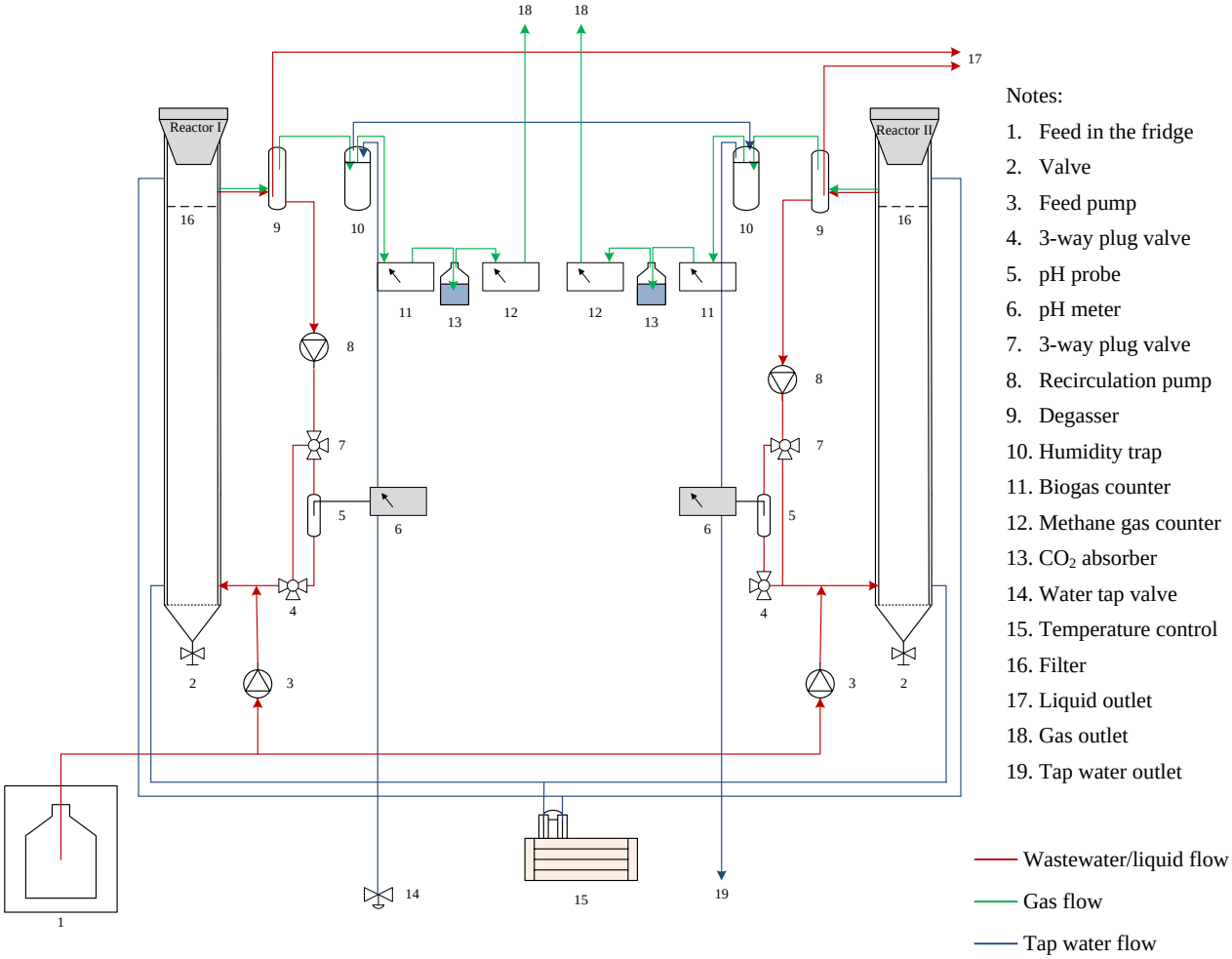


Figure 3.4 *The flow diagram of the laboratory scale UASB Reactor I and Reactor II*

Table 3.2 *The characteristics of equipment used for the UASB Reactor I and Reactor II*

Equipment	Manufacturer	Specification	
Feed and recirculation pump	Ismatec	Type	: peristaltic pump
		Channel	: 4 independent controllable channels
		Model	: Reglo ICC
		Flowrate	: 0 – 43 ml/min
Filters	Sefar	Type	: Sefar® Flourtex
		Model	: 09-1000/45
		Pore size	: 1000 µm
Gas counter	Ritter	Model	: MGC-1 V3.3 PMMA
		Gas flowrate	: 1 ml/h- 1 l/h
		Max. pressure	: 100 mbar
		Min. pressure	: 5 mbar
		Vol. measurement	: Reactor 1 → 3.29 ml (biogas); 3.34 ml (CH_4)
			Reactor 2 → 3.26 ml (biogas); 3.32 ml (CH_4)
		Measuring accuracy	: less than approx. ±1%
Thermo heating circulator	Lauda Alpha	Model	: RA 8 LCK 1907
		Temperature range	: -25 to 100 °C
		Heater capacity:	: 230 V; 50/60 Hz; 1.5 kW
		Max. pressure	: 0.2 bar
		Max. flowrate	: 15 l/min
		Bath volume	: 20 liters
pH meter	VWR	Model	: MU 6100 L (665-0309)
	VWR	pH electrode model	: Phenomenal 221 (662-1161)
	Hanna		Hanna HI1230B (022714BN)

3.2. Starting-up UASB Reactors and Operational Conditions

The first stage of starting up the reactors was to be in steady state condition and the reactors worked hydraulically well. A steady state was achieved in the reactors when the parameters, e.g. the effluent COD and the daily gas production remained constant at the same OLR. Figure 3.5 shows the general operation flowchart of the UASB reactors. This laboratory scale reactors were initially tested with tap water. The goal was to make sure that all necessary devices, instruments and materials were set and installed correctly. Key parameters such as OLR, flow rate, pressure and pH were controlled to ensure that favorable conditions for anaerobic bacteria were maintained during the anaerobic treatment processes. This section describes the starting-up process and operation conditions of all three reactors used in this study.

A sludge volume of 20 – 30% v/v with respect to the UASB reactors was used. The UASB reactor was started-up at low OLR of 1.0 gCOD/l.d equivalent to 30% of the total COD

concentration of wastewater. OLR was increasing gradually until 15.0 gCOD/l.d equivalent to 100% of the COD concentration with HRT 1.4 hour, changes in OLR were made when the reactors was assumed in steady state condition. Dilution was done using tap water that was stripped by N_2 to dissolved oxygen (DO) below 1.00 mg/l (using DO meter WTW Oxi3315) to assure anaerobic treatment process. For an optimum growth of methanogens, pH of the liquid inside the reactors was controlled and maintained at about 7. Buffer, sodium hydrogen bicarbonate ($NaHCO_3$), was added into the reactor in order to bring the pH close to 7.

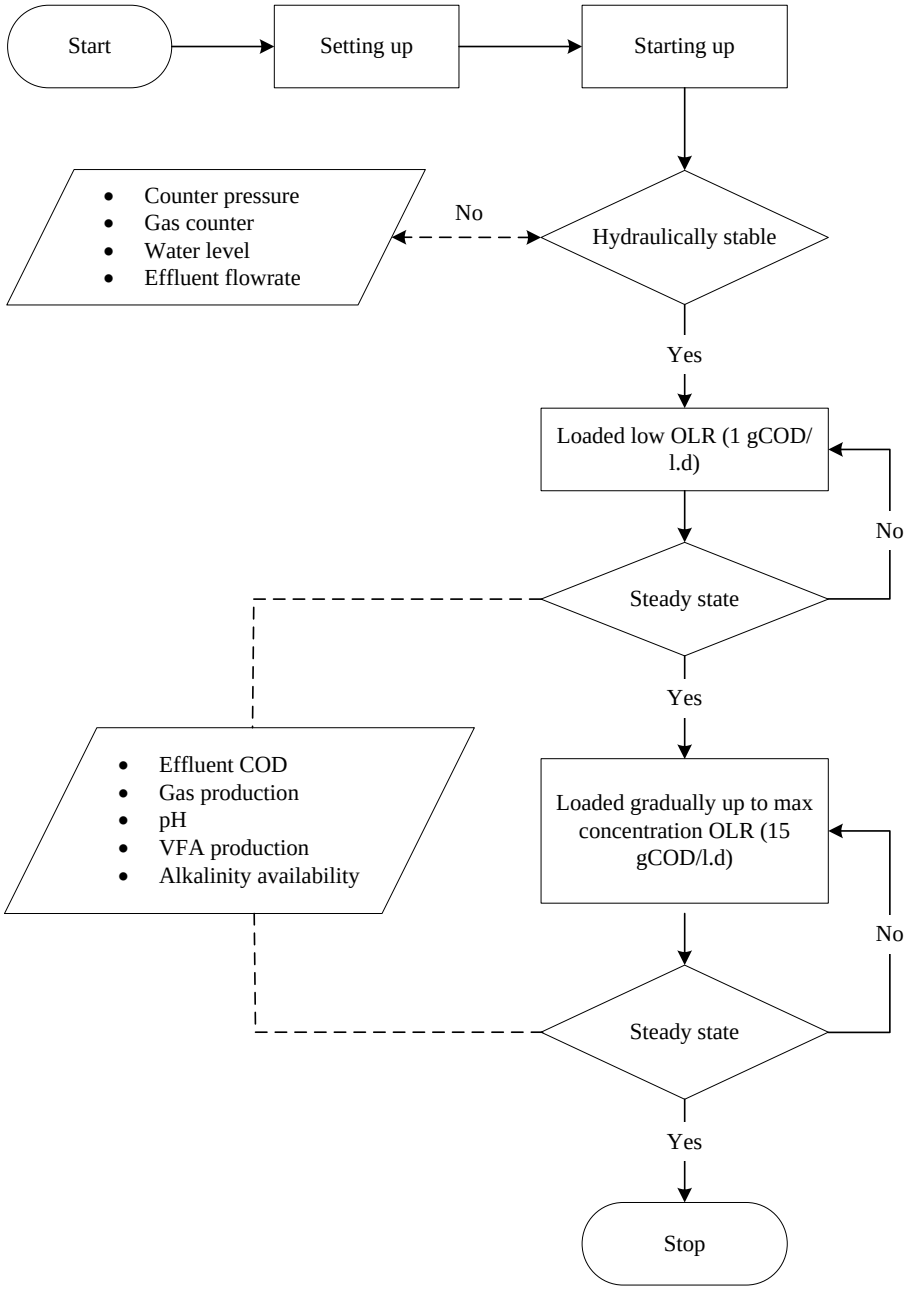


Figure 3.5 *The general operation flowchart*

3.2.1. Starting-up UASB Reactor A and Operation Conditions

Two different experiments were conducted in Reactor A. The first one was reactivation of granules sludge as preliminary tests (from 19th February to 18th March 2016). It aimed to know the operation conditions that would give optimum activity of inactive granules such as up-flow velocity, OLR, and HRT. In the first week of experiment, there was no observed activation of the granules as gas production and COD removal. Nutrients and trace elements were added to stimulate bacterial activity. The compositions of nutrient and trace element that were added during preliminary tests are listed in Table 3.3. The reactivation experiment was conducted until steady state condition. After the granules were considered to be active, the next experiment in which to investigate UASB reactor performance and biogas potential in Reactor A, was readily conducted from 19th March to 26th May 2016 (68 days) under ambient temperature about 20 °C.

Table 3.3 *Nutrient solution composition*

Species	Concentration (g/l)	Species	Concentration (g/l)
Yeast extract	30.00	$NICl_2 \cdot 6H_2O$	0.0423
NH_2CONH_2	21.4316	$CoCl_2 \cdot 6H_2O$	0.0317
KH_2PO_4	5.0696	$Na_2MoO_4 \cdot 2H_2O$	0.0130
KCl	1.4667	$ZnSO_4 \cdot 7H_2O$	0.0273
Na_2SO_4	3.4082	$CuSO_4 \cdot 5H_2O$	0.0039
$CaSO_4 \cdot 2H_2O$	1.3218	$MnCl_2 \cdot 4H_2O$	0.0055
$MgCl_2 \cdot 6H_2O$	1.9303	KI	6.289E-05
$FeSO_4 \cdot 7H_2O$	0.9362	NH_4VO_3	0.000552
		H_3B_3	0.013

3.2.2. Starting-up UASB Reactor I/II and Operation Conditions

The experiment in Reactor I and Reactor II was carried out to investigate UASB reactor performance and analyse biogas potential directly from 22nd April to 3rd June 2016 (42 days) without any reactivation of granules in the first step. The acclimatization of granules in Reactor I and Reactor II was in the first five days of experiment time until the reactors were in steady state condition. Liquid level inside the gas collector was controlled by establishing an equal flow rate for influent and effluent and adjusted counter pressure inside degasser and CO_2 absorber bottles. Frequently the liquid level in the degasser was too high or too low, therefore the gas went out the liquid outlet or the liquid was sucked into the humidity trap and gas counter. This condition could be controlled by adjusting the liquid outlet and degasser position to increase or decrease head until the liquid went to a

normal level. Temperature inside the reactor was fixed and maintained at 25 °C by using a laboratory heating bath and circulator. Addition of water into the heating bath was done regularly to replace water that had been evaporated. To make sure that the flow rate was maintained as same as flow rate shown in the pump display, while the bioreactor was running, the flow rate was measured every day by counting the volume of effluent liquid in the volumetric cylinder per unit time measurement. The flow rate could be adjusted to desirable flow rate.

3.3. Biogas Potential Determination (Batch Test)

One of the main objectives in this project is to investigate the effectiveness of anaerobic treatment of municipal wastewater for generation of biogas (methane). This sub-chapter will describe biogas potential batch test determination methods. In this study, methane gas potential from wastewater was tested using AMPTS II (Automatic Methane Potential Test System II). AMPTS II instrument has been developed by Bioprocess Control (BPC) for online measurements of ultra-low biogas and bio-methane flows produced from the anaerobic treatment of any biological degradable substrate at laboratory scale. The instrument is divided into four main parts: sample incubation unit, CO_2-fixation unit, gas volume measuring device, and AMPTS II software.

The batch test was conducted to test Grødaland wastewater biogas potential using anaerobic sludge from IVAR Sentralrenseanlegg Nord-Jæren as inoculum. This experiment was done (25^{th} Feb – 7^{th} Mar 2016) with some scenario as describe in Table 3.4. Understanding the potential of wastewater to produce methane and its dynamic degradation profile had a significant impact on the choice of wastewater as organic material substrate to treat when producing biogas, as well as provided a better understanding of the quality of the raw gas produced from wastewater treatment process. Biogas quality was measured by calculating the percentage of CH_4 gas production compared to all gas produced. This test result could be compared to the actual amount of gas produced in the UASB reactors.

Table 3.4 *The batch biogas potential test scenario*

Cell	Name	Condition
1	Blank 1-a	50 ml sludge + 450 tap water
2	Blank 1-b	
3	Blank 2-a	200 ml sludge + 300 tap water
4	Blank 2-b	

Cell	Name	Condition
5	Sample 1-a	200 ml sludge + 300 wastewater
6	Sample 1-b	
7	Sample 1-c	
8	Sample 2-a	50 ml sludge + 450 wastewater
9	Sample 2-b	
10	Sample 2-c	
11	Control 1-a	200 ml sludge + 300 ml HAc Solution (1 g/l COD)
12	Control 1-b	
13	Control 2-a	50 ml sludge + 450 ml HAc Solution (1 g/l COD)
14	Control 2-b	

Acetic acid (Product No. 100063, Merck), HAc, was used as control since this acid is readily biodegradable substrate by 1.067 gCOD/gHAc and expected to be fully oxidized. Meanwhile, tap water was used as blank of the tests and $NaHCO_3$ 4.0 g/l was added to maintain the pH. As listed in Table 3.4, blanks and controls tested were duplicated and samples were triplicated to observe the consistency of the measurement. The temperature was set to 37 °C. All of COD values both before and after the test were measured by analytical method that would be described in the following sub-chapter. The biogas quality can be further determined by the proportion of methane in the biogas, and for simplicity it was assumed to contain only CO_2 and CH_4. The tests used CO_2-fixation (absorber) unit that was prepared using NaOH 3 M (Product No. 106498, Merck) and 0.4 % Thymolphthalein pH-indicator solution. The CO_2 produced will be absorbed in alkaline solution. Then, CH_4 went through and was measured online by AMPTS II system as presented in Figure 3.6.

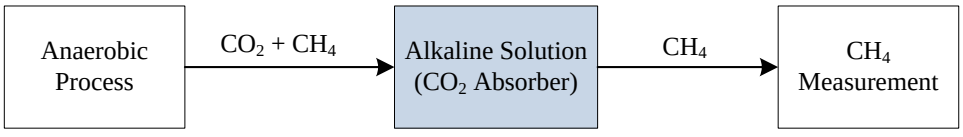


Figure 3.6 *Biogas measurement diagram*

3.4. Analytical Methods

Before doing analytical experiment, it is important to make sure sample to be analysed was homogenised by shaking to make sure sufficient particle and solids distribution was achieved. Commonly, washing and/or diluting were needed in analytical processes. In this study, diluting/washing water used was deionized water of 18 MΩ-cm resistances or higher (DI water type 1). The analytical methods used are described as follows.

3.4.1. pH and Conductivity Measurement

Conductivity was measured using WTW Multi340i. The probe was immersed into the samples until the value was constant. The conductivity unit was also noted correctly as this need to be converted to a unit consistent with the TITRA 5 software in measuring alkalinity and VFA concentration that will be described in the following section. The pH meters as mentioned in the previous sub-chapter, installed in line with the wastewater flow in Reactor I and Reactor II, allowing an instant measurement of pH of the mixed liquor. pH in Reactor A was measured instantly with alkalinity and VFA measurement using TitroLine® 5000 Auto-Titrator. All pH meters were calibrated with buffer standard solutions (4.01 and 7.00) regularly once a week.

3.4.2. COD Measurement

COD analysis was done for feed wastewater and bioreactor effluent, both total and dissolved COD. Dissolved COD tests were conducted every day as parameter control of reactor performance. Total COD tests were done once in a while to observe non-biodegradable COD and biomass produced. To determine dissolved COD, the wastewater was collected in the filtration device using 1.5 µm particle retention of glass microfiber filters (VWR European Ca. No. 516-0876). It is important to compensate the COD value with correction factor of washing/diluting water.

In this study, COD test kits were used to carry out the wastewater analysis. These kits contain digestion and catalyst solutions that, under controlled conditions, react with wastewater samples to be measured. A COD test kit is like a small glass tube (known as vial or reaction cell) on which there is an unique barcode label that is automatically read by a spectrophotometer to identify an appropriate measurement method, and read the COD of samples. The COD tests kits used were Merck Spectroquant® which were Product Number 109773 (100 and 1500 mg/l of COD concentration range) and Product Number 114895 (15 and 300 mg/l of COD concentration range).

The procedures of the COD measurements were started with digesting sample (2.0 ml sample of wastewater for total COD and 2.0 ml sample of filtrated wastewater for dissolved COD) in thermo reactor (Model TR 620), at 148 °C for 2 hours. Then, the next step was cooling the COD vials in metal test tube rack until room temperature about 30 minutes (vials were swirled after 10 minutes of cooling time). Upon reaching room

temperature, test vials were placed in spectrometer (Spectroquant Pharo 300), cell compartment and align mark with orientation mark. The reading was equivalent to COD concentration.

3.4.3. Total Volatile Fatty Acid Alkalinity Measurement

In this study, the instrument used for VFAs and alkalinity measurement was TitroLine® 5000 Auto-titration (Instrument-teknikk AS, Oslo). TitroLine® 5000 system consists of six main components: valve-cover lid and display; probe; dosing unit; titration tip unit; stirrer; and acid/base bottle. VFAs analysis was conducted based on five pH point titration procedures. The analysis was done for the bioreactor effluent. To do so, 20 ml sample was taken and diluted to 50 ml with deionized water (DI water type 1). The diluted sample was then placed on a magnetic stirring for a mixing at a low rotation speed. The initial pH of the distillate was recorded, and the diluted sample was titrated with HCl 0.1 M (Product No. 100317, Merck) to four different selected pH values (6.7, 5.9, 5.2 and 4.3). If pH of sample was less than 6.7, NaOH 0.1 M (Product No. 106498, Merck) was added to the sample until reached 6.7 of pH. Volume of acid added for each titration was recorded, and data recorded were input into computer software (TITRA 5). This software calculated the total VFAs and alkalinity concentration of the sample. The calculated concentrations were expressed as mg acetic acid/l of total VFA and mg $CaCO_3$/l of alkalinity.

3.4.4. Total Phosphorous (TP) and Total Nitrogen (TN) Measurement

Total phosphorous (TP) and total nitrogen (TN) measurement were conducted to investigate nutrient that were contained in wastewater, as phosphorus and nitrogen are macronutrients for the growth of bacteria. This was occasionally done to ensure that the reactor performance was not limited by nutrients. As COD measurement, TP and TN measurement also used spectroquant test kits. The TP tests kit used was Merck Spectroquant® which was Product Number 114729 (0.50 and 5.00 mg/l PO_4^{3-}). Besides, Merck Spectroquant® Product Number 100613 (0.50 and 15.0 mg/l N) was used for TN measurement. TP and TN were also measured in spectrometer (Spectroquant Pharo 300).

The procedures of the TP measurements were started with digesting sample (5.0 ml sample of wastewater and 1 dose of reagent P-1K) in thermo reactor at 120 °C for 30 minutes using a colorimetric method. Then, the next step was cooling the vials in metal test tube rack until room temperature. Upon reaching room temperature, 5 drops of reagent

P-2K and 1 dose of reagent P-3K were added into test vials; the vials were mixed vigorously after adding reagent P-2K and after reagent P-3K. The reaction time is 5 minutes. Lastly, the vials were placed in spectrometer to measure the concentration.

The procedures of the TN measurements were started with digesting sample (10.0 ml sample of wastewater, 1 level of reagent N-1K and 6 drops of reagent N-2K in empty vials) in thermo reactor at 120 °C for 1 hour. Then, the next step was cooling the vials until room temperature. Upon reaching room temperature, 1.0 ml of sample from digested sample and 1 dose of N-3K were added to the test vials; the vials were mixed vigorously after adding reagent N-3K. The reaction time is 10 minutes. Lastly, the vials were also placed in spectrometer to measure the concentration.

3.4.5. Total Solid of Granules (Sludge Blanket)

Total solid of granules measurement was conducted when the sludge was washed out from the reactor to determine the density of solid in the granules. The instrument used in this study was moisture analyser (VWR International No. Product 611-2318), with maximum mass capacity is 160 grams (0.001 grams of accuracy). The standard method (SM 2540 B) was used to determine total solid. The sludge was dried for 1 hour at 105 °C and the evaporated residual would be measured automatically by this instrument. By using this instrument, it is possible to set the desired temperature and drying time values.

4. Results

Results obtained from experiments are presented in this chapter. This chapter is divided into six sub-chapters: (a) biogas potential batch test; (b) preliminary tests during the staring-up of Reactor A; (c) reactor performance including COD removal efficiency, methane production as well as COD balance analysis; (d) pH variability, VFA generation, and alkalinity availability; (e) nutrient availability; (f) and granules density analysis. The presented data and figures have been summarised while the collected raw data are included in the Appendixes.

4.1. Biogas Potential Batch Test

Batch tests of 12 days operations inoculated with waste sludge from the anaerobic reactor at IVAR Sentralrenseanlegg Nord-Jæren, were performed with variable concentration of wastewater and sludge using AMPTS II. The accumulated gas volume data over time is presented in Figure 4.1. The COD removal and methane yield could be calculated by knowing the methane production, COD of the samples before and after the batch test, and COD of the controls (acetic acid) which were needed for each batch test. All measurement and calculation for samples and controls were subtracted by blanks values. The errors were determined using t-distribution analysis with 90% of certainty. The COD removal and methane yield results are summarised in Table 4.1.

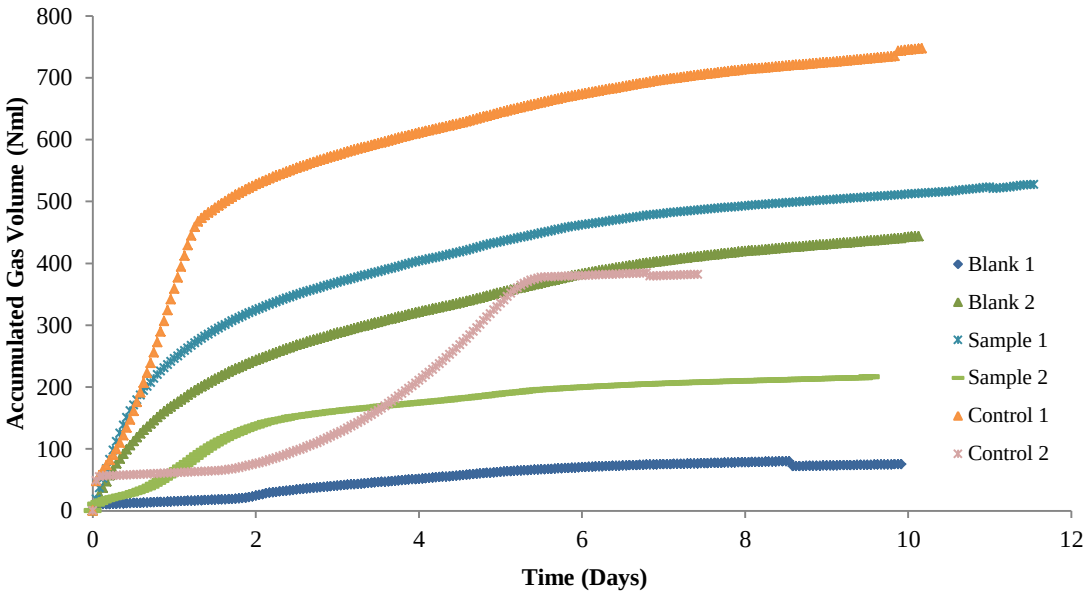


Figure 4.1 *Dynamic degradation profile of biogas potential batch test. Tap water was used as blank; sample was the tested wastewater; and acetic acid (HAc) was used as control.*

Table 4.1 *Biogas potential batch test result*

Name	COD Removal %	Methane Yield gCOD CH_4/gCOD	COD Conversion %	COD Recovery %
Sample 1	70 ±0.6	0.44±0.07	44±7	75±0.5
Sample 2	72±0.2	0.46±0.02	46±2	74±0.7
Control 1	99±0.1	0.98±0.03	98±3	98±0.7
Control 2	95±0.3	0.96±0.02	96±2	100±1.1

The COD removal efficiency and methane yield of wastewater tests were about 70% and 0.4 gCOD CH_4/gCOD respectively, with COD recovery around 70%. Higher COD removal efficiency and methane yield were obtained using acetic acid by up to 99% and 0.98 respectively, with up to 100% of COD recovery. The results showed almost all COD of acetic acid (control) were converted to methane and approximately 40% of COD in wastewater were converted to methane. COD recovery in Table 4.1 was calculated using COD balance equation (2-14). It represented the overall COD balance of which COD conversion to methane and non-biodegradable dissolved COD.

4.2. Preliminary Tests of Reactor A

In the experimental setup of Reactor A, there was no analysis of gas collected and hence gas compositions and concentration was not known. Only the reactor hydraulics was monitored to assure all necessary devices, instruments and materials were set and installed correctly. It was also assumed that the gas being primarily monitored was methane in the second gas counter as sodium hydroxide was added for CO_2 fixation. In the first week of experiment before preliminary tests, there was no observed activation of the granules measured as gas production and COD removal. Nutrients and trace elements were added to stimulate and reactive bacterial activity. When the granules started producing gas, it was note to be day 1 of preliminary tests (reactivation experiment). The reactivation experiment was conducted until steady state condition. COD removal efficiency and gas production rate were measured parameters during this test, in function of up-flow velocity variability.

Figure 4.2 shows the preliminary test results of Reactor A. COD removal and biogas production rate are plotted against time and at different up-flow velocities. OLR were around 110 mgCOD/l.d during this preliminary test. Up-flow velocity applied were 0.5±0.05 m/h, 0.7±0.06 m/h, and 1.0±0.10 m/h resulting in about 82% COD removal for

all HRT's. Gas production is also presented in Figure 4.2. In general, the required HRT to achieve COD removal efficiency up to 80% decreased with the increasing of up-flow velocity.

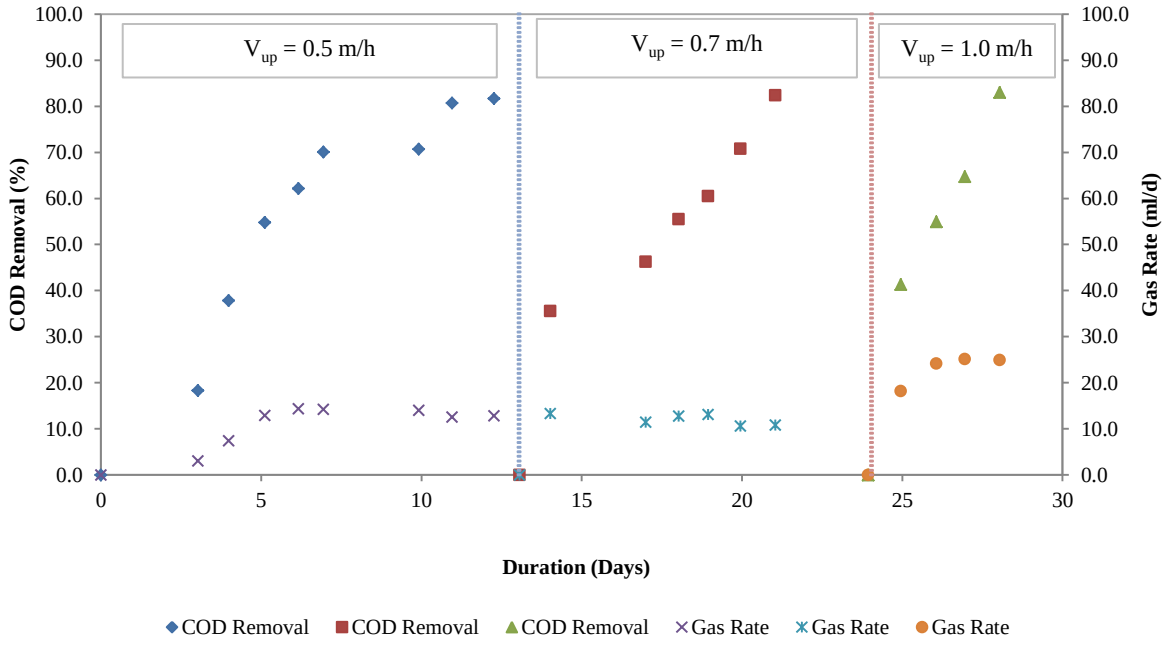


Figure 4.2 *Preliminary test results of Reactor A*

4.3. Reactor Performances

This sub-chapter describes the reactor performance during experiment in Reactor A, Reactor I, and Reactor II. The reactor performances were characterized by COD removal efficiency and COD conversion to methane gas produced.

4.3.1. COD Removal Efficiency

After preliminary tests, Reactor A was operated for 68 days. Following start-up, the operation period of Reactor I/II was 42 days. Figure 4.3 and Figure 4.4 show in- and outlet COD concentrations, and the COD removal efficiency in function of time and OLR. HRT in Reactor A ranged from 24 to 1.4 h, while 5.6 – 1.4 h were applied on Reactor I and Reactor II. As mentioned in previous chapter, the OLR increased gradually from 1.0 g COD/l.d to 15 g COD/l.d. During periods of constant OLR, COD gradually reduced towards, but not reaching steady state. Upon increase in OLR, the effluent COD increased to certain amount resulting in decreased COD removal efficiencies. The lowest COD removal efficiencies was observed at day 55 (28%) and day 22 (5%) in Reactor A and Reactor I respectively, during washing-out of granules.

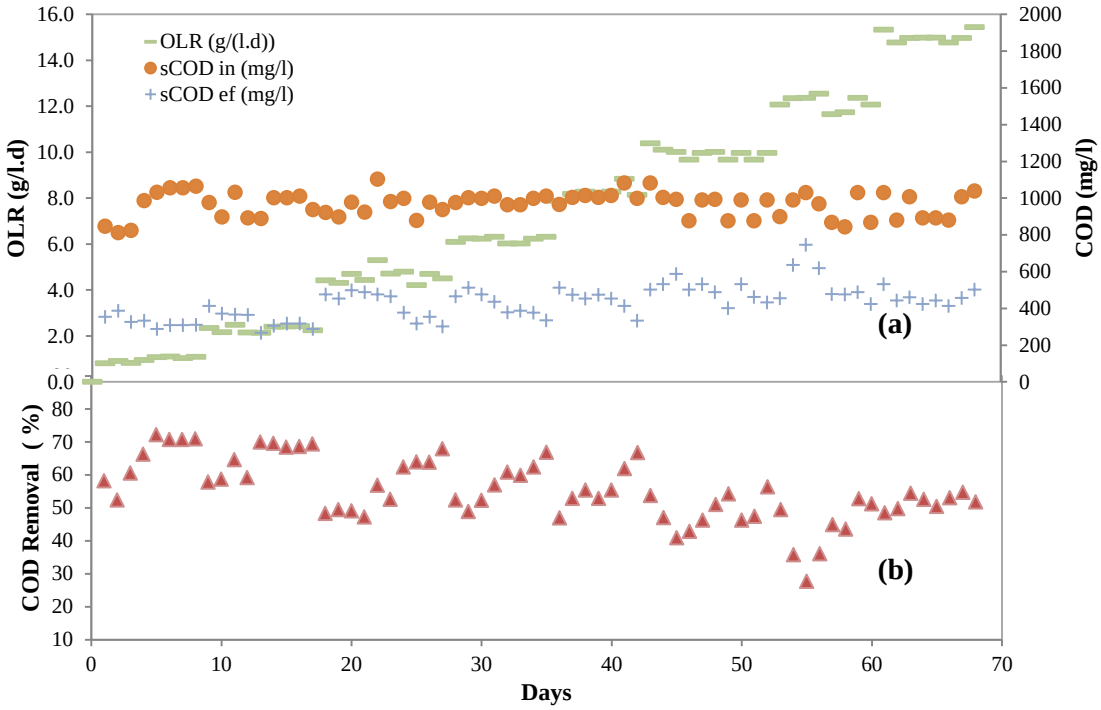


Figure 4.3 *Profiles in Reactor A: (a) OLR, COD inlet and COD outlet and (b) COD removal efficiency*

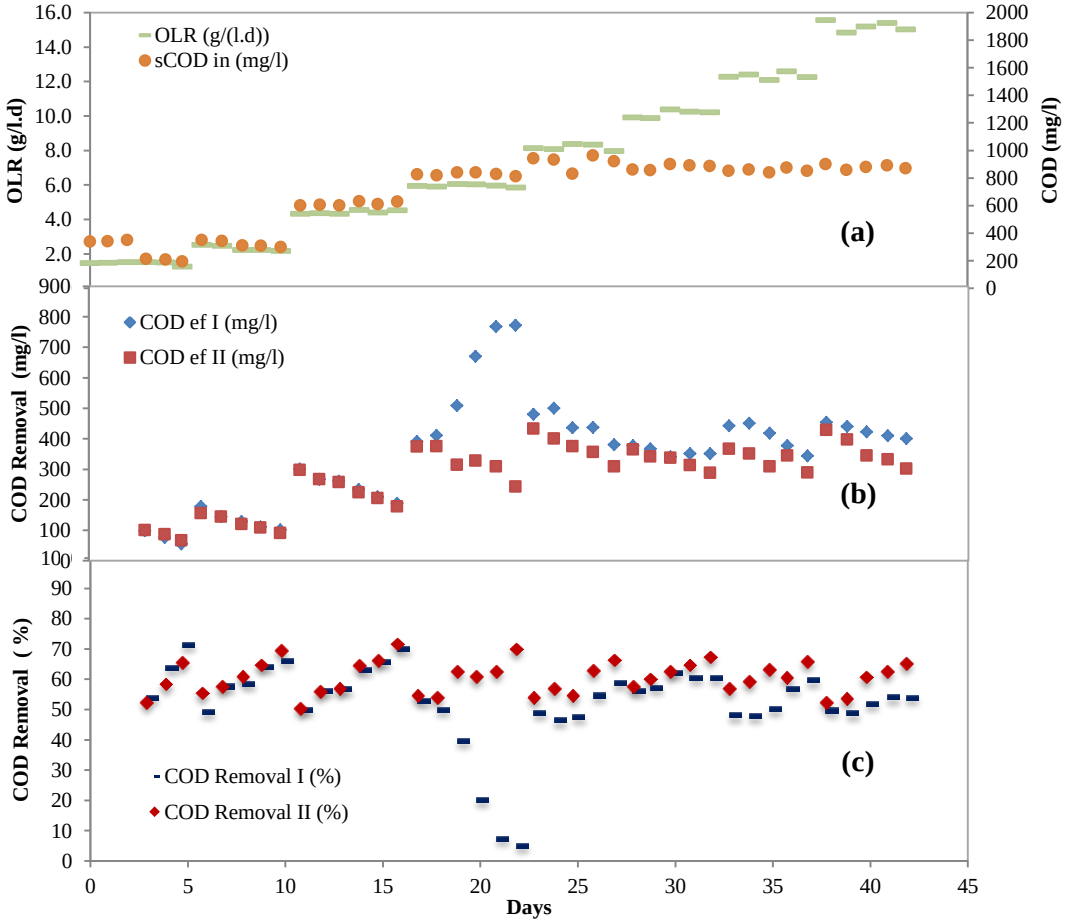


Figure 4.4 *Profiles in Reactor I/II: (a) OLR and COD inlet; (b) COD outlet; and (c) COD removal efficiency*

While Reactor A achieved COD removal efficiency over 70% at 24 h of HRT with 1.1 g COD/l.d of OLR, 70% of COD removal efficiency was achieved in Reactor I at 3.7 h of HRT with 1.3 g COD/l.d of OLR. The optimum biogas potential was occurred in Reactor II; 70% COD removal efficiency was achieved at 3.3 h of HRT with 6.0 g COD/l.d of OLR and 3.0 g COD/l.d of COD removed was converted to methane. The lowest HRT applied was 1.4 h with 15.0 g COD/l.d and the COD removal efficiency achieved were in the range of 48-58%, 49-54% and 52-65% in Reactor A, Reactor I and Reactor II, respectively.

4.3.2. Methane Production

Biogas composition was mainly methane with 70-90% and carbon dioxide with 10-30%. During 30 days of continuous operation of Reactor A, and 20 days in Reactor I/II, the average methane fraction was above 90%. The decreased methane fractions (towards 70 %) in biogas were observed after decreasing buffer ($NaHCO_3$) concentration and at increasing OLR, as presented in Figure 4.5.

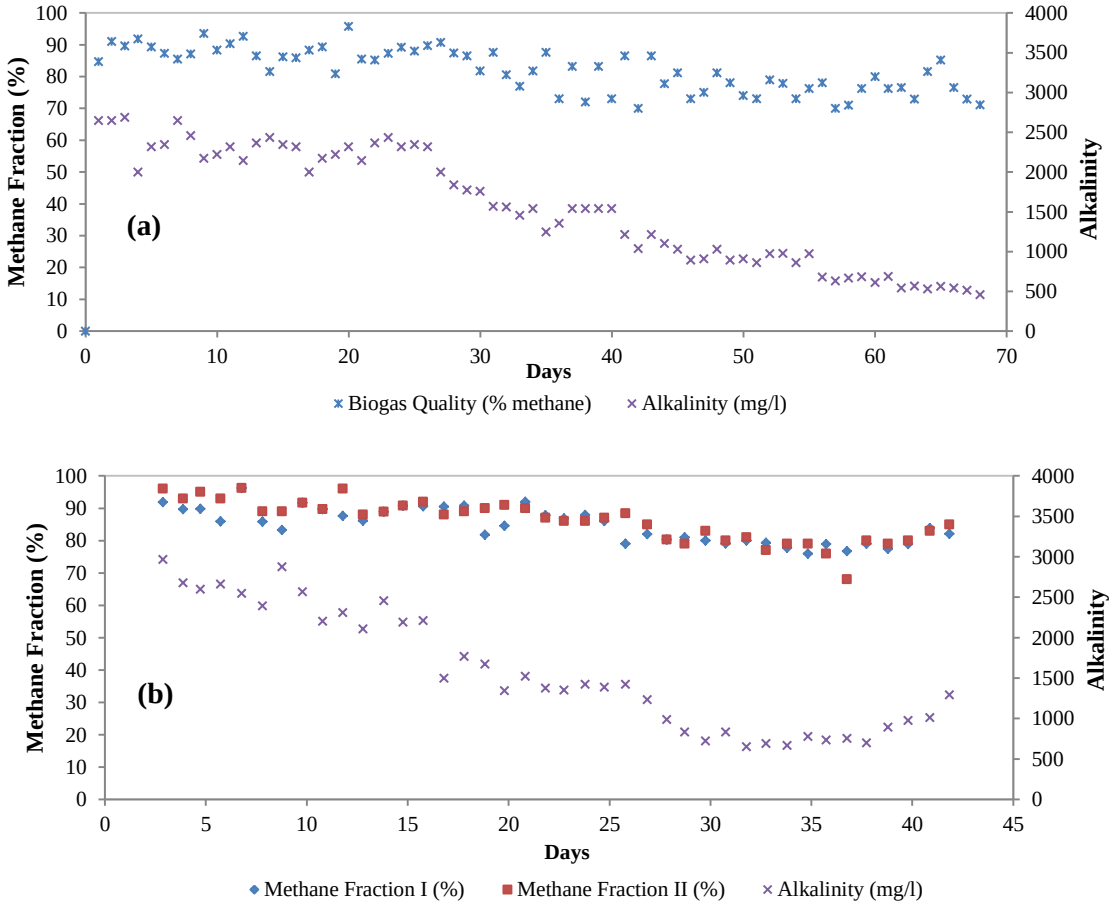


Figure 4.5 *Biogas quality (methane fraction) profiles in (a) Reactor A and (b) Reactor I/II*

Figure 4.6 and Figure 4.7 present the methane production rate of volume specific (l/l.d) in function of operation periods and OLR (gCOD/l.d). Significant decreased methane productions were observed during washing-out the granules from Reactor A (day 55) and Reactor I (day 22). On the other hand, methane production in Reactor II relatively was more stable and higher than methane rate in Reactor A and Reactor I. In general, methane production rate increased with increasing OLR.

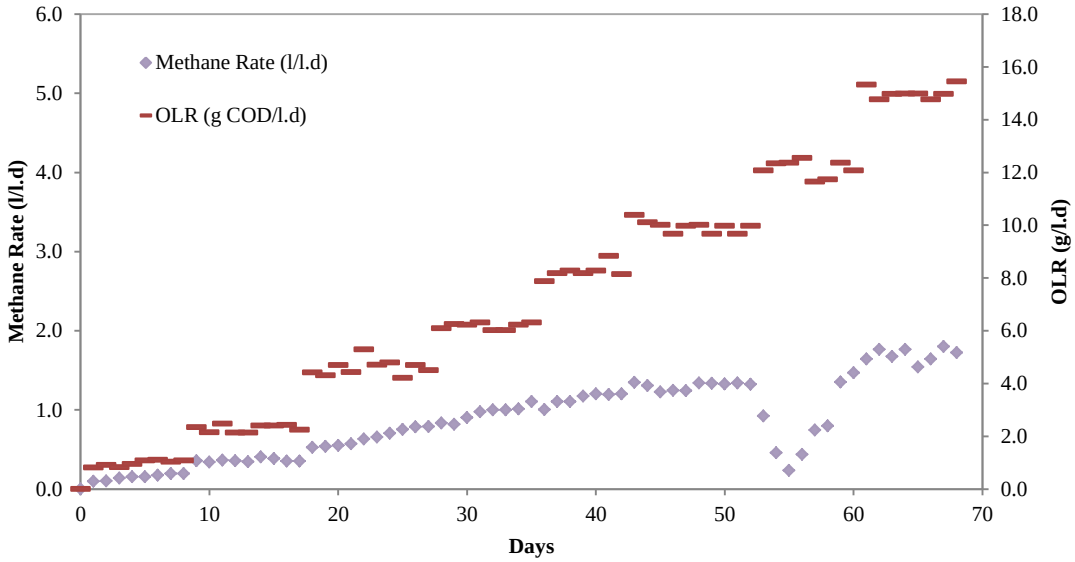


Figure 4.6 *The actual, expected methane production rate, and OLR profiles in Reactor A*

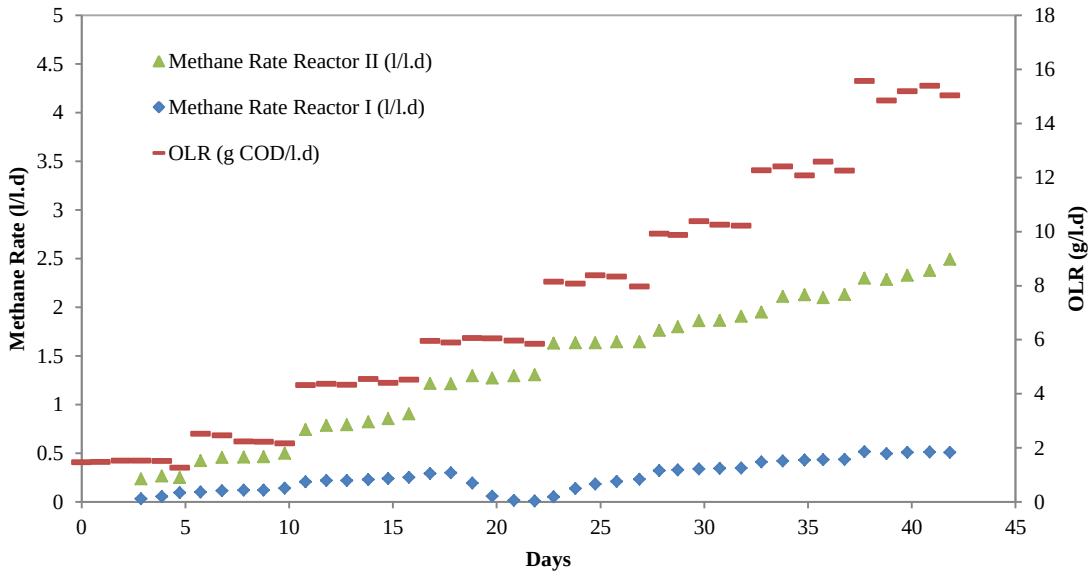


Figure 4.7 *The actual, expected methane production rate, and OLR profiles in Reactor I/II*

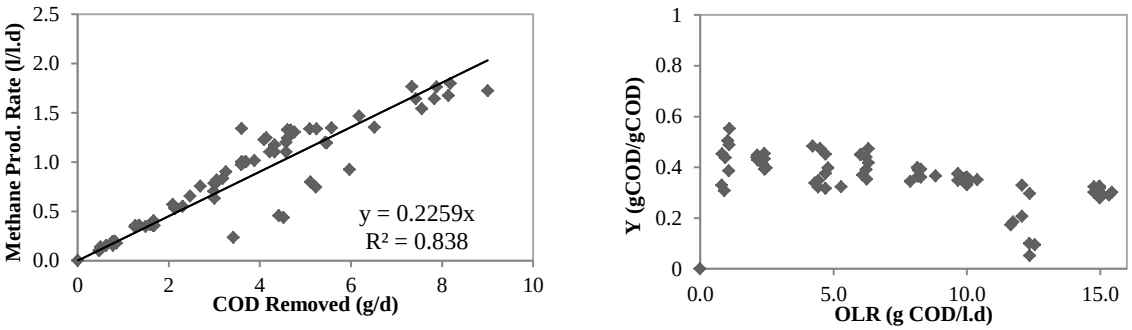
Methane yield was determined graphically in Figure 4.8 from values of methane production rate in function of COD removed. Methane yield in Reactor A was linear by y = 0.2259x and the coefficient regression (R^2) by 0.84. Furthermore, methane yield in Reactor I and Reactor II was also linear by y = 0.2243x (R^2 = 0.838) and y = 0.286x (R^2 = 0.9373) respectively. Methane yields obtained during operation and theoretical methane yield are presented in Table 4.2. The theoretical methane yield was also determined graphically from values of theoretical methane production rate in function of COD removed, as presented in Figure 4.9. From Table 4.2, the observed methane yields were close to the theoretical methane yields. The COD conversion of 62%; 59%; and 75% corresponded to the percentage of COD in wastewater that were converted to methane in Reactor A, Reactor I and Reactor II, respectively.

Table 4.2 *Methane yield and COD conversion to methane at operating condition*

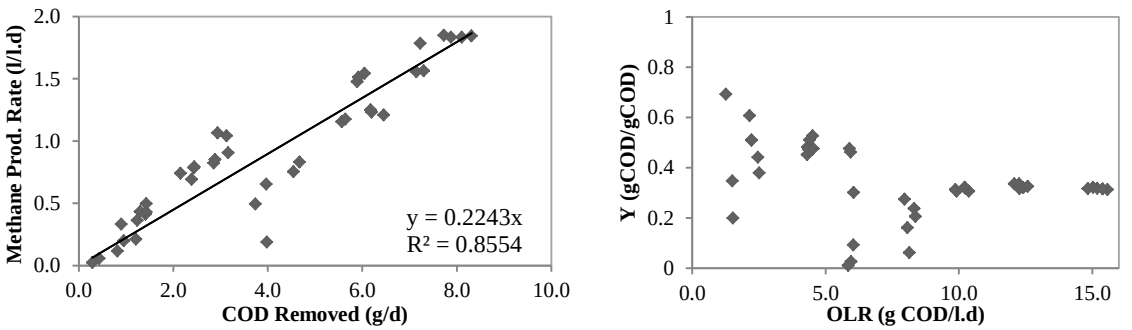
Reactor	Methane Yield l CH_4/$gCOD_{removed}$	Methane Yield gCOD CH_4/$gCOD_{removed}$	Theoretical Methane Yielda[a)] l CH_4/$gCOD_{removed}$	COD Conversion %
Reactor A	0.226	0.62	0.251	62
Reactor I	0.224	0.59	0.258	59
Reactor II	0.286	0.75	0.299	75

a) The theoretical methane yield was also determined graphically from values of theoretical methane production rate in function of COD removed, as presented in Figure 4.9.
Theoretical methane rate = COD removed (1 – methane yield observed)

(a) Reactor A



(b) Reactor I



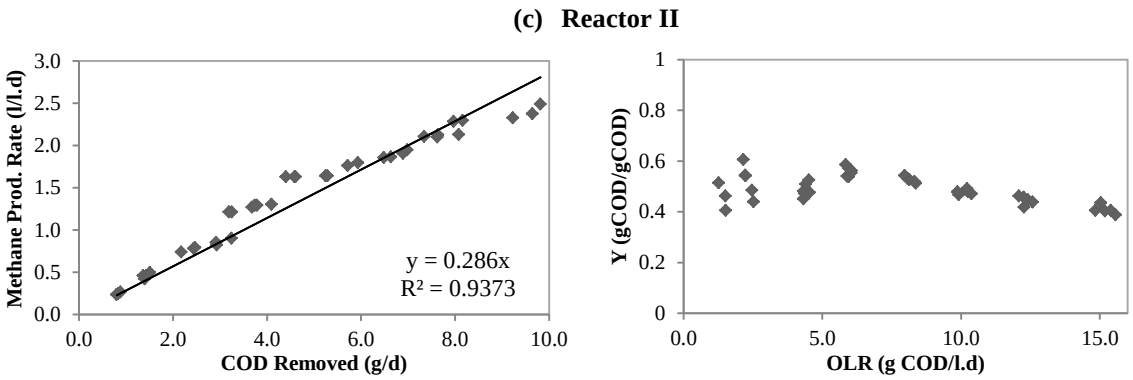


Figure 4.8 *Graphical determination of methane production Reactor A/I/II*

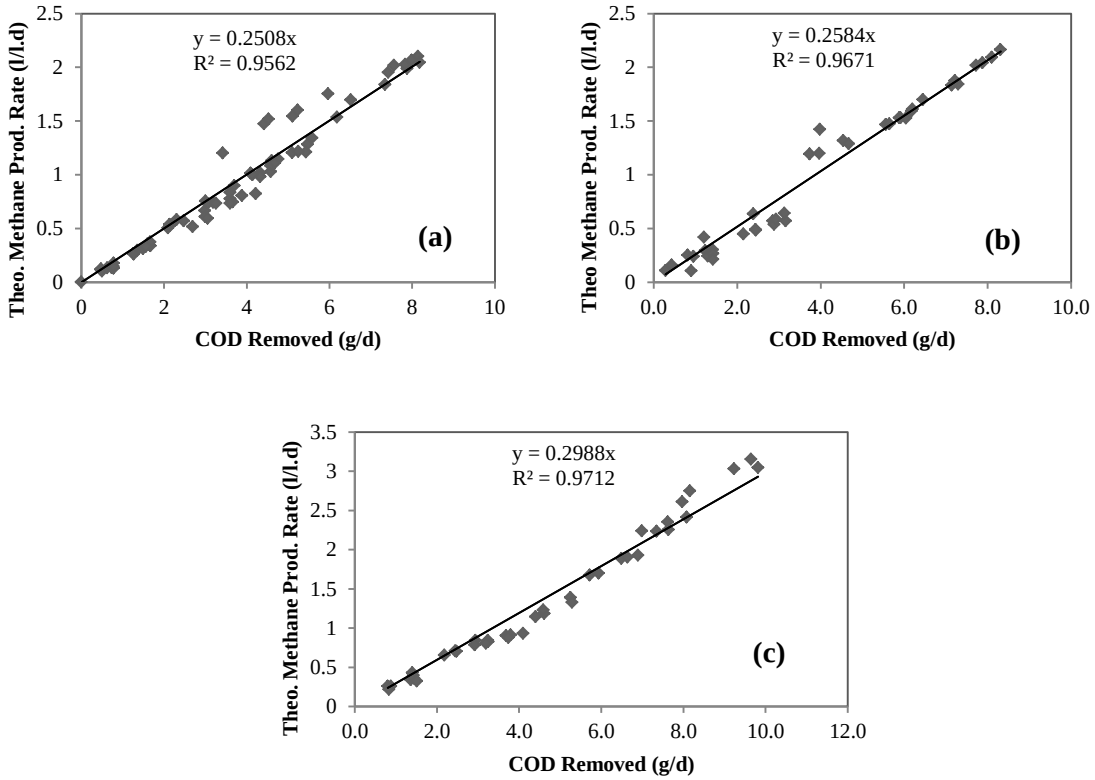


Figure 4.9 *Graphical determination of theoretical methane production (a) Reactor A; (b) Reactor I; and (c) Reactor II*

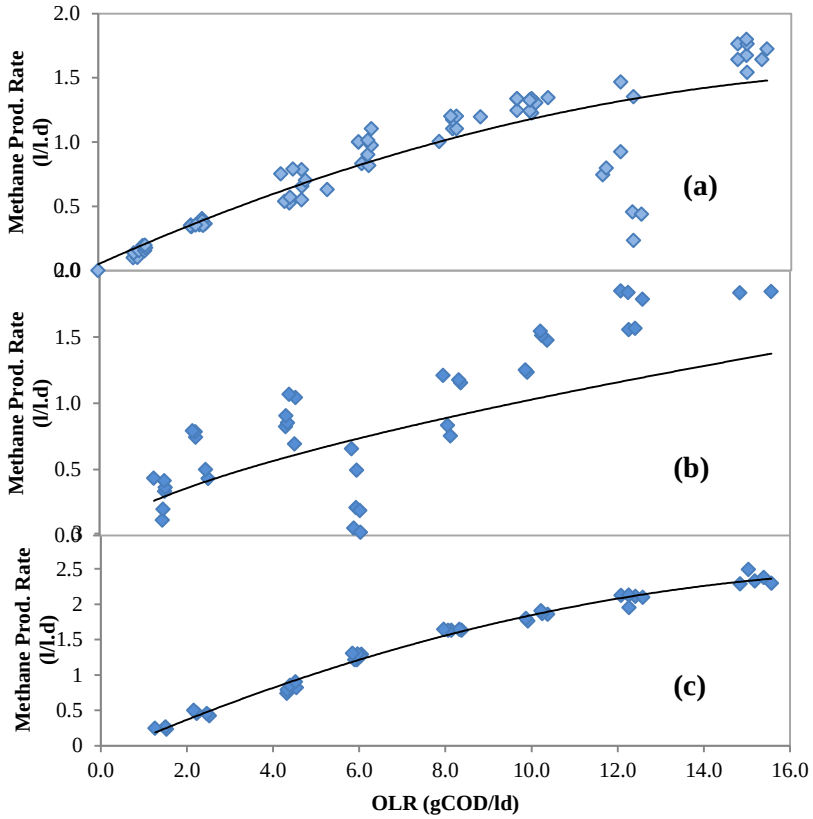


Figure 4.10 *Methane production rate in function of OLR in (a) Reactor A; (b) Reactor I; and (c) Reactor II*

Figure 4.8 also illustrates methane yield profile (gCOD/gCOD) in function of OLR. From Figure 4.8, the significant decreased methane yield productions were also observed during washing-out the granules from Reactor A and Reactor I. Generally, methane yield decreased with increasing OLR. The higher uniformity of methane yield in Reactor II was observed relatively compared to Reactor A and Reactor I at the same OLR. Figure 4.10 shows methane production rate in function of OLR in Reactor A, Reactor I, and Reactor II. The power trend line was applied and the trend lines follows Monod kinetic curve to as presented in Figure 2.7.

4.3.3. COD Balance and Fraction

COD balance and COD fractionation are presented in this sub-chapter. COD balance were calculated based on dissolved COD feed, outlet, and corresponding methane production as COD by 0.37 l CH_4/g COD (20 °C) and 0.38 l CH_4/g COD (25 °C) in Reactor A and Reactor I/II respectively. The COD balance and OLR of Reactor A and Reactor I/II were plotted in Figure 4.11 and Figure 4.12 in function of operating period. The graphs illustrate the COD balance profile with increasing OLR profile during experiment.

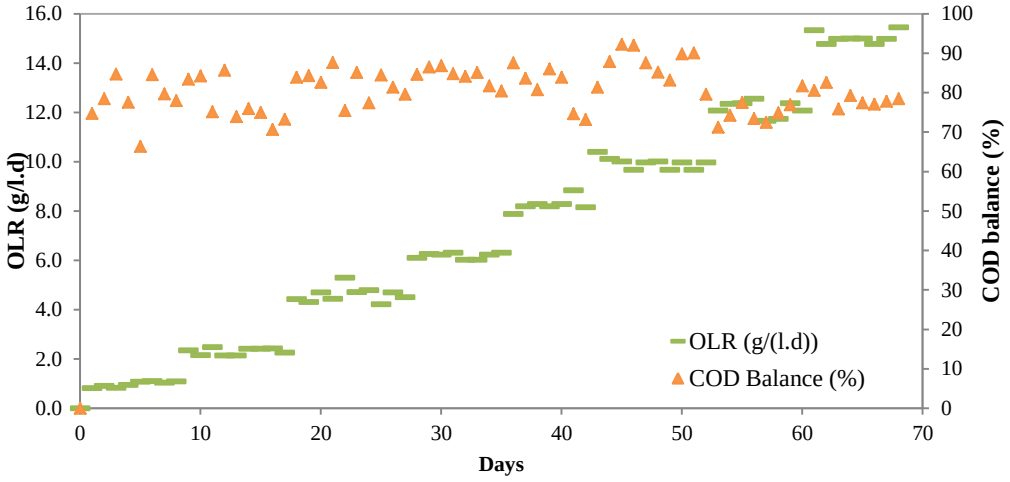


Figure 4.11 *The COD balance and OLR profiles of Reactor A*

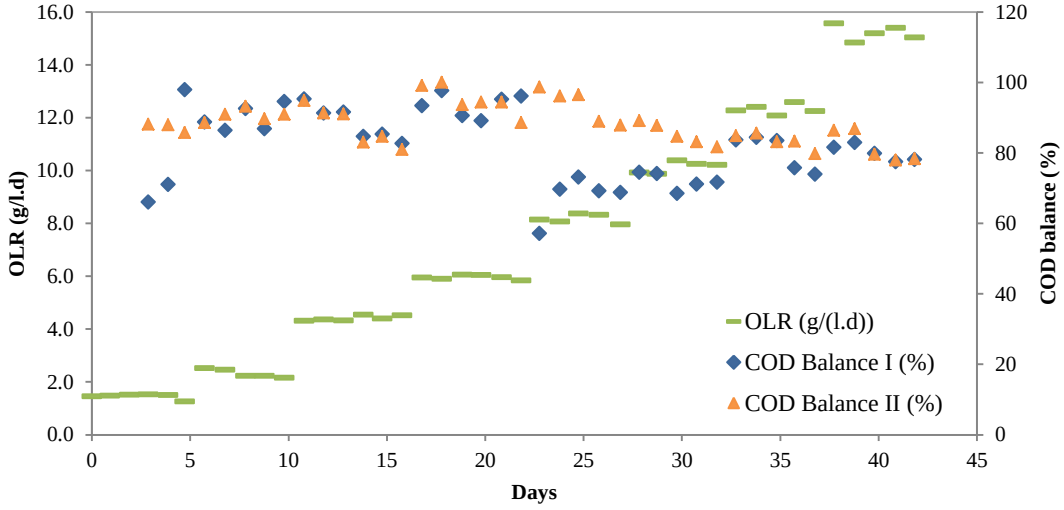


Figure 4.12 *The COD balance and OLR profiles of Reactor I/II*

The greater recoveries of COD were obtained above 90% for periods with high removal efficiencies of organic matter as COD and methane yield when 10.0 g COD/l.d, 6.0 g COD/l.d and 6.0 – 8.0 g COD/l.d of OLR were applied in Reactor A, Reactor I and Reactor II, respectively. The lowest COD recoveries were observed during sludge washed-out. The overall averages of COD recoveries were 80%, 82% and 88% in Reactor A, Reactor I and Reactor II, respectively.

The calculation excluded COD biomass/sludge since COD biomass was not determined. To estimate the biomass produced from the processes, total COD tests were done once in a while to observe particulate fraction in both inlet and outlet of the reactors. Particulate fraction in the outlet compared to the inlet can estimate inert COD fraction and biomass produced. The measurements were conducted four times and every measurement was done

in three consecutive days. Figure 4.13 presents the COD fractionation on Reactor A and Reactor I/II. In general, particulate COD increased by 13.3%, 14.0%, and 11.5% in the outlet of Reactor A, Reactor I and Reactor II respectively. The proportion of influent COD converted to biomass can be also assumed to be 10% [5, 23].

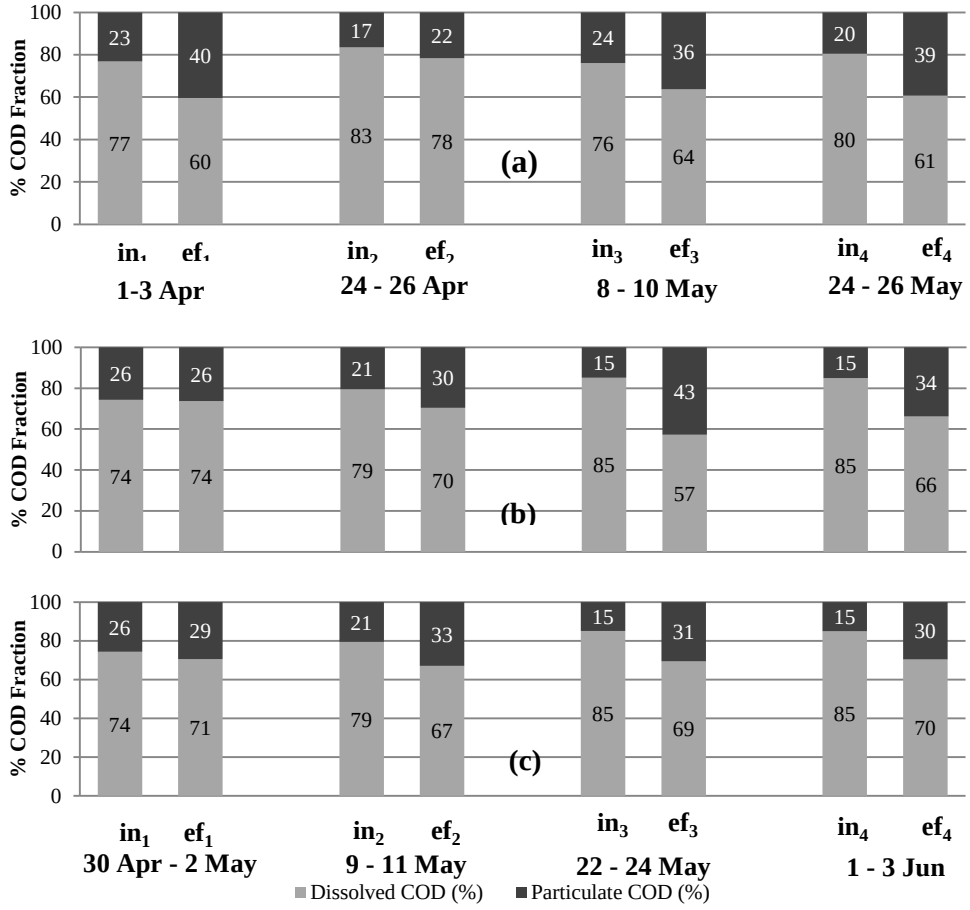


Figure 4.13 *The COD fractionation in (a) Reactor A, (b) Reactor II and (c) Reactor II*

4.4. pH, Alkalinity, and VFA Variability

Figure 4.14 and Figure 4.15 show pH, alkalinity, and VFA variability profiles of Reactor A and Reactor I/II during experiment in function of time and $NaHCO_3$ concentration. The calculated concentrations are expressed as mg acetic acid/l of total VFA and mg $CaCO_3$/l of alkalinity. It is observed an accumulation of VFA up to 721 mg/l, 671 mg/l and 411 mg/l in Reactor A, Reactor I and Reactor II. The gradual drop of $NaHCO_3$ concentration as buffer provoked the accumulation of VFA in the reactors and pH was drop in range 6.4 – 6.8.

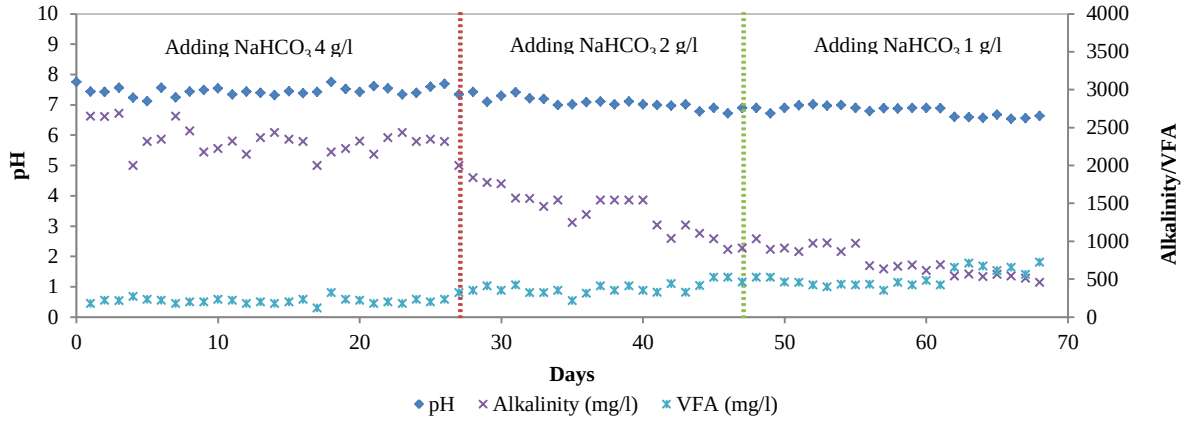


Figure 4.14 *pH, alkalinity, and VFA profiles of Reactor A*

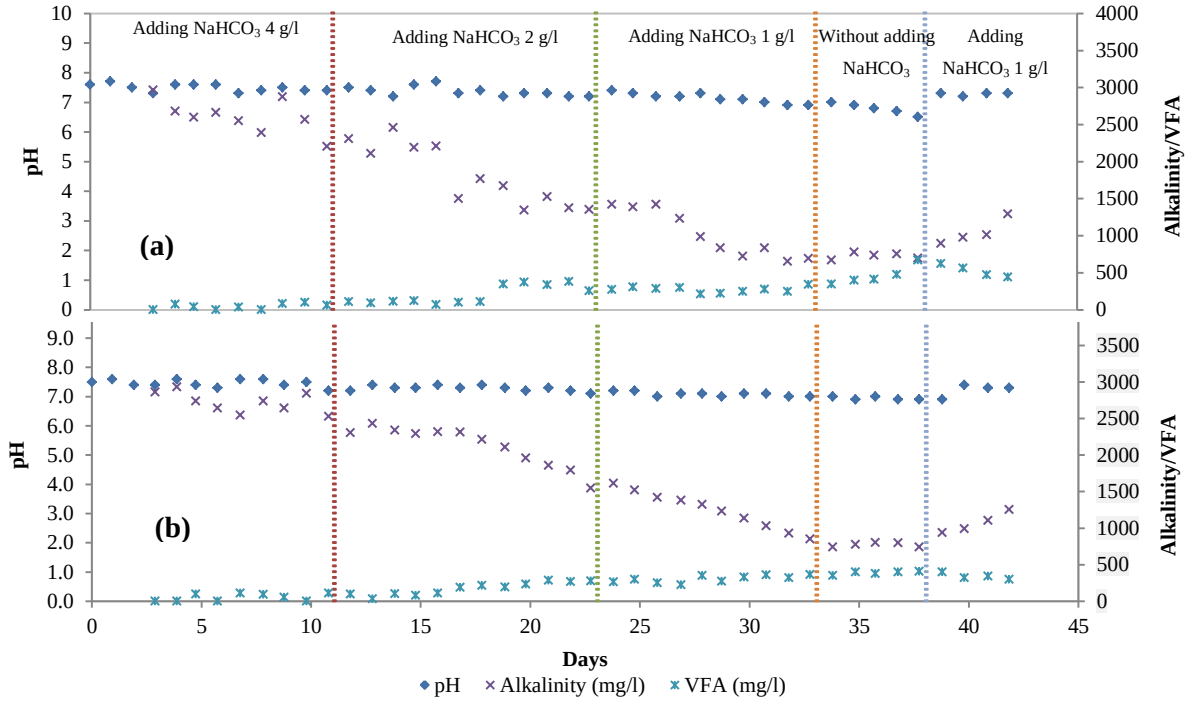


Figure 4.15 *pH, alkalinity, and VFA accumulation profiles of (a) Reactor I and (b) Reactor II*

Alkalinity was recovered more in Reactor I and Reactor compared to Reactor A. Therefore, when 12.0 g COD/l.d of OLR was applied (day 34 to day 38), there was no addition of $NaHCO_3$. At day 39, OLR was increased by 15.0 g COD/l.d and pH in Reactor I dropped to 6.40. Hence $NaHCO_3$ 1 g/l was added to feed wastewater and pH was stabilized around 7.

4.5. Nutrients Availability

Nutrient measurements were conducted once in a while and every measurement was done in three consecutive days. The experiment results are summarised in Table 4.3. The errors

were determined using t-distribution analysis with 90% of certainty. In general, phosphorous and nitrogen in the feed were consumed. The phosphorous concentrations in the outlet were decreased by around 35%, 45% and 37% in Reactor A, Reactor I and Reactor II respectively. Moreover, 44%, 36% and 29% of nitrogen removal were observed in Reactor A, Reactor I and Reactor II respectively.

Table 4.3 *Total phosphorous and total nitrogen availability of Reactor A/I/II*

Date	Total Phosphorous (mg/l)		Total Nitrogen (mg/l)	
	Inlet	Outlet	Inlet	Outlet
Reactor A				
21 – 23 Mar	14.0±0.1	15.3±1.2	26.1±1.9	19.8±1.7
1 – 3 Apr	15.9±0.4	12.8±1.4	23.1±2.2	12.8±1.3
24 – 26 Apr	15.9±1.0	8.8±1.4	25.7±1.7	13.8±1.5
3 – 5 May	17.1±0.4	5.6±1.6	17.7±0.8	7.3±1.6
24 – 26 May	15.8±0.4	8.9±1.9	18.5±1.4	8.7±1.6
Reactor I				
30 Apr – 2 May	15.4±1.2	14.0±0.4	22.8±1.2	16.1±0.7
9 – 11 May	15.7±0.6	7.8±1.8	24.4±1.6	18.5±1.4
22 – 24 May	18.0±0.6	5.8±1.6	17.2±0.5	11.5±1.6
1 – 3 Jun	18.4±1.3	9.4±1.3	18.4±1.3	7.0±0.6
Reactor II				
30 Apr – 2 May	15.4±1.2	15.3±1.2	22.8±1.2	19.8±1.7
9 – 11 May	15.7±0.6	12.8±1.4	24.4±1.6	12.8±1.3
22 – 24 May	18.0±0.6	8.7±1.4	17.2±0.5	12.9±1.0
1 – 3 Jun	18.4±1.3	5.6±1.6	18.4±1.3	13.1±1.5

4.6. Granules Density

Figure 4.16 presents the solid content in the granules that measured after washing out the sludge during experiment. Total solid contents obtained were 265 g/l, 206 g/l and 342 g/l in in Reactor A, Reactor I and Reactor II, respectively.

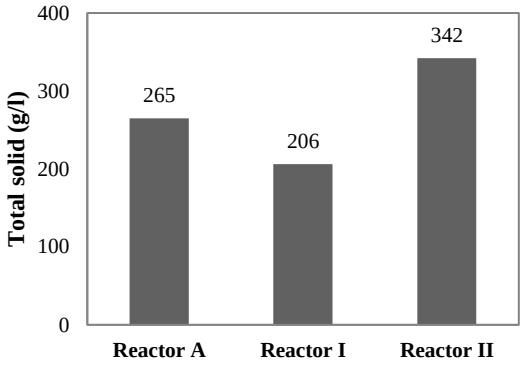


Figure 4.16 *Total solid of the reactors*

5. Discussions

Results obtained from experiments are discussed in this chapter. This chapter is divided into five sub-chapters: (a) reactor acclimatization; (b) reactor performance including COD removal efficiency, methane production, COD balance as well as the comparison between continuous reactors and batch test; (c) environmental factors including pH variability, VFA generation, alkalinity and nutrient availability; (d) economy and energy recovery (e) and hydrodynamic condition that describes overall condition of reactors.

5.1. Reactor Acclimatization

In UASB reactors, the up-flow velocity due to the feed mechanism and the recirculation effluent, as the wastewater was passing the reactor from the bottom side to the top of the reactor. Henze *et al.* (2008) stated that average up-flow velocity in the UASB reactors are in the range of 0.5 – 1.0 m/h. During reactivation of granules in Reactor A, up-flow velocities was applied by 0.5 (±0.05) m/h, 0.7 (±0.06) m/h and 1.0 (±0.10) m/h. The COD removal efficiency up to 80% could be achieved by 1.0 m/h of up-flow velocity in 4 days of HRT, compared to 0.5 m/h and 0.7 m/h, as presented in Figure 4.2. Hence, a 1.0 m/h of up-flow velocity was used during operation in the UASB reactors with a 5:1 ratio, recirculation flow rate and inlet flow rate respectively.

Lopez *et. al.* (2015) observed the influence of the recirculation ratio and found the optimal ratio obtained was 10:1. However, they stated that it would depend on the hydrodynamic and characteristic of the reactor e.g. height/diameter ratio. The up-flow velocity related to mixing and contact between wastewater and granules. For achieving the required sufficient contact between granules and wastewater, the reactors relied on the agitation from the biogas production, the feed distribution and recycling flow at the bottom of the reactors. Mixing should be intensive for generating more contacts, though then it should still be a gentle mode of mixing to avoid the granules from being washed-out or deformed.

The acclimatization of granules in Reactor I and Reactor II was in the first five days of experiment time until the reactors were in steady state condition. Slower bacterial activity in Reactor A was observed during acclimatization periods as the granules had been preserved unfed from May 2015. Lettinga (1995) confirmed an important characteristic of methanogenic granules is its feature that it can be preserved for long periods of time under unfed conditions. This is confirmed by the available practical experiences with this

secondary start-up after idle time. During acclimatisation periods, the granules in Reactor I/II were considered to be relatively more active because these granules had lower food mass ratio (F/M) compared to granules that were used in Reactor A.

F/M refers to the balance between the food supply and the mass of microorganism in the system [13]. After acclimatisation periods, both granules were more active due to lower F/M was developed over time. A low F/M ratio enhanced organic removal efficiency and granulation processes, thereby increasing the biomass retention in the reactor. However, if the F/M ratio becomes too low, cell growth would be limited and granules deformation might be occurred [32, 33]. A high F/M ratio provided a high driving force for metabolic activity and microbial growth and high overall rates of organic matter conversion to methane. However, a excessive value of F/M ratio could disturb the balance between hydrolyzing and methane formation [32, 33], destroyed the process efficiency and the microbial ecology, and result in granules deformation.

5.2. Reactor Performance

In this sub-chapter, reactor performance will be evaluated and analysed by investigating two main parameters; COD recovery and methane production. These two parameters determined the effectiveness of an anaerobic treatment for treating high strength of municipal wastewater under mesophilic condition. In the end of this section, COD balance and the comparison between continuous reactors and batch test also will be analysed.

5.2.1. COD Removal Efficiency

Figure 4.3 and Figure 4.4 show the behavior of COD concentration at the outlet of the reactors and the COD removal efficiency in function of time and OLR, during the period of operation. In general, when the OLR increased, COD removal efficiency decreased; in the same way, when the OLR augmented, perturbations were provoked in the performance of the UASB reactors, affecting the stability, and in consequence the efficiency of the reactor diminished. Therefore, the effluent COD increased to certain amount in every first day of the increased OLR. Then, the reactors were stabilized and the effluent COD started decreasing until steady state condition was achieved. This pattern also described the assimilation of bacterial growth. When increasing the OLR, the concentration of the material was increased, the specific growth rate would increase until the maximum specific growth rate (μ_{max}) was reached. The decreasing F/M was considered to be

occurred over time due to increasing biomass in the Reactors. A low F/M ratio enhanced organic removal efficiency and granulation processes, thereby increasing the biomass retention in the reactor. The F/M ratio would be stable in equilibrium and optimum condition, the bacteria were active and they multiplied rapidly.

The most important instabilities in Reactor A and Reactor I were observed at day 55 (28% of COD removal efficiency) and day 22 (5% of COD removal efficiency) respectively. This was associated with the granules being washed out from the reactor. The mechanisms of washing-out granules will be analysed in the following section (hydrodynamic condition). It also could be caused by the decreasing alkalinity due to the increment in the acidity in the UASB reactors as buffer concentration ($NaHCO_3$) was decreased to 1 g/l. Reactor A was stabilized after day 59 and the efficiencies of COD were above 50%. Moreover, Reactor I achieved COD removal efficiency was above 50% at day 23 after adding new granules to the reactors.

From the result, the average COD removal efficiency decreased with decreasing HRT and increasing OLR. The reason for this decrease in efficiency over decreasing HRT, in spite of increasing the turbulence in the reactor, was that the contact time of wastewater with granules will be decreased. Therefore, less organic matter was utilized. The decreased efficiency also was observed with the increasing OLR. The increasing OLR would increase the biomass growth in the reactors, according to Monod kinetic, the specific growth rate would increase until the maximum specific growth rate (μ_{max}) was reached; this could be associated with the increasing substrate concentrations. Therefore, the increased substrate concentrations resulted in the reduced removal efficiency.

Although very low HRT by 1.4 h and high OLR by 15.0 0 g COD/l.d were applied during experiment, the results show the highest loading capacity (optimum condition) was achieved in Reactor II with 70% of COD removal efficiency at 3.3 h of HRT; 6.0 g COD/l.d of OLR; and 1.3 l CH_4/d (0.6 g COD CH_4/g CODin) was generated. Increasing OLR and decreasing HRT even further could reduce overall efficiency of the reactors both COD removal efficiency and methane production rate.

Similar findings have also been reported in the literature by Rizvi *et.al.* (2013). The results revealed that the COD removal efficiency of UASB reactor treating municipal wastewater was 50 - 73% at HRT of 3 h. Farajzadehha *et. al.* (2012) also reported optimum HRT for influent COD concentration of 1200 mg/l strengthen wastewater was shown to be 4 h with

70-85% of COD removal efficiency. The HRT is also directly related to up-flow velocity in a UASB reactor. Hence, an adequate up-flow velocity and accordingly HRT provides sufficient contact between sludge and wastewater, reduces the formation of gas pockets, disengages the biomass from gas and resultantly enhances COD removal efficiency of the system [34, 35].

5.2.2. Methane Production

Besides the COD removal efficiency, the UASB reactors performance also can be evaluated by investigating methane produced, as CH_4 is equivalent to a certain amount of COD. Figure 4.6 and Figure 4.7 present the significant decreased methane yield productions during washing-out the granules from Reactor A and Reactor I. The mechanisms of washing-out granules will be analysed in the following section (hydrodynamic condition). Methane yield was determined by the linear regression analysis from the experimental data, CH_4 production rate and COD removed, adjusted by the method of least squares. From Figure 4.8, methane yields obtained were 0.226 l CH_4/g COD (0.62 g COD CH_4/g COD removed), 0.224 l CH_4/g COD (0.59 g COD CH_4/g COD removed), and 0.286 l CH_4/g COD (0.75 g COD CH_4/g COD removed) in Reactor A, Reactor I and Reactor II respectively at operating condition.

The observed methane yield were close to the theoretical values; 0.251 l CH_4/g COD, 0.258 l CH_4/g COD and 0.299 l CH_4/g COD. The observed methane yields were equivalent to 0.211 l CH_4/g COD, 0.205 l CH_4/g COD, and 0.262 l CH_4/g COD, respectively at STP (0 °C, 1 atm). At STP, theoretical value corresponds to 0.35 l CH_4/g COD (100% COD conversion) [6, 13]. Besides the errors from the analytical methods, the unforeseen gas leakages, the inaccuracy of the gas counter at low gas flow rates and/or a higher solubility of methane in the wastewater were probably the reasons of methane loss. Frequently, the liquid level in the degasser was too low (even no liquid was observed), therefore the gas would also went out to the outlet. This condition could be controlled by adjusting the liquid outlet and degasser position to increase head until the liquid went to a normal level.

Figure 4.8 also shows the optimum condition at 3.3 h of HRT with 6.0 g COD/l.d of OLR in Reactor II produced maximum methane yield by approximately 0.6 g COD CH_4/ g CODin. Generally, methane yield decreased with decreasing HRT. As described

previously, the contact time of wastewater with granules will be decreased with the decreasing HRT.

Moreover, Figure 4.10 shows methane production rate in function of OLR in Reactor A, Reactor I, and Reactor II. The trend lines follows Monod kinetic curve to as presented in Figure 4.10 due to loading capacity and assimilation of bacterial growth. The increasing OLR would increase the biomass growth in the reactors and reach the maximum growth rate, according to Monod kinetic; this could be associated with the increasing substrate concentrations. Therefore, the increased substrate concentrations resulted in the reduced removal efficiency and methane production. The higher uniformity of methane yield in Reactor II was observed relatively compared to Reactor A and Reactor I at the same OLR. The granules in Reactor II were considered to be relatively more stable due to more granules containing and optimum F/M ratio. As described previously, a low F/M ratio enhanced organic removal efficiency and granulation processes, thereby increasing the biomass retention in the reactor. The F/M ratio would be stable in equilibrium and optimum condition, the bacteria were active and they multiplied rapidly.

Methane production rate increased with OLR, proportional to the amount of organic matter removed in the UASB reactors. Therefore if COD removal efficiencies diminished, methane production rate also decreased; contrarily, if the efficiency increased then the methane production rate also increased. Methane generation was not significant when the UASB reactor was unstable after sludge washed out at day 55 in Reactor A and at day 20 in Reactor I. Besides in Reactor II, methane yield and COD removed were more stable and methane production rate increased gradually with increasing of OLR. These indicators of stability of UASB reactor II were basically associated with biomass retention in the system.

The biogas quality can be further determined by the proportion of methane in the biogas, and for simplicity it was assumed to contain only CO_2 and CH_4. As presented in the configuration of UASB reactors (Figure 3.2 and Figure 3.4), the biogas generated during anaerobic processes passed through a solution of 3 M NaOH in order to absorb and convert the CO_2 present in biogas to Na_2CO_3. No absorbed methane was measured by the second gas counter. From Figure 4.5, during 30 days of experiment periods in Reactor A and 20 days in Reactor I/II, the average methane fraction was above 90% with 10% of CO_2 fraction. The decreased methane fractions in biogas were observed by around 70%

after decreasing buffer ($NaHCO_3$) concentration. It is considered that this condition occurred due to the equilibrium of CO_2 species in the solution within carbonate system [36]. This lower CO_2 fraction also could be caused, by (a) the relatively high solubility of CO_2 in the water and (b) part of the CO_2 produced might become chemically bound in the water phase due to the formation of ammonia in the anaerobic conversion and cations which were present in the wastewater as salts of VFA, SO_4^{2-}, NO_3^- [6].

5.2.3. COD Balance

The anaerobic reactor performance can be monitored by the COD which gives the operator vital information about the functioning of the system. Adequate action can be undertaken before irreversible deterioration occurs. Also, the impact of alternative electron acceptors on the CH_4 production rate can be easily assessed while based on the gas production and effluent COD values, an estimate can be made of the amount of newly grown and entrapped biomass [6]. The overall averages of COD recoveries were above 80% in UASB reactors. The calculation excluded COD biomass/sludge, as COD biomass was not determined. Total COD was conducted to estimate COD fractionation in both inlet and outlet of the reactor. Particulate COD fraction consists of the amount of inert COD and biomass COD produced in the system. The results show particulate COD increased by 13.3%, 14.0%, and 11.5% in the outlet of Reactor A, Reactor I and Reactor II respectively. These values could be associated with COD biomass produced in the reactors. However, a lot of fine fluffy granules were washed-out during experiment especially in Reactor A and Reactor I. Therefore, particulate COD obtained relatively could be altered with the real condition. Hence, assimilation of bacterial growth was demonstrated during experiment as mentioned in previous section. To counter this issue, the proportion of influent COD converted to biomass in this experiment was assumed to be 10% [5, 23] and the COD recoveries obtained would be over 90%.

Henze *et. al.* (2008) mentioned that fat or LCFA-containing substrates resulting very high COD removal efficiencies but low CH_4 production rates which lead to huge gaps in the COD balance. Therefore, the COD balance gaps by around 10% of COD loss from this study can be explained by the high fat containing substrate as several wastewater in IVAR Grødaland are from dairy and animal slaughtering industry; besides the errors from the analytical methods. The unforeseen gas leakages, the inaccuracy of the gas counter at low

gas flow rates and/or a higher solubility of methane in the wastewater were probably the reasons of COD loss as well.

Another frequently cited cause for a COD gap is the loss of electrons when these are linked to oxidized anions like SO_4^{2-} and NO_3^-. Therefore, for closing the COD balance either all reduced gases should be taken into account or the concentration of electron acceptors needs to be measured. Furthermore, soluble COD containing gases like H_2S, will be present in the effluent. Another reason is the entrapment or accumulation of COD in the sludge blanket [6]. However, both ion and biogas specification, and COD biomass/sludge were not conducted in this study.

5.2.4. Batch Test Analysis

The UASB reactors as continuous reactors can be further evaluated by comparing to the biogas potential batch test. Based on Table 4.1, the methane yield of wastewater batch test was about 0.4 gCOD CH_4/gCOD (40% COD conversion to methane) with COD recovery around 70%. The COD recovery represented the overall COD balance of which COD conversion to methane and non-biodegradable dissolved COD. The biogas potential batch tests in closed vessels had generally lower COD reduction and lower gas produced in consequence the COD conversions to methane were also lower compared to the continuous reactors with about 59 – 75% of COD conversion to methane in UASB reactorss. This condition occurred may be due to the different sludge used in the test. The batch test was inoculated with granules from the anaerobic reactor at IVAR Sentralrenseanlegg Nord-Jæren. This granule was considered to have an excessive F/M ratio at day 0 and the operation became substrate limited at the end of test (too low F/M tario). As mentioned previously, an excessive value of F/M ratio could disturb the balance between hydrolyzing and methane formation, destroyed the process efficiency and the microbial ecology, and result in granules deformation. In addition to the previously mentioned, the accumulated inhibitory substances could also be the source of the COD loss in batch test as well, e.g. H_2. Higher H_2 absorption may have stimulated microbial H_2 utilization or generated more reduced anaerobic products [24].

5.3. Environmental Factors

Fermentation process results to formation of various VFAs which are then converted to methane in the methanogenesis stage. During the experiment, the concentration of these

VFAs were measured by the titration method and monitored to ensure that the concentration did not increase beyond an acceptable level, as this would result to accumulation of acids and imbalance in the anaerobic reactor. However, in this experiment, specific measurement and monitoring of various VFAs was not carried out, e.g. propionic acid or butyric acid.

As presented in Figure 4.14 and Figure 4.15, pH varied from initially 7.2 – 7.6 to pH 6.5 – 6.8 at the end of the reaction cycle. Under these conditions, a high fraction of VFA accumulation was expected in the anaerobic processes. The five point titration indicated the total amount of VFA generated in the system as acetic acids. A decrease in pH below 7 in several points during the reactor performance period can be observed; it was provoked basically when the OLR was increased at 10.0 gCOD/l.d, in consequence removal efficiencies of COD dropped to below 50%. This decreasing COD removal efficiency was due to alkalinity in the reactor, which was not sufficient to neutralize the acidity present in the inlet of the reactors. In these cases, the addition of $NaHCO_3$ allowed to reach the stability of the UASB reactors operation. $NaHCO_3$ 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. The addition of sodium bicarbonate, especially during starting up, is imperative for maintenance of pH around 7 and for keeping the stability of the system [19, 23].

Generally, the decreased alkalinity led to pH drops due to VFA accumulation in the reactors. An important accumulation of VFAs was observed at day 68 in Reactor A, directly related to a change in the OLR; at the same time a decrease of alkalinity and pH was observed. Furthermore, COD conversion to methane was inhibited; COD removal efficiency and/or methane production decreased, especially occurred in Reactor A of which the decreasing COD removal efficiency below 50%. Meanwhile, Reactor I and Reactor II had relatively stable alkalinity. During the first 10 days of Reactor I and Reactor II operation, the addition of external alkalinity by 4 g/l of $NaHCO_3$ was necessary to assure the stability of the reactor. The concentration of buffer gradually decreased. From day 33, due to the recirculation, it was possible to recover alkalinity generated from the anaerobic process, that is carbonate system, the CO_2 reacted with water to form carbonic acid (H_2CO_3); H_2CO_3 is a diprotic acid and dissociates in to steps to bicarbonate (HCO_3^-) and then to carbonate (CO_3^{2-}) [36], therefore the addition of $NaHCO_3$ was not

necessary anymore. Gradual increments in OLR and recirculation assured the stabilization of the UASB reactors operation.

At day 38, pH in Reactor I dropped to 6.2 and VFA was generated by 671 mg/l as acetic acid. To stabilize alkalinity in Reactor I, 1 g/l of $NaHCO_3$ was added at day 38 and pH started to increase. However, Reactor II was stable and the removal efficiency of COD in kept constant above 50%. Stable reactor performance in Reactor II, as shown by constant COD removal efficiency and low VFA concentration when buffer was not added, was related to high degree of sludge retention inside the reactor. The accumulation of VFAs in different periods could be associated with the increased OLR and the overload supply of VFAs in the feed, or with a possible inhibition of methanogenic bacteria by the environmental factors and/or inhibitory substances [13]. The make-up of the different anaerobic intermediates products may be couples to environmental condition such as the high concentration of H_2. The presence of H_2 is frequently used to regulate the generation of acetic, propionic and butyric acid [6, 13].

Besides pH, alkalinity and VFA variability, nutrient availability in the reactors was also important to assure bioreactor performance. From nutrient availability analysis, phosphorous and nitrogen in the feed were consumed for assimilation of bacterial growth. It is considered as macronutrient for biomass growth in the reactors. Benefield, F. D. *et. al.* (1980) has stated that for microorganisms, nutrients (a) provide the material required for synthesis of cytoplasmic material, (b) serve as an energy source for cell growth and biosynthetic reactions, and (c) serve as acceptors for the electrons released in the energy-yielding reactions.

Reddish filamentous precipitates were also observed in the reactors which might be inorganic ferric (Fe) precipitate [6, 13]. As several wastewater in IVAR Grødaland are from animal slaughtering industry, high blood containing, therefore high iron (Fe) concentration will be expected in feed wastewater. Wastewater high in sulphate can be prone to sulphide generation [13]. Sulphide reacts with metal cations including Fe, to form highly insoluble precipitates. The concentration of sulphide is pH-dependent. At acid pH only this metal sulphides of very low solubility can be precipitated e.g. HgS, As_2S_3, CdS, CuS and PbS, whereas at a more alkaline Ph, ZnS, FeS, NiS, and MnS form precipitates [37]. 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 [18].

5.4. Economy and Energy Recovery

In this section, economic and energy recovery will be discussed. Estimation of electricity generation from the methane produced in this experiment will be presented. Anaerobic treatment systems are more expensive to construct but less expensive to operate than aerobic treatment systems. From an economic point of view, the specific methane amounts and the biogas quality from anaerobic treatment are important process parameters related to economic and energy recovery [6, 38].

Henze, M. *et. al.* (2008) and Bruns, E. *et. al.* (2010) have stated the production of about 13.5 MJ CH_4 energy/kg COD removed to methane gives 1.5 kWh electricity by assuming 40% electric conversion efficiency and the rest turns into heat. From the experiment, at operation condition, methane yield of Reactor II was 0.286 l CH_4/g COD removed (0.75 g COD CH_4/g COD removed). Optimum biogas potential (70% of COD removal efficiency) was achieved in Reactor II at 3.3 h of HRT with 6.0 g COD/l.d of OLR and 4.1 g COD/l.d was removed. Therefore, 3.0 g COD/l.d of COD removed was converted to methane. The average loading of IVAR Grødaland is approximately 5000 m^3 per day; in consequence 15000 kg COD/d of methane will be produced. Energy generation will be 202500 MJ giving 81000 MJ (22.5 MWh/d) of electricity and 121500 MJ of heat which can be used for other purposes e.g. aeration and heating applications [38, 39]. The detailed energy recovery calculation is presented in Appendix 6.

5.5. Hydrodynamic Condition

This section describes overall condition of reactors including granulation and granules characteristic and its behaviours affect the reactor performances in Reactor A and Reactor I/II as well as evaluated design and operational condition during experiment.

Granules volumes of 20-30% v/v with respect to the UASB reactors were used. During staring-up period of Reactor II, granules volume of 50% v/v was used and some fine granules were washed-out to approximately 30% v/v. Different size and density of granules occurred in the reactors. Total solid contents obtained were 265 g/l, 206 g/l and 342 g/l in Reactor A, Reactor I and Reactor II respectively. Granules size was observed

during experiment; larger granules were applied in Reactor II by roughly 1.0 – 2.5 mm of granules size compared to 0.5 – 1.5 mm of granules size in Reactor A and Reactor I.

The results showed a distinct decrease in the granules diameter during operation of reactors. Generally, the average grain size of granules was reduced from 2.5 to 1.5 mm approximately in Reactor II and 1.5 to 1.0 mm in Reactor A and Reactor II. Severe deterioration of granules occurred in Reactor I. Granules became smaller fine particles by approximately 0.5 mm of granules size in day 19 and were easily washed-out from the reactor. Furthermore, about 90% of granules were washed-out from Reactor I at day 22. In consequence, COD removal efficiency and methane production decreased significantly. At day 23, the new bigger granules were added to Reactor II; three days of acclimatization were needed until the reactor was stabilized and COD removal efficiency was above 50%.

van Lier (1996) has observed small granules has higher mass transfer due to higher substrate affinity to the granules. However, smaller granules has higher specific activity, they appear to be weaker and more easily washed-out from the system [40]. Due to the imposed specific up-flow velocity, segregation of granules occurred, big granules accumulated in the lower part as a stationary bed while fine fluffy granules presented in the upper part of the reactors.

Sludge blanket expansion frequently occurred in Reactor I and Reactor II due to the buoyancy of accumulated and entrapped biogas, and liquid friction due to up-flow from inlet and recirculation flow rate. It was used to lift the recirculation of granules over the lower part of the reactor, which could result in eliminated dead zone and improved contact between wastewater and sludge. However, it should still be a gentle mode of mixing for avoiding too much granules to be washed-out or deformed and high sludge retention cannot be accomplished, as occurred in Reactor I at day 19 to day 22. Furthermore, too narrow or too high height/diameter ratio in Reactor I and Reactor II were also exacerbated. The expansion could be also caused by the attachment of fast growing filamentous acidogenic bacteria in the granules. Moreover, the growth of acidogenic bacteria enhances granules flotation and leads to excessive expansion of the sludge blanket, consequently to low sludge retention in the system [7].

On the other hand, there was no excessive sludge blanket expansion in Reactor A during experiment due to lower height/diameter ratio compared to Reactor I and Reactor II. As mentioned previously, using taller reactors (or a high height/diameter ratio) combined with

effluent recirculation where a high superficial velocity is beneficial for better mixing and contact between wastewater and granules. In tall reactors, the gas loading and the hydrostatic pressure at the bottom can be higher than in short reactors [7, 28].

Reactor I and Reactor II demonstrated reactors with high height/diameter ratio. This study identified the internal mixing to be sub-optimal in Reactor I and Reactor II due to dead spaces in the bottom part of the reactors. This condition could be caused by the inlet and recirculation liquid was not distributed well as the liquid came into the reactors from the bottom side, not from the very end bottom part. Re-design of laboratory scale reactors, or increased recirculation flow distribution should be considered.

6. Conclusions

During acclimatization periods in the Reactor A, which was 1 month, the variability of up-flow velocity was applied. The results showed the COD removal efficiency up to 80% could be achieved by 1.0 m/h of up-flow velocity in 4 days of HRT. Hence, a 1.0 m/h of up-flow velocity was used during operation in the UASB reactors with a 5:1 ratio, recirculation flow rate and inlet flow rate respectively. During acclimatization periods, the granules in Reactor I/II were found to be more active with more granules containing compared to Reactor A.

COD removal efficiency reached a maximum of above 70% in UASB reactors. At the lowest HRT applied (1.4 h) with 15.0 g COD/l.d of OLR, the COD removal efficiency was in the range of 48-65%. The optimum biogas potential was occurred in Reactor II; 70% COD removal efficiency was achieved at 3.3 h of HRT with 6.0 g COD/l.d of OLR and 3.0 g COD/l.d of COD removed was converted to methane. Overall methane yields obtained were 0.226 l CH_4/g COD, 0.224 l CH_4/g COD, and 0.286 l CH_4/g COD in Reactor A, Reactor I and Reactor II, respectively, at operating condition. The observed methane yield were close to the theoretical values; 0.251 l CH_4/g COD, 0.258 l CH_4/g COD and 0.299 l CH_4/g COD. Under these conditions, approximately 22.5 MWh/d of electricity and 121500 MJ/d of heat could be recovered at IVAR Grødaland which has approximately 5000 m^3/d of average hydraulic loading and an OLR of 6.0 g COD/l.d. Methane generation was not significant when the UASB reactor was unstable after sludge washed out at day 55 in Reactor A and at day 20 in Reactor I.

The continuous UASB reactors can be further evaluated by comparing to the biogas potential batch test. The methane yield of wastewater batch tests were about 0.4 gCOD CH_4/gCOD (40% COD conversion to methane) with COD recovery around 70%. Generally, the batch test had lower COD reduction and lower gas produced in consequence the COD conversions to methane were also lower compared to the continuous reactors with about 59 – 75% of COD conversion to methane and above 80% of COD recovery in Reactor A, Reactor I, and Reactor II. The COD recovery represented the overall COD balance of which COD conversion to methane and non-biodegradable dissolved COD. From UASB reactors experiment, the wastewater is 59 – 75% anaerobically convertible to methane.

During experiment, alkalinity concentration had affected the methane fraction in biogas. The average methane fraction was above 90% when adding $NaHCO_3$ by 4 g/l. The decreased methane fractions were observed by around 70% after decreasing $NaHCO_3$ concentration to 1 g/l. A decrease in pH below 7 in several points was provoked basically when the OLR was increased at 10.0 gCOD/l.d of OLR, in consequence removal efficiencies of COD was dropped to below 50%. An important accumulation of VFAs was observed at day 68 in Reactor A. Meanwhile, Reactor I and Reactor II had relatively stable alkalinity. From day 33, it was possible to recover alkalinity due to the equilibrium of CO_2 species in the solution within carbonate system and recirculation. Therefore, the addition of $NaHCO_3$ was not necessary anymore with above 60% of COD removal efficiency.

Nutrient availability in the reactors was also important parameters. From results, phosphorous and nitrogen in the feed were assimilated for bacterial growth. Reddish filamentous precipitates were also observed in the reactors which might be inorganic ferric precipitates, as several wastewater in IVAR Grødaland are from animal slaughtering industry, high blood containing. Therefore, high Fe concentration will be expected in feed wastewater.

The reactor design and operational condition were also important factors affecting reactor performance. Generally, the average grain size of granules was reduced from 2.5 to 1.5 mm approximately in Reactor II and 1.5 to 1.0 mm in Reactor A and Reactor II. Smaller granules appeared to be weaker and more easily washed-out from the system. Sludge blanket expansion frequently occurred in Reactor I and Reactor II due to the buoyancy of the accumulated and entrapped biogas, and the liquid friction due to up-flow from inlet and recirculation flow rate. In addition, this study identified the internal mixing to be sub-optimal in Reactor I and Reactor II due to dead spaces in the bottom part of the reactors. Re-design of laboratory scale reactors, or increased recirculation flow distribution should be considered.

Anaerobic treatment systems using UASB reactor for treating high strength municipal wastewater represents a feasible and an attractive alternative as pre-treatment for SBR units at IVAR Grødaland by reduction of the SBR inlet total COD to about 300-400 mg/l and conversion organic matter into economically valuable products as methane with 1.3 l CH_4/d of specific volume methane production.

7. Recommendations

The results are within expectations, but more conclusions could be provided by further studies and experimentations such as temperature effect, VFA, ions and biogas specification, and granulation process study. This experiment was conducted under mesophilic conditions which were in range of 20 - 25 °C. It could be interesting to investigate the effect of temperature variability to reactor performance. Treating the wastewaters at initial temperatures would be beneficial because of reduced resources. Psychrophilic experiment condition will be beneficial for some high latitudes countries such as Norway, while thermophilic experiment can be applied in some countries in Middle East e.g. Saudi Arabia as some researches have been done and promising results have already been reported in its studies [21, 40].

The presence and concentration of other possible gases e.g. H_2S, could be also significant in anaerobic processes. These gases could have been detected if there was an analysis on the biogas specification from reactor system as well as defined ions e.g. SO_4^{2-}, and VFAs e.g. butyric acid specification. These analyses can describe the effect of each species of gas/ion/VFA to reactor performances whether stimulatory or inhibitory substances. Mathematical modelling to verify the stoichiometry and kinetics from this experiment using ADM1 (Anaerobic Digestion Model No.1) also can be conducted. The obtained results show the start-up and the performance of reactors were governed by several parameters such as size and quality of the granules. Therefore, size distribution of granular sludge studies also will be advantageous to observe development of the granulation process.

Despite the COD removal efficiencies achieved from 50 – 70% in this study, a significant fraction of organic matter is still remained in the effluent. In field application, a post-treatment requires to be considered to remove residual COD. If limiting concentrations for nitrogen and phosphorous also have to be achieved, further treatment steps such as nitrification, denitrification and biological or chemical phosphate removal must be considered. Hence, anaerobic treatment by UASB reactors represents a feasible and attractive alternative for energy/carbon efficient pre-treatment for SBR units at IVAR Grødaland by reduction of the SBR inlet total COD to about 300-400 mg/l and conversion organic matter into economically valuable products as methane with 1.3 l CH_4/d of

specific volume methane production. This pre-treatment can minimize the oxygen demand and surplus sludge formation in a subsequent aerobic post-treatment stage.

Although very low HRT by 1.4 h and high OLR by 15.0 0 g COD/l.d were applied during experiment, the results show the highest loading capacity (optimum condition) was achieved in Reactor II with 70% of COD removal efficiency at 3.3 h of HRT; 6.0 g COD/l.d of OLR; and 1.3 l CH_4/d (0.6 g COD CH_4/g CODin) of methane production was generated. Increasing OLR and decreasing HRT even further could reduce overall efficiency of the reactors both COD removal efficiency and methane production rate.

As described in previous chapter, this study identified the internal mixing to be sub-optimal in Reactor I and Reactor II due to dead spaces in the bottom part of the reactors. Re-design of laboratory scale reactors, or increased recirculation flow distribution should be considered. In order to use the entire reactor volume efficiently, a better liquid distribution was also required. The feed mechanism and the recirculation effluent should pass the reactor from the bottom to the top of the reactor linearly with the height of the reactor, which is from the very end bottom of reactors. Different feed inlet devices, more feed inlet points per square meter, or higher superficial velocities have been also proposed as solutions.

8. References

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Appendixes

Appendix 1: Biogas Potential Batch Test Data

Appendix 2: Reactivation of Granules Data in Reactor A

Appendix 3: Daily Data of Reactor A, Reactor I and Reactor II

Appendix 4: Total COD Analysis Data

Appendix 5: Nutrient Analysis Data

Appendix 6: Energy Recovery Calculation

Appendixes

Appendix 1: Biogas Potential Batch Test Data

The biogas potential batch test data are calculated and summarised in Table A.1.

Table A.1 *Recapitulation of biogas potential batch test data*[a)]

Name	sCOD (Day 0) mg/l	sCOD (Day 11) mg/l	CH_4 Produced ml	COD CH_4 mg	Methane Produced ml	COD Removal %	Methane Yield g COD/g COD	Methane Conversion %	COD Recovery %
Sample 1	540±4.1	163.5±2.1	83.82±13.1	239.48±37.5	83.8±3.1	70±0.6	0.44±0.07	44±7	75±0.5
Sample 2	810±8.8	225.8±4.4	130.70±6.7	373.43±19.2	130.7±6.7	72±0.2	0.46±0.02	46±2	74±0.7
Control 1	600±18.9	2.5±0.6	205.10±12.0	586.00±34.3	205.1±12.0	99.5±0.1	0.98±0.03	98±3	98±0.7
Control 2	900±12.6	45.5±3.2	303.20±11.4	866.29±32.5	303.2±11.4	95±0.3	0.96±0.02	96±2	100±1.1

a) The errors were determined using t-distribution analysis with 90% of certainty.

Appendix 2: Reactivation of Granules Data in Reactor A

The granules reactivation test data are calculated and summarised in Table A.2.

Table A.2 *Recapitulation of granules reactivation data in Reactor A*

Date	Duration (hours)	Ph	T (°C)	Gas (ml)	Gas Rate (ml/d)	$CaCO_3$ (mg/l)	HAc (mg/l)	SCODin (mg/l)	SCODef (mg/l)	SCOD removal (%)	OLR (mg/l.d)	V_{up} [a)] (m/h)
19-Feb	0.0	7.82	20.5	0	0.0	2917.2	130.5	1023	856	0.0		
22-Feb	72.8	7.72	20	9.3	3.1	2765	123.4	810	699	18.3		
23-Feb	95.7	7.75	20	16.4	7.4	2787.3	143.6	775	532	37.9		
24-Feb	122.8	7.65	20.5	31	12.9	2678.6	265.2	662	387	54.8		
25-Feb	148.0	7.49	21	46.1	14.4	2678.3	298.6	625	324	62.1	69.8	0.5±0.05
26-Feb	166.8	7.51	20	57.2	14.2	2598.7	262.2	597	256	70.1		
29-Feb	238.0	7.49	20.5	98.9	14.0	2432.8	289.9	581	251	70.7		
1-Mar	263.0	7.56	20.5	112	12.6	2278.2	199.2	480	165	80.7		
2-Mar	294.5	7.62	20.5	128.9	12.9	2376.8	176	457	157	81.7		
3-Mar	313.5	7.69	20	0	0.0	3072.1	201.2	1200	987	0.0		
4-Mar	336.5	7.42	20	12.8	13.4	2890.7	234.3	773.5	678	31.3		
7-Mar	408.0	7.32	20	47	11.5	2652.2	208.6	645	542	45.1		
8-Mar	432.8	7.51	20.5	60.2	12.8	2787.2	167.9	534	487	50.7	112.5	0.7±0.06
9-Mar	454.8	7.45	20	72.2	13.1	2598.3	198.6	474	343	65.2		
10-Mar	479.0	7.59	21	82.9	10.6	2354.7	187.9	351	234	76.3		
11-Mar	505.0	7.42	20.5	94.6	10.8	2235.6	219	211.5	159	83.9		
14-Mar	574.5	7.75	20	0	0.0	2770.5	209	1099	783	0.0		
15-Mar	599.0	7.43	20.5	18.6	18.2	2678.6	166.2	645	365	53.4		
16-Mar	625.5	7.52	20	45.3	24.2	2500.7	112.9	495	398	49.2	111.9	1.0±0.10
17-Mar	647.0	7.56	20.5	67.8	25.1	2309.6	187.4	388	265	66.2		
18-Mar	673.0	7.42	21	94.8	24.9	2321.8	198.2	187	139	82.2		

a) The errors were determined using t-distribution analysis with 90% of certainty.

Appendix 3: Daily Data of Reactor A, Reactor I and Reactor II

Reactor A

The daily measurement data of Reactor A are calculated and summarised in Table A.3.

Table A.3 *Recapitulation of daily measurement of Reactor A*

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate (l/l.d)[a)]	COD Removed (g/l.d)
1	7.43	846	0.8	24	2647.9	178.9	354	58	0.10	85	75	0.33	0.12	0.47
2	7.42	812	0.9	24	2645.9	221.9	387	52	0.10	91	78	0.31	0.12	0.47
3	7.56	823	0.8	24	2688.5	215.7	325	61	0.14	90	85	0.45	0.10	0.50
4	7.23	986	0.9	24	1998.3	271	333	66	0.15	92	78	0.44	0.13	0.63
5	7.12	1032	1.1	24	2315.9	231.9	287	72	0.15	89	66	0.39	0.18	0.78
6	7.56	1056	1.1	24	2345.9	221.9	309	71	0.18	87	84	0.55	0.13	0.78
7	7.24	1056	1.0	24	2647.9	178.9	309	71	0.19	86	80	0.50	0.13	0.73
8	7.43	1064	1.1	24	2456.4	198.7	310	71	0.20	87	78	0.49	0.15	0.77
9	7.49	976	2.3	10	2173.8	198	412	58	0.36	93	83	0.41	0.29	1.35
10	7.54	897	2.2	10	2220.2	231.9	371	59	0.34	88	84	0.43	0.27	1.26
11	7.34	1031	2.5	10	2317.8	221.9	365	65	0.36	90	75	0.40	0.36	1.60
12	7.44	892	2.1	10	2145.7	178.9	364.5	59	0.35	93	86	0.45	0.26	1.27
13	7.39	888	2.1	10	2364.5	198.7	267	70	0.35	86	74	0.44	0.31	1.49
14	7.32	1001	2.4	10	2432.3	178.9	305	70	0.40	82	76	0.45	0.34	1.67
15	7.45	1002	2.4	10	2346.7	198.7	317	68	0.38	86	75	0.43	0.35	1.64
16	7.38	1010	2.4	10	2315.9	231.9	317	69	0.35	86	71	0.39	0.37	1.66
17	7.42	937	2.2	10	1998.3	118	287	69	0.35	88	73	0.43	0.33	1.56
18	7.75	921	4.4	5	2173.8	321.8	476	48	0.53	89	84	0.32	0.54	2.14
19	7.52	897	4.3	5	2220.2	231.9	453	49	0.54	81	84	0.34	0.52	2.13
20	7.42	978	4.7	5	2317.8	221.9	498	49	0.55	96	83	0.32	0.58	2.30
21	7.62	923	4.4	5	2145.7	178.9	487	47	0.57	85	88	0.35	0.50	2.09
22	7.54	1102	5.3	5	2364.5	198.7	476	57	0.63	85	75	0.32	0.75	3.00
23	7.34	980	4.7	5	2432.3	178.9	465	53	0.65	87	85	0.38	0.57	2.47

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate (l/l.d)[a)]	COD Removed (g/l.d)
24	7.39	998	4.8	5	2315.9	231.9	376	62	0.70	89	77	0.40	0.67	2.99
25	7.59	878	4.2	5	2346.7	198.7	317	64	0.75	88	84	0.48	0.52	2.69
26	7.69	978	4.7	5	2315.9	231.9	354	64	0.78	90	81	0.45	0.61	3.00
27	7.34	937	4.5	5	1998.3	321.8	301	68	0.79	91	80	0.47	0.59	3.05
28	7.42	976	6.1	3.8	1838	351	465	52	0.83	87	85	0.37	0.74	3.19
29	7.1	1001	6.2	3.8	1773	412	512	49	0.82	86	86	0.35	0.73	3.05
30	7.3	998	6.2	3.8	1756	351	476	52	0.90	82	87	0.39	0.73	3.26
31	7.41	1011	6.3	3.8	1567	421	435	57	0.97	88	85	0.42	0.78	3.59
32	7.21	964	6.0	3.8	1562	321.8	378	61	1.00	81	84	0.45	0.75	3.66
33	7.19	964	6.0	3.8	1456	321.8	387	60	1.00	77	85	0.45	0.73	3.60
34	6.99	998	6.2	3.8	1542	351	376	62	1.01	82	82	0.44	0.80	3.88
35	7.01	1011	6.3	3.8	1247	216	335	67	1.10	88	80	0.47	0.82	4.22
36	7.09	965	7.9	2.9	1354	312.9	512	47	1.00	73	87	0.34	0.90	3.70
37	7.11	1003	8.2	2.9	1542	412	473	53	1.10	83	84	0.36	1.02	4.32
38	7.01	1014	8.3	2.9	1542	352	453	55	1.10	72	81	0.36	1.08	4.58
39	7.11	1003	8.2	2.9	1542	412	473	53	1.17	83	86	0.39	0.98	4.32
40	7.01	1014	8.3	2.9	1542	352	453	55	1.20	73	84	0.39	1.03	4.58
41	6.989	1082	8.8	2.9	1214	325	412	62	1.19	86	75	0.37	1.28	5.47
42	6.97	998	8.1	2.9	1036	438	332	67	1.20	70	73	0.40	1.21	5.43
43	7.01	1082	10.4	2.5	1214	325	501	54	1.35	86	81	0.35	1.34	5.58
44	6.78	1003	10.1	2.4	1102	413	531	47	1.31	78	88	0.35	1.15	4.76
45	6.9	993	10.0	2.4	1029	523	587	41	1.22	81	92	0.33	1.01	4.09
46	6.72	876	9.7	2.2	891	523	501	43	1.25	73	92	0.35	1.00	4.14
47	6.9	989	10.0	2.4	910	461	532	46	1.24	75	88	0.34	1.13	4.61
48	6.9	993	10.0	2.4	1029	523	487	51	1.34	81	85	0.36	1.21	5.10
49	6.72	876	9.7	2.2	891	523	401	54	1.34	78	83	0.37	1.22	5.24
50	6.9	989	10.0	2.4	910	461	532	46	1.33	74	90	0.36	1.09	4.61
51	6.98	876	9.7	2.2	861	456	461	47	1.34	73	90	0.37	0.83	3.60
52	7.01	989	10.0	2.4	973	421	432	56	1.32	79	80	0.36	1.11	4.68
53	6.97	898	12.1	1.8	977	398	454	49	0.92	78	71	0.21	1.75	5.97
54	6.987	989	12.3	1.9	862	431	635	36	0.46	73	74	0.10	1.47	4.42

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate (l/l.d)[a)]	COD Removed (g/l.d)
55	6.9	1030	12.4	2.0	973	421	745	28	0.23	76	77	0.05	1.20	3.42
56	6.79	968	12.5	1.9	678	431	619	36	0.44	78	73	0.09	1.52	4.52
57	6.89	867	11.7	1.8	632	352	478	45	0.75	70	72	0.17	1.60	5.23
58	6.88	843	11.7	1.7	667	456	476	44	0.80	71	75	0.18	1.54	5.11
59	6.9	1030	12.4	2.0	683	421	487	53	1.35	76	77	0.30	1.70	6.52
60	6.9	867	12.1	1.7	612	481	423	51	1.47	80	82	0.33	1.54	6.18
61	6.89	1030	15.3	1.6	687	421	531	48	1.64	76	81	0.29	1.95	7.43
62	6.6	879	14.8	1.4	542	654	442	50	1.76	77	83	0.32	1.84	7.34
63	6.59	1006	15.0	1.6	565	709	459	54	1.67	73	76	0.30	2.10	8.14
64	6.57	892	15.0	1.4	532	673	423	53	1.76	82	79	0.32	1.99	7.88
65	6.67	892	15.0	1.4	561	612	442	50	1.54	85	77	0.28	2.02	7.56
66	6.534	879	14.8	1.4	542	654	413	53	1.64	77	77	0.30	2.03	7.83
67	6.56	1006	15.0	1.6	513	562	456	55	1.80	73	78	0.32	2.04	8.18
68	6.63	1038	15.4	1.6	456	721	501	52	1.72	71	78	0.30	2.06	7.99

a) The theoretical methane rate (l/l.d) was calculated using equation below.

$$\text{The theoretical methane rate} = \text{COD removed } (1 - \text{ methane yield observed})$$

Reactor I

The daily measurement data of Reactor I are calculated and summarised in Table A.4.

Table A.4 *Recapitulation of daily measurement of Reactor I*

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate[a)] (l/l.d)	COD Removed (g/l.d)
1	7.7	341	1.5	5.6										
2	7.5	351	1.5	5.6										
3	7.3	212	1.5	3.3	2965	0	98	54	0.12	92	66	0.20	0.25	0.82
4	7.6	209	1.5	3.3	2678	76	76	64	0.20	90	71	0.35	0.24	0.96
5	7.6	195	1.3	3.7	2598	39	56	71	0.33	90	98	0.69	0.11	0.90
6	7.6	350	2.5	3.3	2664	0	178	49	0.36	86	89	0.38	0.29	1.24
7	7.3	342	2.5	3.3	2548	34	145	58	0.41	96	86	0.44	0.30	1.42
8	7.4	310	2.2	3.3	2391	0	129	58	0.43	86	93	0.51	0.24	1.30
9	7.5	309	2.2	3.3	2874	83	111	64	0.43	83	87	0.51	0.27	1.43
10	7.4	300	2.2	3.3	2567	98	102	66	0.50	92	95	0.61	0.21	1.43
11	7.4	600	4.3	3.3	2203	60	301	50	0.74	90	95	0.45	0.45	2.15
12	7.5	606	4.4	3.3	2308	109	267	56	0.78	88	91	0.47	0.49	2.44
13	7.4	601	4.3	3.3	2109	90	261	57	0.79	86	92	0.48	0.48	2.45
14	7.2	631	4.5	3.3	2457	114	234	63	0.82	89	85	0.48	0.57	2.86
15	7.6	611	4.4	3.3	2193	121	210	66	0.85	91	85	0.51	0.54	2.89
16	7.7	628	4.5	3.3	2210	69	189	70	0.90	91	83	0.53	0.57	3.16
17	7.3	826	5.9	3.3	1500	98	391	53	1.04	90	93	0.46	0.64	3.13
18	7.4	819	5.9	3.3	1769	109	411	50	1.07	91	98	0.48	0.59	2.94
19	7.2	841	6.1	3.3	1672	347	509	39	0.69	82	91	0.30	0.64	2.39
20	7.3	839	6.0	3.3	1345	372	671	20	0.21	85	89	0.09	0.42	1.21
21	7.3	828	6.0	3.3	1524	339	768	7	0.06	92	95	0.03	0.16	0.43
22	7.2	812	5.8	3.3	1376	379	772	5	0.02	88	96	0.01	0.11	0.29
23	7.2	942	8.1	2.8	1354	256	481	49	0.19	87	57	0.06	1.42	3.98
24	7.4	934	8.1	2.8	1423	271	501	46	0.49	88	70	0.16	1.19	3.74
25	7.3	831	8.4	2.4	1387	309	437	47	0.65	86	73	0.21	1.20	3.97

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate[a)] (l/l.d)	COD Removed (g/l.d)
26	7.2	964	8.3	2.8	1422	287	438	55	0.75	79	69	0.24	1.32	4.54
27	7.2	922	8.0	2.8	1234	298	381	59	0.83	82	69	0.27	1.29	4.67
28	7.3	861	9.9	2.1	987	213	378	56	1.15	80	75	0.31	1.47	5.56
29	7.1	857	9.9	2.1	834	222	367	57	1.17	81	74	0.31	1.47	5.64
30	7.1	901	10.4	2.1	723	247	341	62	1.21	80	69	0.31	1.70	6.45
31	7	890	10.3	2.1	835	276	352	60	1.23	79	71	0.32	1.61	6.20
32	6.9	887	10.2	2.1	652	248	351	60	1.25	80	72	0.32	1.59	6.17
33	6.9	852	12.3	1.7	693	341	443	48	1.48	79	84	0.32	1.53	5.89
34	7	862	12.4	1.7	669	347	451	48	1.51	78	84	0.32	1.53	5.92
35	6.9	839	12.1	1.7	779	398	419	50	1.54	76	84	0.34	1.53	6.05
36	6.8	874	12.6	1.7	735	410	378	57	1.55	79	76	0.33	1.83	7.14
37	6.7	851	12.3	1.7	753	474	344	60	1.56	77	74	0.34	1.84	7.30
38	6.5	901	15.6	1.4	698	671	454	50	1.85	79	82	0.31	2.02	7.72
39	7.3	859	14.8	1.4	893	623	441	49	1.78	77	83	0.32	1.88	7.22
40	7.2	879	15.2	1.4	978	561	423	52	1.83	79	80	0.32	2.04	7.88
41	7.3	891	15.4	1.4	1012	473	410	54	1.84	84	78	0.32	2.16	8.31
42	7.3	870	15.0	1.4	1293	441	401	54	1.83	82	78	0.32	2.09	8.10

a) The theoretical methane rate (l/l.d) was calculated using equation below.

$$\text{The theoretical methane rate} = \text{COD removed } (1 - \text{methane yield observed})$$

Reactor II

The daily measurement data of Reactor II are calculated and summarised in Table A.5.

Table A.5 *Recapitulation of daily measurement of Reactor II*

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate [a)] (l/l.d)	COD Removed (g/l.d)
1	7.6	341	1.5	5.6										
2	7.4	351	1.5	5.6										
3	7.4	212	1.5	3.3	2863	0	101	52	0.24	96	88	0.41	0.26	0.80
4	7.6	209	1.5	3.3	2934	0	87	58	0.26	93	88	0.46	0.26	0.88
5	7.4	195	1.3	3.7	2742	97	67	66	0.25	95	86	0.51	0.22	0.83
6	7.3	350	2.5	3.3	2642	0	156	55	0.42	93	89	0.44	0.43	1.40
7	7.6	342	2.5	3.3	2548	109	145	58	0.45	96	91	0.49	0.40	1.42
8	7.6	310	2.2	3.3	2742	95	121	61	0.46	89	93	0.54	0.34	1.36
9	7.4	309	2.2	3.3	2642	54	109	65	0.46	89	90	0.54	0.36	1.44
10	7.5	300	2.2	3.3	2846	0	91	70	0.50	92	91	0.61	0.33	1.50
11	7.2	600	4.3	3.3	2531	109	298	50	0.74	90	95	0.45	0.66	2.17
12	7.2	606	4.4	3.3	2308	98	267	56	0.78	96	91	0.47	0.71	2.44
13	7.4	601	4.3	3.3	2431	30	258	57	0.79	88	91	0.48	0.71	2.47
14	7.3	631	4.5	3.3	2341	101	224	65	0.82	89	83	0.48	0.85	2.93
15	7.3	611	4.4	3.3	2294	82	206	66	0.85	91	85	0.51	0.79	2.92
16	7.4	628	4.5	3.3	2321	113	178	72	0.90	92	81	0.53	0.85	3.24
17	7.3	826	5.9	3.3	2313	190	375	55	1.22	88	99	0.54	0.83	3.25
18	7.4	819	5.9	3.3	2213	218	376	54	1.21	89	100	0.54	0.81	3.19
19	7.3	841	6.1	3.3	2109	192	315	63	1.29	90	94	0.56	0.91	3.79
20	7.2	839	6.0	3.3	1961	233	328	61	1.27	91	94	0.55	0.91	3.68
21	7.3	828	6.0	3.3	1861	287	309	63	1.29	90	94	0.57	0.88	3.74
22	7.2	812	5.8	3.3	1792	267	243	70	1.30	87	89	0.59	0.93	4.10
23	7.1	942	8.1	2.8	1548	278	433	54	1.63	86	99	0.53	1.14	4.40
24	7.2	934	8.1	2.8	1612	264	401	57	1.63	86	96	0.53	1.19	4.61
25	7.2	831	8.4	2.4	1523	298	376	55	1.63	87	97	0.51	1.23	4.59

Day	pH	sCODin (mg/l)	OLR (g/l.d)	HRT (h)	$CaCO_3$ (mg/l)	HAc (mg/l)	sCODef (mg/l)	COD removal (%)	Methane Rate (l/l.d)	Methane Fraction (%)	COD Balance (%)	Y (g COD CH_4/g COD)	Theo. Methane Rate [a)] (l/l.d)	COD Removed (g/l.d)
26	7	964	8.3	2.8	1421	251	357	63	1.64	88	89	0.52	1.39	5.24
27	7.1	922	8.0	2.8	1380	223	310	66	1.64	85	88	0.54	1.33	5.29
28	7.1	861	9.9	2.1	1325	351	365	58	1.76	80	89	0.47	1.68	5.71
29	7	857	9.9	2.1	1234	272	342	60	1.80	79	88	0.48	1.70	5.93
30	7.1	901	10.4	2.1	1137	332	338	62	1.86	83	85	0.47	1.89	6.49
31	7.1	890	10.3	2.1	1032	361	314	65	1.86	80	83	0.48	1.91	6.64
32	7	887	10.2	2.1	932	321	289	67	1.91	81	82	0.49	1.93	6.89
33	7	852	12.3	1.7	851	363	367	57	1.95	77	85	0.42	2.24	6.98
34	7	862	12.4	1.7	741	352	352	59	2.11	79	86	0.45	2.24	7.34
35	6.9	839	12.1	1.7	779	398	309	63	2.13	79	83	0.46	2.26	7.63
36	7	874	12.6	1.7	801	378	345	61	2.10	76	83	0.44	2.36	7.62
37	6.9	851	12.3	1.7	798	401	290	66	2.13	68	80	0.46	2.42	8.08
38	6.9	901	15.6	1.4	743	411	429	52	2.30	80	86	0.39	2.75	8.16
39	6.9	859	14.8	1.4	941	398	398	54	2.29	79	87	0.41	2.61	7.97
40	7.4	879	15.2	1.4	991	323	345	61	2.33	80	80	0.40	3.03	9.23
41	7.3	891	15.4	1.4	1104	342	333	63	2.38	83	78	0.41	3.16	9.64
42	7.3	870	15.0	1.4	1253	298	302	65	2.49	85	78	0.44	3.05	9.82

a) The theoretical methane rate (l/l.d) was calculated using equation below.

$$\text{The theoretical methane rate} = \text{COD removed } (1 - \text{methane yield observed})$$

Appendix 4: Total COD Analysis Data

Reactor A

The total COD measurement data of Reactor A are calculated and summarised in Table A.6.

Table A.6 *Recapitulation of COD fractionation in Reactor A*

Date	TCODin (mg/l)	SCODin (mg/l)	%SCODin	%PCODin	TCODef (mg/l)	SCODef (mg/l)	%SCODef	%PCODef
1-Apr	1212	888	73.27	26.73	447	267	59.73	40.27
2-Apr	1321	1001	75.78	24.22	509.5	305	59.86	40.14
3-Apr	1231	1002	81.40	18.60	535	317	59.25	40.75
Average [a)]			76.81±7.85	23.19±7.85			59.62±0.61	40.38±0.61
24-Apr	1221	965	79.03	20.97	709	512	72.21	27.79
25-Apr	1123	1003	89.31	10.69	576	473	82.12	17.88
26-Apr	1235	1014	82.11	17.89	562	453	80.60	19.40
Average [a)]			83.48±9.95	16.52±9.95			78.31±10.06	21.69±10.06
8-May	1351	989	73.21	26.79	798	532	66.67	33.33
9-May	1208	876	72.52	27.48	713	461	64.66	35.34
10-May	1199	989	82.49	17.51	723	432	59.75	40.25
Average [a)]			76.07±10.50	23.93±10.50			63.69±6.71	36.31±6.71
24-May	1237	879	71.06	28.94	692	413	59.68	40.32
25-May	1292	1006	77.86	22.14	652	412	63.19	36.81
26-May	1123	1038	92.43	7.57	659	390	59.18	40.82
Average [a)]			80.45±20.59	19.55±20.59			60.68±4.12	39.32±4.12

a) The errors were determined using t-distribution analysis with 80% of certainty.

Reactor I and Reactor II

The total COD measurement data of Reactor I and Reactor II are calculated and summarised in Table A.7.

Table A.7 *Recapitulation of COD fractionation in Reactor I and Reactor II*

Date	Feed				Reactor I				Reactor II			
	TCODin	**SCODin**	**%SCODin**	**%PCODin**	**TCODef**	**SCODef**	**%SCODef**	**%PCODef**	**TCODef**	**SCODef**	**%SCODef**	**%PCODef**
30-Apr	401	310	77.31	22.69	157	129	82.17	17.83	167	121	72.46	27.54
1-May	407	309	75.92	24.08	175	111	63.43	36.57	155	109	70.32	29.68
2-May	431	300	69.61	30.39	135	102	75.56	24.44	132	91	68.94	31.06
Average [a)]			74.28±7.74	25.72±7.74			73.72±17.92	26.28±17.92			70.57±3.34	29.43±3.34
9-May	1021	826	80.90	19.10	512	391	76.37	23.63	550	375	68.18	31.82
10-May	983	819	83.32	16.68	606	411	67.82	32.18	529	376	71.08	28.92
11-May	1135	841	74.10	25.90	762	509	66.80	33.20	507	315	62.13	37.87
Average [a)]			79.44±9.02	20.56±9.02			70.33±9.91	29.67±9.91			67.13±8.61	32.87±8.61
22-May	1151	901	78.28	21.72	598	341	57.02	42.98	461	338	73.32	26.68
23-May	1008	890	88.29	11.71	600	352	58.67	41.33	458	314	68.56	31.44
24-May	999	887	88.79	11.21	624	351	56.25	43.75	435	289	66.44	33.56
Average [a)]			85.12±11.18	14.88±11.18			57.31±2.33	42.69±2.33			69.44±6.65	30.56±6.65
1-Jun	1037	879	84.76	15.24	595	379	63.70	36.30	463	345	74.51	25.49
2-Jun	992	891	89.82	10.18	562	378	67.26	32.74	455	333	73.19	26.81
3-Jun	1083	870	80.33	19.67	542	365	67.34	32.66	476	302	63.45	36.55
Average [a)]			84.97±8.95	15.03±8.95			66.10±3.93	33.90±3.93			70.38±11.40	29.62±11.40

a) The errors were determined using t-distribution analysis with 80% of certainty.

Appendix 5: Nutrient Analysis Data

The nutrient analysis data of Reactor A, Reactor I, and Reactor II are calculated and summarised in Table A.8.

Table A.8 *Recapitulation of nutrient analysis data in Reactor A, Reactor I, and Reactor II*

	Ractor A (mg/l)					**Feed I/II (mg/l)**		**Reactor I (mg/l)**		**Reactor II (mg/l**	
Date	**TP in**	**TP out**	**TN in**	**TN out**	**Date**	**TP in**	**TN in**	**TP out**	**TN out**	**TP out**	**TN out**
21-Mar	14.1	15.1	25.7	19.2	30-Apr	15.6	22.3	14.2	15.9	15.1	19.2
22-Mar	14.0	15.0	26.9	20.2	1-May	15.0	23.1	14.1	16.0	15.0	20.2
23-Mar	14.0	15.7	25.8	20.0	2-May	15.8	23.0	13.9	16.3	15.7	20.0
Average [a)]	14.0±0.1	15.3±1.2	26.1±1.9	19.8±1.7	Average [a)]	15.4±1.2	22.8±1.1	14.0±0.4	16.1±0.7	15.3±1.2	19.8±1.7
1-Apr	15.9	13.0	22.3	12.3	9-May	14.9	24.8	7.8	18.9	13.0	12.3
2-Apr	16.0	12.3	23.2	13.2	10-May	15.1	24.7	8.3	18.0	12.3	13.2
3-Apr	15.8	13.1	23.8	12.8	11-May	15.3	23.8	7.1	18.5	13.1	12.8
Average [a)]	15.9±0.4	12.8±1.4	23.1±2.2	12.8±1.3	Average [a)]	15.1±0.6	24.4±1.6	7.8±1.8	18.5±1.4	12.8±1.4	12.8±1.3
24-Apr	15.6	8.2	26.1	14.2	22-May	17.9	17.3	5.2	10.9	8.2	12.9
25-Apr	15.8	9.1	25.0	13.2	23-May	18.1	17.0	6.3	12.0	9.1	13.2
26-Apr	16.2	9.0	25.9	13.9	24-May	17.7	17.2	6.0	11.7	9.0	12.5
Average [a)]	15.9±1.0	8.8±1.4	25.7±1.7	13.8±1.5	Average [a)]	17.9±0.6	17.2±0.5	5.8±1.6	11.5±1.6	8.8±1.4	12.9±1.0
3-May	17.1	5.2	18.0	7.9	1-Jun	18.9	18.9	9.9	6.8	5.2	13.7
4-May	17.2	5.4	17.6	7.0	2-Jun	18.0	18.4	9.0	7.2	5.4	12.7
5-May	16.9	6.2	17.5	7.0	3-Jun	18.4	18.0	9.3	6.9	6.2	13.0
Average [a)]	17.1±0.4	5.6	17.7	7.3	Average [a)]	18.4±1.3	18.4±1.3	9.4±1.3	7.0±0.6	5.6±1.6	13.1±1.5
24-May	15.8	9.3	19.0	8.1							
25-May	16.0	9.1	18.0	9.1							
26-May	15.7	8.1	18.5	9.0							
Average [a)]	15.8±0.4	8.9±1.9	18.5±1.4	8.7±1.6							

a) The errors were determined using t-distribution analysis with 90% of certainty.

Appendix 6: Energy Recovery Calculation

Energy recovery was calculated under optimum biogas potential condition in Reactor II, as shown below.

Table A.9 *Parameter conditions used in energy recovery calculation*

Parameter	Mark(s)
Convertion[a)]	13.5 MJ CH_4 energy/kg COD removed to methane gives 1.5 kWh electricity by assuming 40% electric conversion efficiency and the rest turns into heat
OLR[b)]	6.0 g COD/l.d
HRT [b)]	3.3 H
COD removal efficiency [b)]	70%
Methane yield [c)]	0.286 l CH_4 / COD (0.75 g COD CH_4/g COD removed)
COD Removed [b)]	4.1 g COD/l.d (3.0 g COD/l.d was converted to methane)
Q[d)]	5000 m^3/d
Electricity generation [e)]	81000 MJ/d (22.5 MWh/d)
Heat generation [f)]	121500 MJ/d

a) Conversion was referred to Henze *et.al* (2008).

b) The data was taken from daily measurement Reactor II in Table A.5 (day 22).

c) Methane yield was determined graphically in Figure 4.8 from values of methane production rate in function of COD removed.

d) The value is the approximate average hydraulic loading at IVAR Grødaland.

e) Electricity generation was calculated using equation below.

$$Electricity\ (kWh/d) = COD removed\ to\ CH_4\ x\ Q_V\ x\ 1.5\ kWh = 3.0 \frac{kgCOD}{m^3.d} x\ 5000\ m^3 x\ 1.5\ kWh$$

$$Electricity\ (kWh/d) = 22500\ kWh/d = 22.5\ MWh/d$$

$$Electricity\ (MJ/d) = \frac{40\%\ x\ 22500\ kWh/d}{1.5\ kWh} x 13.5\ MJ = 81000\ MJ/d$$

f) Heat generation was calculated using equation below.

$$Heat\ (MJ/d) = \frac{60\%\ x\ 22500\ kWh/d}{1.5\ kWh} x 13.5\ MJ = 121500\ MJ/d$$