Universitetet
i Stavanger

FACULTY OF SCIENCE AND
TECHNOLOGY

MASTER'S THESIS

Study programme/specialisation:

Environmental Technology/ Water Science and Technology

Spring semester 2017

Open/~~Confidential~~

Author:

Ayesha Akhtar

(signature of author)

Programme coordinator:

Roald Kommedal

Supervisor(s): **Leif Ydstebø**

Title of master's thesis:

Anaerobic treatment of industrial wastewater under psychrophilic condition (10 °C) using Upflow Anaerobic Sludge Blanket (UASB) reactor, Lab studies.

Credits:

30

Keywords:

anaerobic wastewater treatment, UASB reactor, psychrophilic temperature, COD removal efficiency, COD recovery, methane production

Number of pages: 59
+ Front Pages: 08
+ Appendixes: 09
+ Memory stick

Stavanger June 15, 2017

Anaerobic Treatment of Industrial Wastewater under Psychrophilic Condition (10 °C) using Upflow Anaerobic Sludge Blanket (UASB) Reactor, Lab Studies.

Master's Thesis

Ayesha Akhtar

Environmental Technology Study Programme

Water Science and Technology

Department of Mathematics and Natural Science

University of Stavanger

2017

Abstract

The main objective of this study was to investigate the performance of UASB reactors for their efficiency of removing COD and producing methane gas at psychrophilic temperature, 10 °C by treating high strength municipal wastewater ranged from 500 – 1600 mg COD/l, received from the effluent from DAF unit at IVAR Grødaland WWTP. The study also included to investigate if UASB treatment would be a better option to apply prior to SBR unit at Grødaland WWTP for the removal of dissolved COD. All the thesis work was done on the laboratory scale at University of Stavanger. Two In-house designed Upflow Anaerobic Sludge Blanket, UASB reactors Reactor I and Reactor II that had been in operation for a year were used in this study. The organic loading rate (OLR) was variable in both reactors throughout the experimental period. The flow rate in Reactor II was purposely increased, 20 % to investigate its effect on methane gas production and COD removal efficiency. The gas meters were not functioning properly during parts of the study, therefore, the gas production values were estimated from Day 24 in Reactor I and from Day 28 in Reactor II, for calculations. The hydraulic retention time (HRT) in Reactor I and Reactor II varied from 6 – 14 h and 5 – 14 h, respectively. The pH in Rector I was between 7.2 – 7.8 while in Reactor II it was between 7.2 – 7.6 throughout the experimental period. The results obtained indicate a significant amount of methane production and COD removal efficiency. The maximum methane production achieved at psychrophilic temperature in Reactor I was 0.21 l CH_4/l.d, which is 0.37 l CH_4/g COD at OLR of 1.0 g/l.d and in Reactor II maximum methane production achieved was 0.23 l CH_4/l.d which is 0.29 l CH_4/g COD at OLR of 1.8 g/l.d. The average COD recovery achieved in both the reactors was 78 % with average COD removal efficiency of 56 % at 10 °C. There was a little deviation in results due to the equipment error otherwise, the results obtained indicate that anaerobic treatment using UASB technology to treat high strength wastewater to remove dissolved COD prior to SBR unit at IVAR Grødaland WWTP would be an attractive and feasible option even at psychrophilic temperature. By using a longer HRT and sludge retention time (SRT) and appropriate gas measuring meters the economically valuable methane gas can be produced and achieved.

Keywords: *anaerobic wastewater treatment, UASB reactor, psychrophilic temperature, COD removal efficiency, COD recovery, methane production*

Acknowledgements

I would thank several persons because without their contribution and support this thesis would not have been possible. First of all, I would like to express my sincere gratitude to my supervisor, Dr. Leif Ydstebø for his continuous and great support and help throughout my thesis work. And for his enormous knowledge which helped me to understand the subject better.

I am grateful to Assoc. Prof. Roald Kommedal for his help in the lab during my research, despite his busy schedule. I would like to thank laboratory engineer, Hans Kristian and all the lab employees at the University of Stavanger for their great support throughout my work. I am also thankful to Ph.D. student, Anders Wold, for sharing his knowledge about UASB reactor with me.

A big thanks to all my classmates and lab-mates in Environmental Technology Master Programme, for all the fun and discussions we have had in two years and specially in the lab during this thesis work. Thanks to my lab-mates especially Ricky, Azin, Zola, Sori and all others. I also thank Anissa Sukma Safitri for her great help and sharing her experience about UASB reactor. I am thankful to all my friends specially Sagal and Quratulain for giving me motivation through their kind words to do my work

Last but not the least a heartiest thanks and respect to my parents, my sisters and my brother for their prayers and support throughout my work. And a very special thanks to someone very special to me for his love and support during my thesis. Thank you. This is all yours.

Stavanger, June 2017

Ayesha Akhtar

Table of Contents

List of Figures and Tables

List of Abbreviations

AnWT	Anaerobic Wastewater Treatment
IVAR	*Interkommunalt Vann Avløp og Renovasjon*
WWTP	Wastewater Treatment Plant
SBR	Sequencing Batch Reactor
DAF	Dissolved Air Flotation
UASB	Upflow Anaerobic Sludge Blanket
COD	Chemical Oxygen Demand
OLR	Organic Loading Rate
HRT	Hydraulic Retention time
SRT	Sludge Retention time
TSS	Total Suspended Solids
EGSB	Expanded Granular Sludge Blanket
VFA	Volatile Fatty Acid
LCFA	Long Chain Fatty Acid

1. Introduction

The liquid waste – wastewater – is the water supply of community after it has been used in a variety of applications. From the viewpoint of source of generation, wastewater is defined as a combination of liquid or water-carried wastes removed from residence, institutions and industries together with groundwater, surface water and storm water as may be present. The untreated wastewater contains pathogenic microorganisms, nutrients and toxic compounds or compounds that potentially may be mutagenic or carcinogenic. For these reasons, the immediate nuisance-free removal of wastewater from its sources of generation, followed by treatment and reuse or dispersal into the environment is necessary to protect the public health and the environment [1].

Water quality issues arise when increasing amount of treated wastewater are discharged to water bodies that are eventually used as water supplies. Prior to about, 1940 most municipal wastewater was generated from domestic sources. After 1940, as industrial development grew in United States, increasing amount of wastewater have been discharged to municipal collection systems [1]. Methods of wastewater treatment in which the removal of contaminants is brought about by chemical or biological reactions are known as unit processes. Biological treatment is an important part of any wastewater treatment plant that treats wastewater from either municipality or industry having soluble organic substrates or a mix of two types of wastewater sources [2].

1.1 Scope of work

This Master thesis is a part of the project in cooperation with IVAR and the experiments were conducted in the 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. The company's vision is to secure region's most competitive water, wastewater and waste services. In this study, the experiment was performed using two anaerobic UASB reactors for treating effluent wastewater from dissolved air flotation (DAF) at Grødaland wastewater treatment plant (WWTP). The Grødaland WWTP receives wastewater from several sources mainly industrial, as animal destruction, dairy and chicken slaughtering, municipal wastewater of 3000 houses and food industry.

The whole process as shown in Figure 1.1 consists of pre-treatment with sand and fat removal followed by dissolved air flotation unit with the addition of polymer and a subsequent biological stage using sequential batch reactor (SBR).

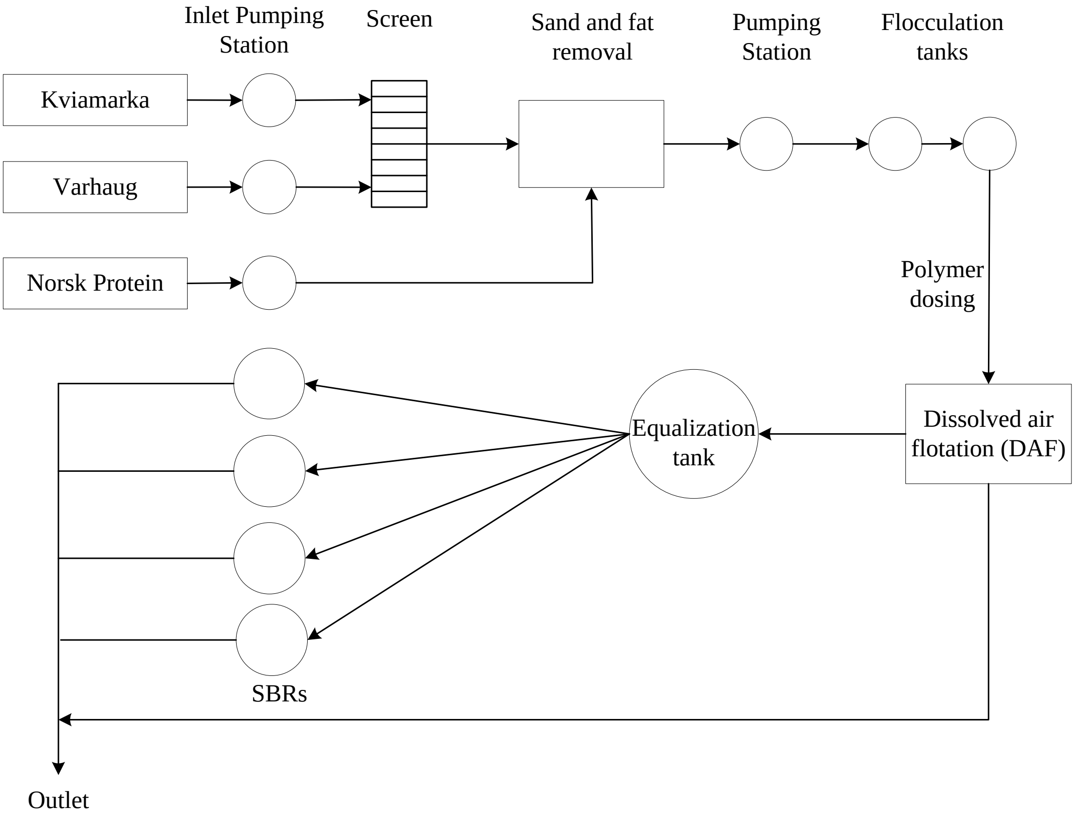


Figure 1.1 The process configuration of Grødaland wastewater treatment plant [23].

The flotation units consist of two tanks each with 48 m^2 surface area designed for maximum 7.5 m/h surface loading. At maximum load 200 l/s of wastewater passes through the plant. 1000 m^3 equalization tank equalizes the load to the biological unit. The biological SBR unit consists of four reactors each of 750 m^3, serving both as bioreactor and settling tanks [23].

The average flow and composition of wastewater in and out of the DAF unit is shown in Table 1.1.

Table 1.1 The average flow and composition of wastewater.

Parameters	**Inlet**	**Outlet**
SS (mg/L)	510	196
Dissolved COD (mg/L)	959	825
Total COD (mg/L)	1862	1184
BOD (mg/L)	1501	927
Total Phosphorous (mg/L)	25,9	23,1
Conductivity (µS/cm)	1938	1904
pH	7.73	7.54
Flow rate (m^3/d)	4728	4330

The wastewater which Grødaland WWTP receives presents high fat content. The variations in the composition and characteristics of wastewater is observed during the day and during the year which make the treatment performance difficult, especially the biological stage. With the addition of polymer (2ml/L), DAF unit removes approximately 61 % of suspended solids (SS). The removal of SS can be increased to 80 % by increasing the polymer dose. On the other hand, the DAF unit cannot remove high fractions of dissolved COD. Only 14 % of dissolved COD is removed in this stage, so high concentrations of dissolved COD affect the performance of SBR leading to high oxygen demand and poor sludge sedimentation. A way to solve this problem is to remove dissolved COD prior to SBR unit through anaerobic granular sludge treatment using UASB reactor. This has been done on the laboratory scale at University of Stavanger using UASB reactors at mesophilic conditions i.e. at 20 – 25 °C and the tests have shown satisfactory results (COD removal efficiency above 70 %).

1.2 Thesis objective

This thesis work is the continuation of investigating the removal of dissolved COD i.e. COD removal efficiency from the effluent of DAF unit from Grødaland WWTP at laboratory scale using UASB reactors at psychrophilic conditions i.e. at 10 °C. Also, the main objective of this study was to investigate the efficiency of UASB reactor to treat the municipal wastewater and convert the organic matter into methane gas.

1.3 Thesis outline

The title of this thesis is: **Anaerobic treatment of industrial wastewater under psychrophilic conditions (10 °C) using Upflow Anaerobic Sludge Blanket (UASB) reactor, Lab studies.**

The thesis is divided into following chapters:

- Introduction
- Literature review and Background knowledge
- Materials and Methods
- Results
- Discussion
- Conclusion
- Future recommendations for further research

2. Literature review and theoretical background

This chapter describes the literature review and theoretical background knowledge of anaerobic treatment of wastewater as well as the microbiology and stoichiometry of anaerobic processes.

2.1 Anaerobic wastewater treatment

The fermentation process in which organic material is degraded and biogas (composed of mainly methane and carbon dioxide) is produced, is referred to as anaerobic digestion process. Anaerobic digestion processes occur in many places where organic material is available and reduction potential is low and zero oxygen. Anaerobic treatment can be conducted in plain systems, and this process can be applied at any scale and at any place. The amount of excess sludge produced in anaerobic treatment is very small and well stabilized, even having a market value when so called granular anaerobic sludge is produced in the bioreactor. Furthermore, useful energy in the form of biogas is produced instead of high-grade energy consumed.

In contrast to anaerobic treatment, aerobic treatment is generally characterized by high operational costs, while a large fraction of waste is converted to another type of waste. Aerobic treatment, as the conventional activated sludge process yields about 50 % of sludge from COD converted, which requires further treatment, e.g. anaerobic treatment before it is reused or disposed off. Figure 2.1 shows the fate of carbon and energy in both aerobic and anaerobic wastewater treatment, assuming the oxidation of 1 kgCOD requires 1 kWh of aeration energy [3].

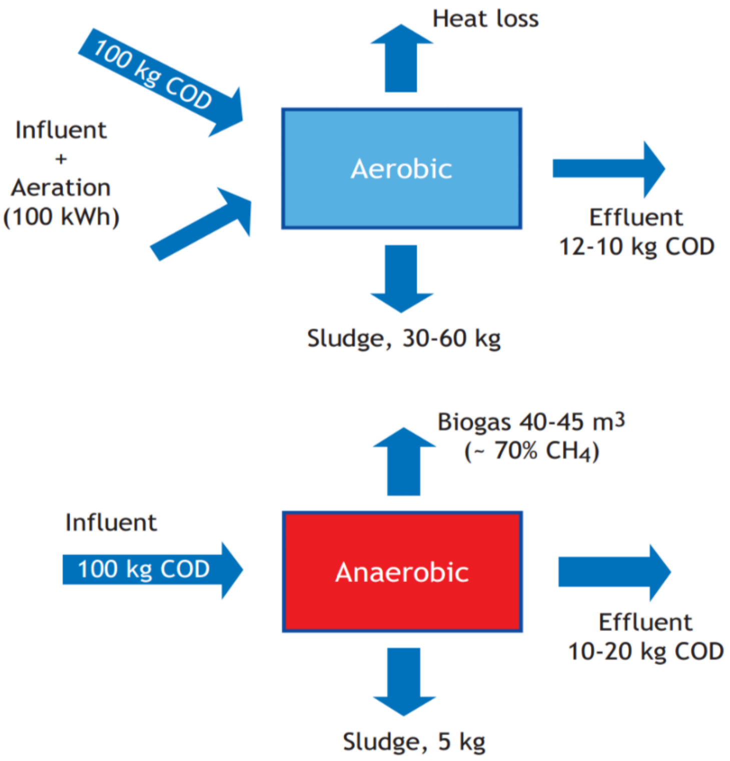


Figure 2.1 Fate of carbon and energy in aerobic (above) and anaerobic (below) wastewater treatment [3].

Table 2.1 shows some advantages and disadvantages of anaerobic wastewater treatment over aerobic treatment [4].

Table 2.1 Advantages and disadvantages of anaerobic process.

Advantages	Disadvantages
• Low production of solids, about 3 to 5 times lower than that in aerobic processes • Low energy consumption, usually associated with an influent pumping station leading to very low operational costs • Low land requirement • Production of methane, a highly calorific fuel gas • Application in small and large scale • Low nutrients consumption	• Anaerobic microorganisms are susceptible to inhibition by a large number of compounds • Process start-up can be slow in the absence of adapted seed sludge • Some of the post treatment is usually necessary • The biochemistry and microbiology of anaerobic digestion are complex and still require further studies • Possible generation of effluents with unpleasant aspect • Unsatisfactory removal of nitrogen, phosphorous and pathogens

Figure 2.2 shows the gradual increase in the number of anaerobic high-rate reactors from the mid-seventies [3]. It shows the anaerobic treatment became a popular option to treat wastewater.

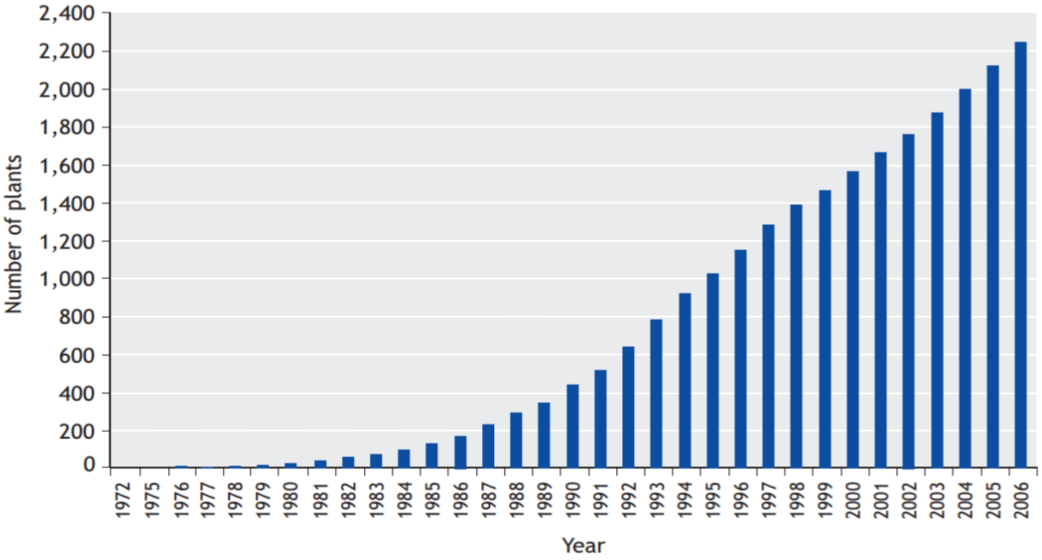


Figure 2.2 Increased in the number of installed anaerobic high-rate reactors in the period 1972-2006 [3].

The big challenge in anaerobic wastewater treatment is to demonstrate the feasibility for sewage treatment especially in moderate climates [10]. Since 1980s, several investigations have been done in the field of AnWT at several laboratories worldwide. The results obtained show that anaerobic treatment of domestic sewage is an attractive and feasible option for moderate climates (Table 2.2). Table 2.3 shows that different reactors which treat sewage anaerobically are efficient even under psychrophilic condition. For example, 60 – 70 % COD removal efficiency of treating sewage could be obtained using the UASB reactor with the process temperature of < 20°C and longer HRT (>8 h). Hydraulic retention time or HRT is the average length of time that a compound, for example, water remains in a unit or a bioreactor

When treating the domestic sewage at low temperatures using UASB reactor inoculated with granular sludge, the accumulation of suspended solids (SS) occurs which deteriorates the methanogenic activity and the performance of the reactor. Therefore, SS should be removed from the influent wastewater prior to UASB treatment. At low temperatures, long SRTs is required resulting in the long HRTs [11]. Solid retention time or SRT is the average period of time during which the sludge remains in the system.

Physical and biological processes are of great importance for the treatment of sewage. The first step of anaerobic treatment and conversion of domestic sewage is the physical removal of particulate organics through settling, adsorption and entrapment in the sludge bed of UASB reactor. The hydrolysis of retained particulates is considered to be the rate-limiting step in the digestion process and it requires long hydraulic and sludge retention times depending on the temperature applied. Degradation of these retained particulates in anaerobic reactors is affected under psychrophilic conditions due to the decrease in hydrolysis rate with decrease in process temperature. The hydrolysis rate of lipids and proteins decrease with decrease in temperature. The anaerobic hydrolysis rate of these particles depends on the available surface area and the size of the particles [12].

Table 2.2. The anaerobic treatment of sewage under psychrophilic conditions (<20°C) [10].

Reactor type	Influent	Concentration ($gCOD/dm^3$)	OLR ($kgCOD/m^3d$)	Temperature (°C)	HRT (h)	COD Removal Efficiency %
UASB	Raw	0.3	0.6	8 – 20	12	67
UASB	Raw	0.4	1.2	11 – 12	8	30 - 50
AF	Raw	0.53	1.8	13 – 15	6	35 – 55
UASB	Raw	0.19 – 1.18	0.6 – 3.5	12 – 20	7 – 8	30 – 75
UASB	Raw	0.465	0.62	12 – 18	18	73
FB	Raw	0.76	8.9	10	1.7 – 2.3	53 – 85
EGSB	Pre-settled	0.3	4.5	9 – 11	2.1	20 – 48
UASB+EGSB	Raw	0.32 – 0.51	1.6 – 2.5	8 – 13	5	45 – 57
AF+AH	Raw	0.46 – 0.53	0.9 – 1.1	13	12	70
UASB	Raw	0.15 – 0.6	0.8 – 3.1	13 – 25	4.7	64 – 70

2.2 Microbiology of anaerobic conversion

The anaerobic degradation pathway of organic matter is a multi-step process. The anaerobic process of organic matter degradation proceeds in four stages as follows:

I. Hydrolysis
II. Acidogenesis
III. Acetogenesis
IV. Methanogenesis

The anaerobic digestion process involves a complex web, in which organic matter is degraded by a wide variety of microorganisms. The microbial consortia involved jointly convert complex organic matter and mineralize it into methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3), hydrogen sulphide (H_2S) and water (H_2O). The degradation processes are described below in detail [3].

2.2.1 Hydrolysis

The first step in anaerobic degradation consists of hydrolysis of the polymers because bacteria are unable to take up particulate organic matter. It is the process where enzymes excreted by fermentative bacteria convert complex, undissolved material into less complex, dissolved compounds which can pass through cell walls and membranes of the fermentative bacteria. During the enzymatic hydrolysis process, proteins are hydrolyzed to amino acids, polysaccharides to simple sugars and lipids to long chain fatty acids (LCFA). This process is very sensitive to temperature and temperature fluctuations. The products of the hydrolysis are the substrates for acidogenic bacteria. Hydrolysis is considered to be the rate limiting step during the anaerobic digestion of complex substrates. However, this is not due to the lack of the enzyme activity but to the availability of the free accessible surface area of the particles and the overall structure of the solid substrate. In dilute wastewaters such as low temperature domestic sewage, hydrolysis may determine the overall process and thereby, determining the required reactor design. It must be noted that 45 – 75 % of domestic sewage, and 80 % of primary sludge consists of suspended matter. The main biopolymers in sewage are proteins, carbohydrates and lipids [3].

2.2.2 Acidogenesis

It is the process where the hydrolysis products which are dissolved compounds (amino acids, simple sugars and LCFAs) are diffused inside the bacterial cells through the cell membrane and are converted by fermentative bacteria into a number of simple compounds which are then excreted. Acidogenesis is very common reaction and is performed by a large number of hydrolytic and non-hydrolytic microorganisms. The compounds produced during this process include volatile fatty acids (VFAs), alcohols, lactic acid, CO_2, H_2, NH_3 and H_2S, as well as new cell material. Therefore, the fermentative organisms are usually designated as acidifying or acidogenic microorganisms and the process is called acidogenesis. Table 2.3 shows different acidogenic reactions starting from sucrose and generating amounts of VFAs, HCO_3^-, H_2 and H^+. The type of final products depends upon the conditions in the reactor medium. Table 2.3 shows that the $\Delta G°$ of the less energetic acidogenic reactions with the sucrose as substrate, depends on the H_2 concentration. If H_2 is removed by H_2 scavenging bacteria such as methanogens the final product is acetate. However, if H_2 accumulates in the reactor the more reduced forms for example lactate, propionate, butyrate and alcohols are the final products [3].

Table 2.3. Acidogenic reactions with sucrose as the substrate and the corresponding free energy change (ΔG°) at 25°C. [3].

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

The ΔG° of acidifying reactions is the highest of all anaerobic reactions which results in ten to twentyfold higher bacterial growth rates and fivefold higher bacterial yield and conversion rates compared to methanogenesis (Table 2.4). That is the reason that anaerobic reactors are subject to souring i.e. a sudden pH drop when overloaded with organic compounds. When alkalinity is consumed by the acids produced the pH in the reactor starts to drop which leads to the accumulation of non-dissociated VFAs resulting in the inhibition of methanogenesis [3].

The acidogenic reactions of amino acids generally follows the Stickland reaction, in which an amino acid is de-ammonified by anaerobic oxidation yielding VFA and H_2 together with the reductive de-ammonification of other amino acid which consumes the produced H_2. In both these reactions NH_3 is produced which is an electron acceptor that leads to increase in pH resulting in no net proton production and no pH drop in the reactor. NH_3 reacts with H^+ resulting in OH^- in excess that reacts with CO_2 and forms HCO_3^-, this is reaction producing alkalinity in anaerobic digestion.

Table 2.4. Average kinetic properties of acidogenesis and methanogenesis [3].

Process	Conversion rate gCOD/gVSS.d	Yield gVSS/gCOD	K_s mgCOD/l	μ_m 1/d
Acidogenesis	13	0.15	200	2.00
Methanogenesis	3	0.03	30	0.12
Overall	2	0.03 – 0.18	-	0.12

2.2.3 Acetogenesis

The process where fermentation products generated in acidogenesis are converted into acetate, hydrogen and carbon dioxide, as well as new cell material is called acetogenesis. Among these metabolized products H_2, CO_2 and acetate are used by methanogenic bacteria [4]. CO_2 is utilized by hydrogenotrophic methanogens. This phase is also called intermediary acid production phase. A large amount of H_2 is produced when acetic and propionic acid are formed which causes the pH of aqueous medium to decrease. There is an association between H_2-producing acetogenic bacteria and H_2-consuming methanogenic bacteria which regulates the H_2 level in the environment [3]. This is of great importance because these reactions are thermodynamically unfavorable. In anaerobic digestion, methanogenic bacteria utilize H_2 so rapidly that the hydrogen partial pressure drops below 10^{-4} atm, which is enough for the occurrence of acetogenic reactions (Figure 2.3).

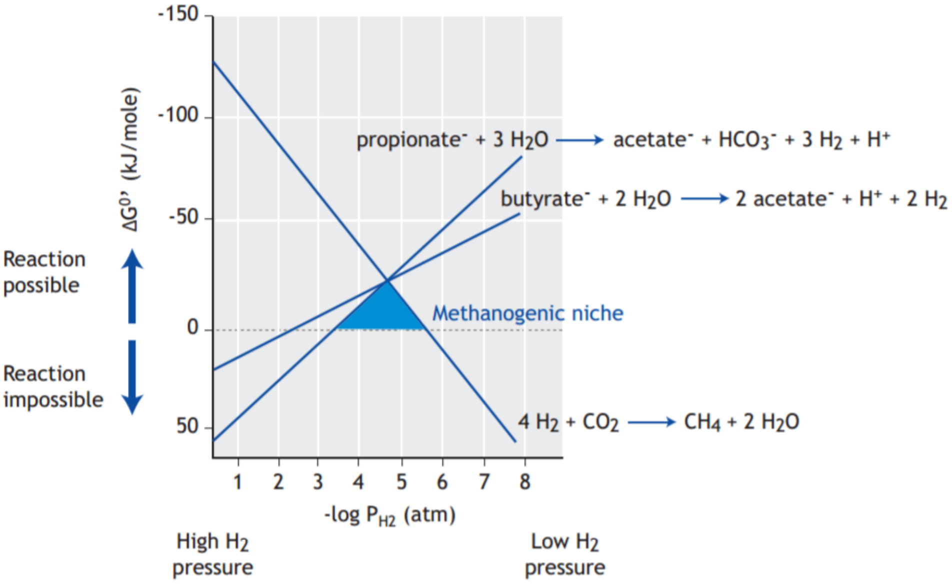


Figure 2.3. Free energy change as a function of H2 partial pressure [3].

The coupling of the generation and use of H_2 is called interspecies hydrogen transfer. In a properly functioning methane-producing reactor the partial hydrogen pressure is usually between 10^{-4} – 10^{-6}. At this low hydrogen concentrations, the degradation of butyrate, ethanol and propionate becomes exergonic which yield energy for the acetogenesis reaction to occur [3].

2.2.4 Methanogenesis

Methanogenesis is the final step in anaerobic digestion where acetate, formic acid, methanol and H_2 are converted into methane and CO_2. It is this stage of anaerobic digestion where influent COD is converted to gaseous form. Methanogens are divided into two main groups, one that forms methane from acetic acid and methanol and the others that form methane from hydrogen and carbon dioxide as follows [4]:

- Aceticlastic methanogens
- Hydrogenotrophic methanogens

About 70 % of methane produced comes from acetate and the rest generates from hydrogen and CO_2. Aceticlastic methanogens have very low growth-rate which explains why the anaerobic reactors require a longer start-up time and higher sludge concentrations. Hydrogenotrophic microorganisms on the other hand have a higher or maximum growth rate which maintains the stability of high rate anaerobic systems under varying conditions [3]. The overall acetotrophic and hydrogenotrophic reactions are shown in Table 2.5.

Table 2.5. Important methanogenic reactions and free energy change.

Step	**Reaction**	**ΔG° (kJ/mol)**
Acetotrophic methanogenesis	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-31
Hydrogenotrophic methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	-131

Temperature primarily affects the rate of methanogenesis. The maximum specific growth rate of methanogens increases linearly with the increase in temperature from 30 °C to 60 °C. The activity of methanogens is slower at the lower temperatures. A consistent amount of methane can be produced from a given mass of manure by increasing retention time at low temperature because the ultimate methane production is independent on temperature but depends upon the specific substrates [25]. The methane production by methanogens increase with the increase in temperature from 5 °C to 40 °C [26].

The maximum specific growth rate (μ_{max}) of methanogens is independent of waste or treatment process but dependent on the temperature. So, with the decrease in temperature the maximum growth decreases shown in Figure 2.4, which shows the linear regression of data with the following relationship [27]:

$$\mu_{max} = 0.012\,(T) - 0.086 \qquad (1)$$

The μ_{max} of mixed anaerobic microorganisms at 10 °C is estimated to be 0.031 1/d and the dimensionless parameter K, which indicates the degree of inhibition for methanogens is estimated to be 102 [28]. The higher K values corresponds to the environments that inhibit the methanogens [25]. Under psychrophilic temperature, the methanogens are inhibited by the higher loading rate as well as the very low temperature [28].

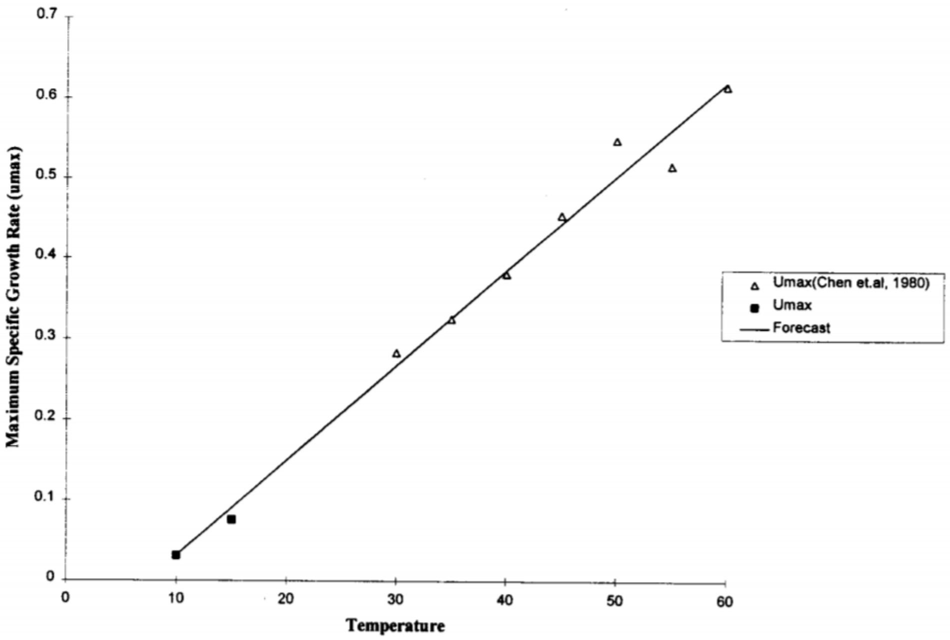


Figure 2.4 The relationship of temperature and maximum specific growth rate of methanogens [27].

The above mentioned anaerobic reactions, overall metabolic pathways and the microorganisms involved in anaerobic digestion is shown in Figure 2.5.

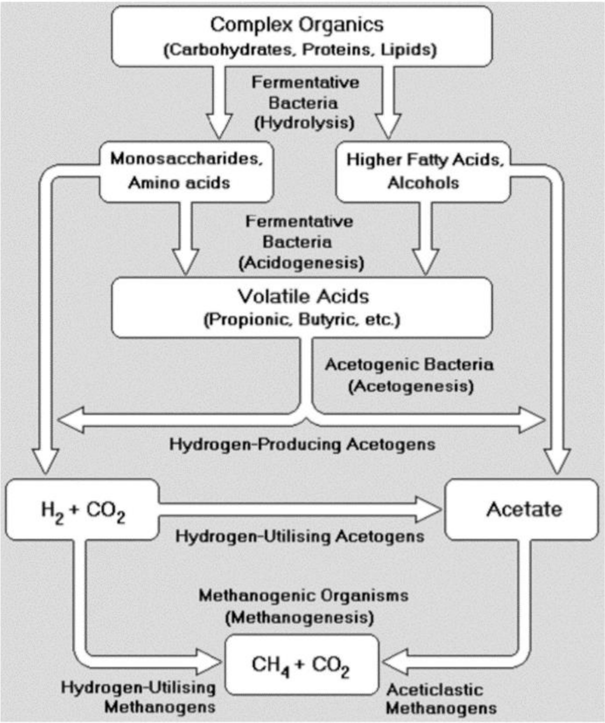


Figure 2.5 The metabolic pathways and microbial groups involved in anaerobic digestion [4].

The overall composition of the biogas produced during the anaerobic digestion varies according to the environmental conditions in the reactor. The ratio of carbon dioxide and methane in the biogas depends upon the characteristics of the organic compound to be degraded. In the anaerobic treatment of domestic sewage, the typical methane to carbon dioxide ratio in biogas is 70 – 80 % and 20 – 30 % respectively. The methane produced is quickly separated from the liquid phase due to its low solubility in water while carbon dioxide on the other hand is more soluble in water and it leaves the anaerobic system/reactor partly as gas and partly dissolved in the liquid effluent [4].

2.3 Stoichiometry and Energetics

The organic compounds in the wastewater are characterized by parameters such as COD and BOD. This sub-chapter describes the stoichiometry and the energetics of anaerobic wastewater treatment.

2.3.1 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) involves the oxidation of organic compounds in the presence of acidic dichromate solution heated at 148 °C for 2 hours. The number of electrons donated by the dichromate to the organic compounds in the test is expressed as oxygen equivalents in gO_2/m^3 or mgO_2/l. The electron equivalents of oxygen can be determined by noting that 1 mole of oxygen weighs 32 g and contains 4 electron equivalents. Thus 1 electron equivalent (eeq) corresponds to 8 gCOD shown in Eq. 2. [13].

$$1\ eeq = 8\ gCOD \tag{2}$$

The theoretical COD (thCOD) of a substrate can be determined by writing a balanced equation in which O_2 is added and the compound is mineralized to its end products with ammonia remaining in its NH_3 (III) oxidation state shown in Eq. 3. The equation shows the thCOD of a substrate which contains C, H, O, N. The thCOD can deviate from measured COD if the compound does not react in the COD test [13].

$$C_nH_aO_bN_c + \frac{1}{2}(2n + 0.5a - 1.5c - b)O_2 \rightarrow nCO_2 + cNH_3 + \frac{a - 3c}{2}H_2O \tag{3}$$

The thCOD can be calculated by the mineralization of glucose shown in Eq. 4:

$$\begin{array}{l} C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \\ \quad 180\ g \quad 192\ g \end{array} \tag{4}$$

Therefore, 1 g glucose represents 1.067 g thCOD/g glucose (192/180). The thCOD per unit mass is different for different organic compounds e.g. for fats thCOD is 2.8 g COD/ g fat and for proteins it is 1.5 g COD/ g protein. The COD reflects the energy potential of the compounds. For organic substrates, the thCOD/gVSS ratio varies greatly according to the degree of reduction of the substrate. The value 1.42 gCOD/gVSS is considered typical for the municipal biological wastewater treatment [13]. The lower the oxidation state i.e. more negative of the carbon atom in an organic compound the more oxygen can be bound to that compound which results in its higher COD value [3].

2.3.2 COD Balance

COD balance is a useful parameter for evaluating the adequate operation and control of the anaerobic system or process. The COD that enters the anaerobic reactor system end up in the final product CH_4 minus the COD that is incorporated in the new bacterial cell mass and effluent wastewater [3]. A perfect mass balance can be made using COD as a parameter (Eq. 5).

$$COD_{in} = COD_{out} \tag{5}$$

The Eq. 5 can be expanded to various outlets of the anaerobic reactor system i.e. COD of effluent, gas and sludge as shown in Figure 2.6.

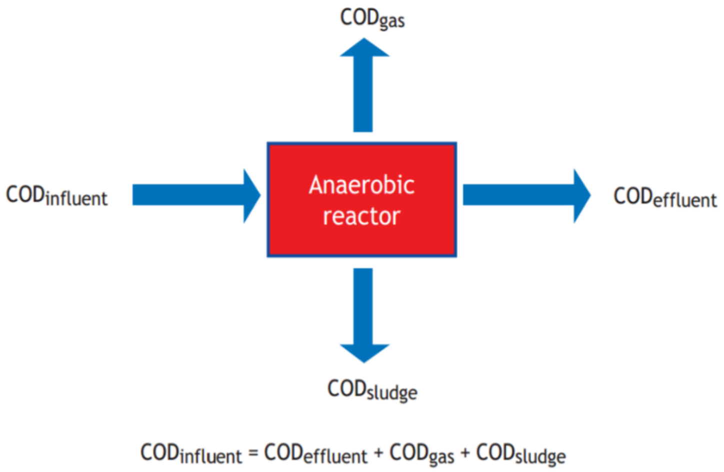


Figure 2.6. COD balance of an anaerobic reactor.

Sometimes there are gaps in the COD balance. One cause of this COD gap is the accumulation of COD in the sludge bed which drastically change the stoichiometric value of 1.42 gCOD/gVSS. This is the case when treating fat or low chain fatty acids (LCFA) containing wastewater. With these substrates, the COD removal efficiency is very high but the CH_4 production rate is very low which leads to a huge gap in COD balance, which indicates long-term operational problems. The accumulation of solids results in failing of the anaerobic process. So, by taking the COD balance as a tool to assess the reactor performance adequate actions can be made to avoid deterioration of the anaerobic processes.

2.3.3 Methane Production

The total amount of CH_4 produced in an anaerobic process is related to the amount of organic matter present in the wastewater and its degradation efficiency. Based on the influent characteristics i.e. the flow rate, the COD concentration and the biodegradability of COD, the production rate of methane gas can be estimated.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad (6)$$

Eq. 6 shows that at standard temperature and pressure (STP) 22.4 m^3 of CH_4 requires 2 moles of O_2 (COD) for its complete oxidation to CO_2 and H_2O which equals 64 kg of COD. So, theoretically 1 kg of COD can be converted into 0.35 m^3 CH_4/ kg COD (as 22.4m^3/64kg COD).

3. Environmental factors affecting anaerobic treatment

This chapter explains the different environmental factors e.g. temperature, pH and alkalinity, salinity, metals, hydraulic and organic loading rates affecting the anaerobic process and also the causes of these environmental variations to the anaerobic systems.

3.1 Causes of environmental variations

The anaerobic treatment system can be exposed to different environmental conditions. For example, in the case of sewage, the cyclic nature of human activities leads to a variable sewage production over the day [14]. Secondly, the sewage network often has one or more pumping stations, which convey the sewage at larger flow than average. Thirdly tourist areas increase their populations during holidays which leads to high flow rate variations during the year. Another cause of environmental variation to anaerobic process is that the operational procedures at the treatment plant can result in increased hydraulic and organic loads which causes variable environmental condition and when it is necessary to stop one of the anaerobic reactors for maintenance, the other reactors must cope with the flow rate [15].

Anaerobic digestion is therefore, susceptible to the strict control of environmental conditions, as the process requires an interaction between fermentative and methanogenic bacteria. Special attention should be given to the methanogenic microorganism as they are considered highly vulnerable to the changes in the environmental conditions [16]. So, in order to keep the stable environmental condition, the loading rate into the reactor must be kept constant. The main environmental factors which affect the anaerobic wastewater treatment are: pH, alkalinity, temperature, metals and salinity, and they are discussed below in detail.

3.2 pH and Alkalinity

The alkalinity and pH are both closely related to each other and these two factors are equally important for the operation and maintenance of the anaerobic process. pH affects the anaerobic process in two ways [17]:

I. Directly: affecting, for example, the enzyme activity by changing their protein structure, which may occur drastically as a result of change in pH.

II. Indirectly: affecting the toxicity of different compounds.

III. pH also affects the precipitation in the reactor e.g. the high pH results in $CaCO_3$ precipitation.

Anaerobic processes are sensitive to pH and inhibitory substances. The methanogens have optimum pH in the range between 6.6 and 7.4 for growth. A pH value below 6.0 and above 8.3 must be avoided as it can inhibit the methanogenic activity so a pH value near neutral is preferred [4]. Because of the high CO_2 content in the gases developed in the anaerobic processes, a high alkalinity is needed to assure pH near neutrality. An alkalinity concentration in the range of 3000 to 5000 mg/l $CaCO_3$ is often found. For sludge digestion alkalinity is produced by the breakdown of protein and amino acids to produce NH_3, which combines with CO_2 and water to form alkalinity as $NH_4(HCO_3)$. For industrial wastewater applications, especially for waste containing carbohydrates, it is necessary to add alkalinity for pH control. Several chemicals that can be used to control the pH or to increase the buffering capacity of an anaerobic process are sodium bicarbonate, lime and sodium carbonate [1]. Table 3.1 shows values of optimum pH ranges for the degradation of different substrates [17].

Table 3.1. Optimum pH ranges for the degradation of different substrates.

Substrate	Optimum pH
Formiate	6.8 to 7.3
Acetate	6.5 to 7.1
Propionate	7.2 to 7.5

Regarding the stability of the process, the fact that the acid-producing bacteria or acidogens are much less sensitive to pH than the methanogens, as the acidogenic bacteria are very active, even for pH values as low as 4.5 [4] while methanogens are more sensitive to pH and toxic compounds in anaerobic treatment. The main consumer of alkalinity in the anaerobic digester is CO_2. Due to its partial pressure, CO_2 solubilizes and produces carbonic acid which consumes alkalinity [1].

3.3 Temperature

The temperature greatly affects the economics and feasibility of anaerobic wastewater treatment. Reactor temperatures of 25 to 35 °C are generally preferred to support optimal biological reaction rates and to provide stable treatment. Generally, COD concentrations of greater than 1500 to 2000 mg/L are needed to produce sufficient quantities of methane to heat the wastewater without an external fuel source. Anaerobic treatment can be applied at lower temperatures and has been sustained at 10 to 20 °C in suspended and attached growth reactors. At lower temperatures, slower reaction rates occur and longer SRTs, larger reactor volumes and lower organic COD loadings are needed [1]. At temperature in the range of 10 to 20 °C, the degradation of long chain fatty acids is often rate limiting. If long chain fatty acids accumulate, foaming may occur in the reactor. When higher SRTs are needed, the solids loss in an anaerobic reactor can become critical limiting factor. Anaerobic reactor produces more dispersed, less flocculant solids than aerobic systems, with effluent TSS concentrations for suspended growth processes in the range of 100 to 200 mg/L. For dilute wastewaters, the effluent TSS concentrations will limit the possible SRT of the process and the treatment potential.

There are three temperature ranges that can be associated with microbial growth in most biological processes that are [4]:

- Psychrophilic range: between 4 and approximately 15 °C
- Mesophilic range: between 20 and approximately 40 °C
- Thermophilic range: between 45 and 70 °C and above

In each of these three-temperature ranges, three temperature values are usually used to characterize the growth of microorganism species as follows:

- Minimum temperature below which growth is not possible
- Optimum temperature at which growth is maximum
- Maximum temperature above which growth is not possible

Figure 3.1 shows these temperature values [4]. The overlapping growth temperature ranges in Figure 3.1 indicate that there is not a clear boundary between these classic groups of psychrophilic, mesophilic and thermophilic methanogens [10].

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 temperature of 37 °C. However, some reactions such as hydrogenotrophic sulfate reduction, hydrogenotrophic methane production and acetate formation from hydrogen and bicarbonate require less energy. In general, lowering the operational temperature leads to decrease in the specific growth and substrate utilization rates but it might also lead to an increased net biomass yield of methanogenic population or acidogenic sludge [10]. Methanogens are considered to be the most sensitive by lower temperature/ temperature variations.

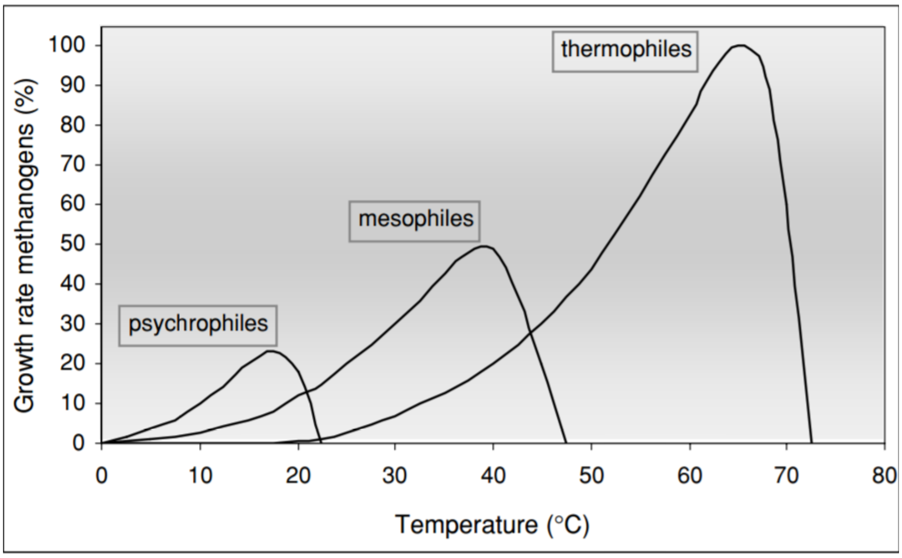


Figure 3.1. Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens.

Most of the anaerobic digesters have been designed in the mesophilic range, although their operation is possible in thermophilic range [4]. At temperature exceeding that of the maximum growth, the decay rate exceeds the bacterial growth rate which results in decrease in specific sludge activity and reactor efficiency [15].

A drop in the temperature affects the physical and chemical properties of wastewater which in turn affects the design and operation of the treatment system. For example, the solubility of gaseous compounds increase as the temperature decreases below 20 °C. Therefore, the increased solubility of CO_2 indicates that a slightly lower reactor pH might prevail under psychrophilic conditions [10].

3.4 Effect of Salinity

Several organic and inorganic compounds can be toxic or inhibitory to anaerobic process, although the resulting effect of addition of most of them may vary from stimulating to toxic. Toxicity by salts is usually associated with cations, and not with the anion of the salts. Cation toxicity assessment indicates the following increasing order of inhibition, based on the molar concentration: Na^+ (0.32 M), NH_4^+ (0.25 M), K^+ (0.15 M), Ca^{2+} (0.11 M) and Mg^{2+} (0.08 M). If some cation is found at inhibiting concentration in the influent sewage, inhibition can be reduced if an antagonistic ion is either present or added to the system. Sodium and potassium are best antagonistic for this purpose, if they are used in stimulating concentrations as shown in Table 3.2. Antagonistic elements are usually added by means of chloride salts [4].

Table 3.2. The stimulating and inhibiting concentrations of some cations.

	Concentration (mg/L)		
Cation	Stimulating	Moderately inhibiting	Strongly inhibiting
Calcium	100 to 200	2,500 to 4,500	8,000
Magnesium	75 to 150	1,000 to 1,500	3,000
Potassium	200 to 400	2,500 to 4,500	12,000
Sodium	100 to 200	3,500 to 5,500	8,000

3.5 Effect of Metals

The metals and elements such as chromium, chromates, nickel, zinc, copper, arsenic and cyanides are classified as highly toxic inorganic toxins. The presence of low concentrations of copper, zinc and nickel in soluble state is considered highly toxic, and these salts are associated with most of the toxicity problems caused by the metals in anaerobic treatment [4]. The concentrations of most toxic metals that can be tolerated in anaerobic treatment are related to the concentrations of sulfide available to be combined with the metals and form insoluble sulfide salts that have no adverse effect. One of the most effective procedures to control toxicity by metals is the addition of sufficient amount of sulfide to precipitate the metals. Approximately

1.8 to 2.0 mg/L of metals is precipitated as metallic sulfides by the addition of 1.0 mg/L of sulfide (S^{2-}). This phenomenon is a good alternative for the treatment of industrial effluents containing metals [4].

3.6 Effect of variation in hydraulic and organic loading rate

In anaerobic digestion, a balance exists between primary processes (hydrolysis and acidogenesis) and the conversion of acid products into methane and CO_2 by acetogens and methanogens [18]. The strong variations in flow rates and concentrations may adversely affect the efficiency of an anaerobic reactor [19]. The effect of fluctuations in hydraulic and organic load generally depends on the HRT, SRT, intensity and duration of variations, sludge properties and reactor design. The accumulation of VFAs can be a typical reactor response during overloading and during sudden variations in hydraulic and organic loading rates (OLR). OLR is the amount of COD in g/l applied per day to the UASB or anaerobic reactors. Such highly undesirable situations could lead to the production of CO_2 and H_2 gas in the biogas. The partial pressure of H_2 gas in the reactor might increase to values exceeding 10^{-4} atm, which may cause a shift in the metabolic pathways. When slowly growing methanogens cannot rapidly remove H_2 produced by H_2 producing bacteria this may result in the inhibition of degradation of propionate, butyrate and lactate. Another effect during a situation of stress is the drastic change in biogas production rates and composition [15].

UASB reactors are robust systems with regard to COD removal efficiency and pH stability when exposed to shock loads. However, it cannot attenuate the imposed fluctuations in the influent COD. It describes that either a secondary treatment unit is needed to retain the expelled sludge occurring as a result of the hydraulic shock load, or prior to the shock a sufficient amount of sludge needs to be discharged from the reactor [20]. The OLR applied to the reactor depends on the influent concentration, flow rate and reactor volume and also on HRT shown in Eq. 7. where Q is the flow rate in l/d and V is the reactor volume in m^3 and HRT is in days.

$$OLR = \frac{COD_{in} \times Q}{V} = \frac{COD_{in}}{HRT} \qquad (7)$$

OLR is measured in g/l.d and since it is a function of different parameters (Eq. 7) so the effect of it on the reactor performance is contradictory i.e. increasing the influent concentration and hence OLR up to a certain limit will increase the removal efficiency. And increasing the flow rate (hence OLR) reduces the HRT and leads to decrease in reactor efficiency. Thus, in order to characterize UASB's performance in treating sewage, the OLR has to be analyzed in combination with HRT and COD_{in} [20].

4. Anaerobic Wastewater Treatment Systems

This chapter describes the general concept and the operating conditions of UASB and EGSB reactors. It also explains the applications of UASB reactor to treat the municipal wastewater.

4.1 Upflow Anaerobic Sludge Blanket (UASB) Reactor

One of the most notable developments in anaerobic treatment process technology was the upflow anaerobic sludge blanket (UASB) reactor in the late 1970s in the Netherlands by Lettinga and his coworkers.

The basic UASB reactor is shown in Figure 4.1. As shown in Figure 4.1, the influent wastewater is distributed at the bottom of the reactor and travels in an upflow mode through the sludge blanket. Critical elements of the UASB reactor design are the influent distribution system, the gas-solids separator, and the effluent withdrawal design [1].

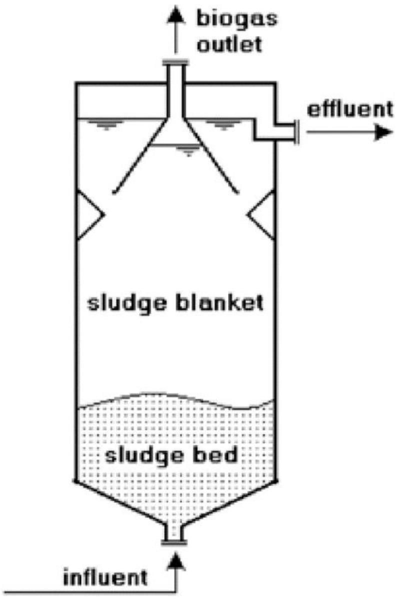


Figure 4.1 Schematic representation of an UASB reactor [4].

Modifications to the basic UASB design include adding a settling tank as shown in the Figure 4.2 (Left) or the use of packing material at the top of reactor as shown in Figure 4.2 (Right). Both modifications are intended to provide better solids capture in the system and to prevent the loss of large amount of UASB solids due to process upset or changes in reactor sludge blanket characteristics and density [1].

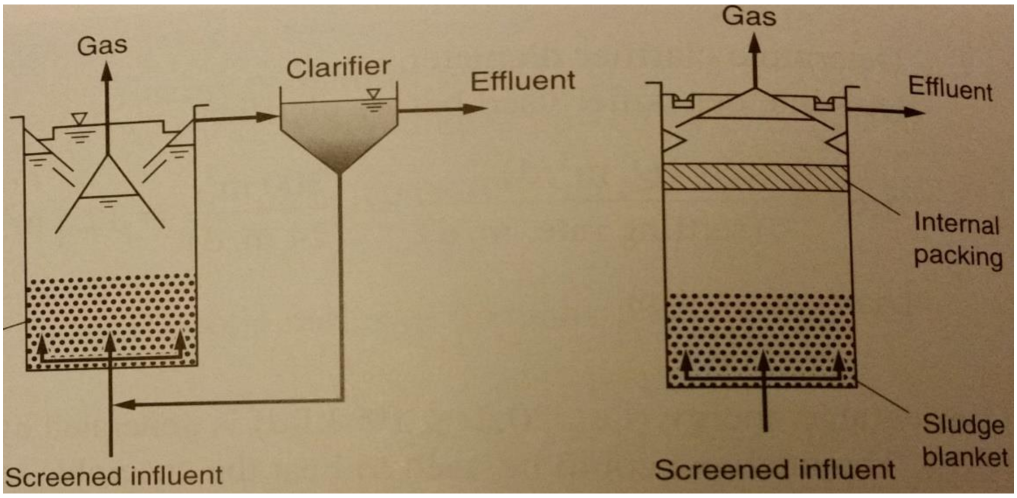


Figure 4.2. (Left) UASB reactor with sedimentation tank and sludge recycle, (Right) UASB reactor with internal packing and fixed-film attached growth, placed above the sludge blanket [1].

The key feature of UASB reactor that allows the use of high volumetric COD loadings compared to the other anaerobic processes is the development of a dense granulated sludge. Several months may be required to develop the granulated sludge, and the seed is often supplied from other facilities to accelerate the system start-up. The development of granulated sludge solids is affected by the wastewater characteristics. Granulation is successful with high carbohydrate or sugar wastewater but less with wastewaters high in proteins [1].

UASB wastewater treatment systems represent a proven sustainable technology for a wide range of very different industrial effluents, including those containing toxic or inhibitory compounds. The process is also feasible for treatment of domestic wastewater with temperatures as low as 14–16 °C and likely even lower. In UASB process sludge is not exposed to heavy mechanical agitation. For achieving the required sufficient contact between sludge and wastewater the conventional UASB system relies on the agitation brought about by the biogas production and even feed distribution at the bottom of the reactor [7]. The produced biogas causes hydraulic turbulence as it moves upward trough the reactor, providing adequate mixing within the system and eliminating the need for mechanical mixing. Granule retention is facilitated by the presence of three phase separators (also known as the gas – liquid – solid separator) at the top of the reactor where the water phase is separated from the sludge solids and gas. The high biomass concentration within the UASB reactor results in rapid transformation of the contaminant, allowing for the application of high loading rates [21].

L. Seghezzo (1998) 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 % at 20 °C and 70 – 55 % at 13 – 17 °C. He concluded that the UASB concept was a simple, compact, and inexpensive technology for sewage treatment, even at relatively low temperatures [8]. Based on research carried out in Netherlands on different UASB reactors (0.120, 0.240, 6 and 20 m^3), de Man et al. (1986) concluded that anaerobic treatment of raw domestic sewage having COD of 500 – 700 mg /l can be accomplished at 12 – 18 °C applying HRTs of 7 – 12 h with total COD and BOD removal efficiencies of 40 – 60 %, and 50 – 70 % respectively [7].

However, sludge-wastewater contact was found to considerably affect the treatment efficiency, especially at low temperatures when the gas mixing was poor. Better SS removals were achieved using shallow reactors. Efficiencies up to 80 % were obtained at 10 – 20 °C when treating sewage from a separated sewer system (domestic wastewater separated from rain-water) with a granular bed reactor. Higher upflow velocities, like those applied in expanded granular sludge bed (EGSB) reactors, induce a better sludge – wastewater contact and the removal efficiency of soluble substrates is likely to increase. Figure 4.3 shows a schematic representation of EGSB reactor.

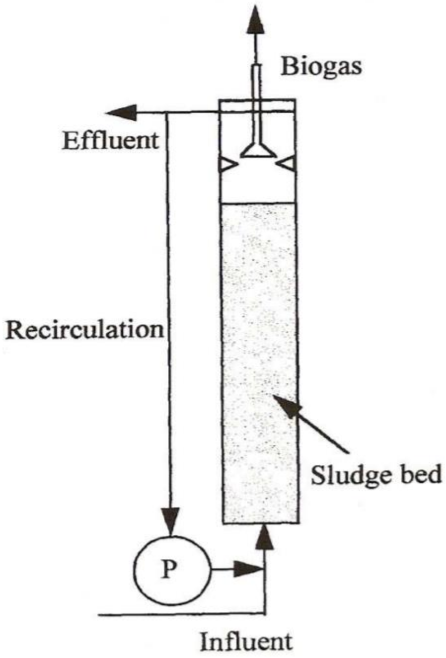


Figure 4.3. Schematic diagram of EGSB reactor [9].

The EGSB reactor is the family of UASB reactor. With a high recycle ratio, the upflow of this reactor is typically maintained higher than 6 m/hr. While the general range of the UASB reactor is 0.5 to 1.0 m/hr. These high upflow velocities can be achieved by applying effluent recycle and using tall reactors. Additionally, due to the high velocity, granules are expended and the hydraulic mixing is intensified as to also give granules more chances to contact with wastewater. Thus, this reactor can treat high-strength organic wastewater (up to loading rate about 30 $kg/m^3 \cdot d$). The definitive feature of EGSB reactor is the rapid upflow velocity. It enables the reactor to separate dispersed sludge from mature granules in the reactor [9].

There was a research result of treating the slaughterhouse wastewater by the EGSB reactor. In this study, removal efficiencies of COD, TSS, and fats were 67 %, 90 %, and 85 %, respectively. And, there was no accumulation of fats in the reactor [9].

5. Materials and Methods

This chapter explains the materials and methodology of the anaerobic treatment of industrial wastewater from Grødaland WWTP for the removal of dissolved COD and generation of methane gas, using UASB reactor. The key feature of UASB that allows the use of high volumetric COD loadings compared to the other anaerobic processes is the development of a dense granulated sludge. Two UASB reactors (Reactor I and II), were used for investigating the UASB reactor performance and their potential to remove COD and to produce biogas. This chapter also includes the operational and maintenance procedures and the other analytical methods used in this study. All the laboratory tests were performed and conducted at the University of Stavanger and the wastewater used was brought from the Grødaland WWTP.

5.1 Configuration of UASB reactors

In the laboratory scale two UASB reactors were used to assure the high anaerobic treatment of wastewater shown in Figure 5.1 and 5.2. The two reactors Reactor I and II were already set and were in operation for a year in the laboratory at University of Stavanger at temperature around 20 – 25 °C. The granules used in these two reactors were made from mixed sources as: (1) pulp and paper industry treating cellulose and lignin containing (Norske Skog, Moss), (2) agriculture pilot plant treating swine and cow manure supernatant (farm in Skien, Norway), (3) hydrocarbon oil containing wastewater at Bamble Industrial Park, Telemark [22].

These two UASB reactors, each having a volume of 1 L, were connected to the feed wastewater via a peristaltic pump with adjustable flow rate and to the gas counters. The wastewater from Grødaland WWTP was stored in the fridge in a 30 L plastic container to assure the stable composition of wastewater in the feed. The reactor effluent (treated water) and biogas (methane + CO_2) went out of the reactors through the filter to the gas trap/collector due to the liquid height, pressure and suction force given by the recirculation pump. The filter is installed to avoid the granules or biomass to be washed out with effluent to clog the pump or tubes. The schematic diagram of Reactors I and II is shown in Figure 5.2 [22].

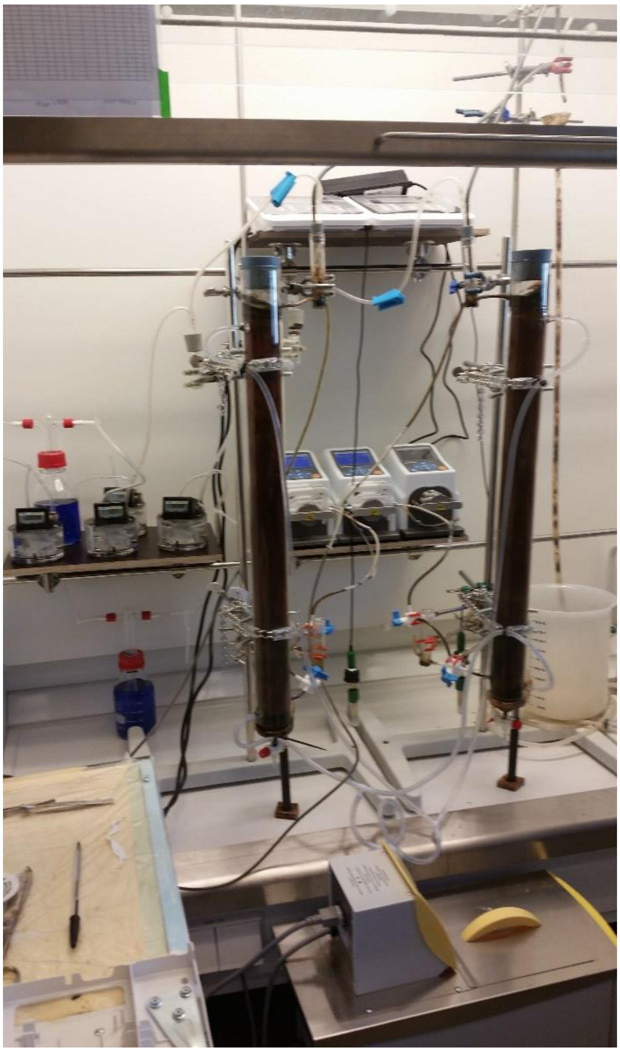
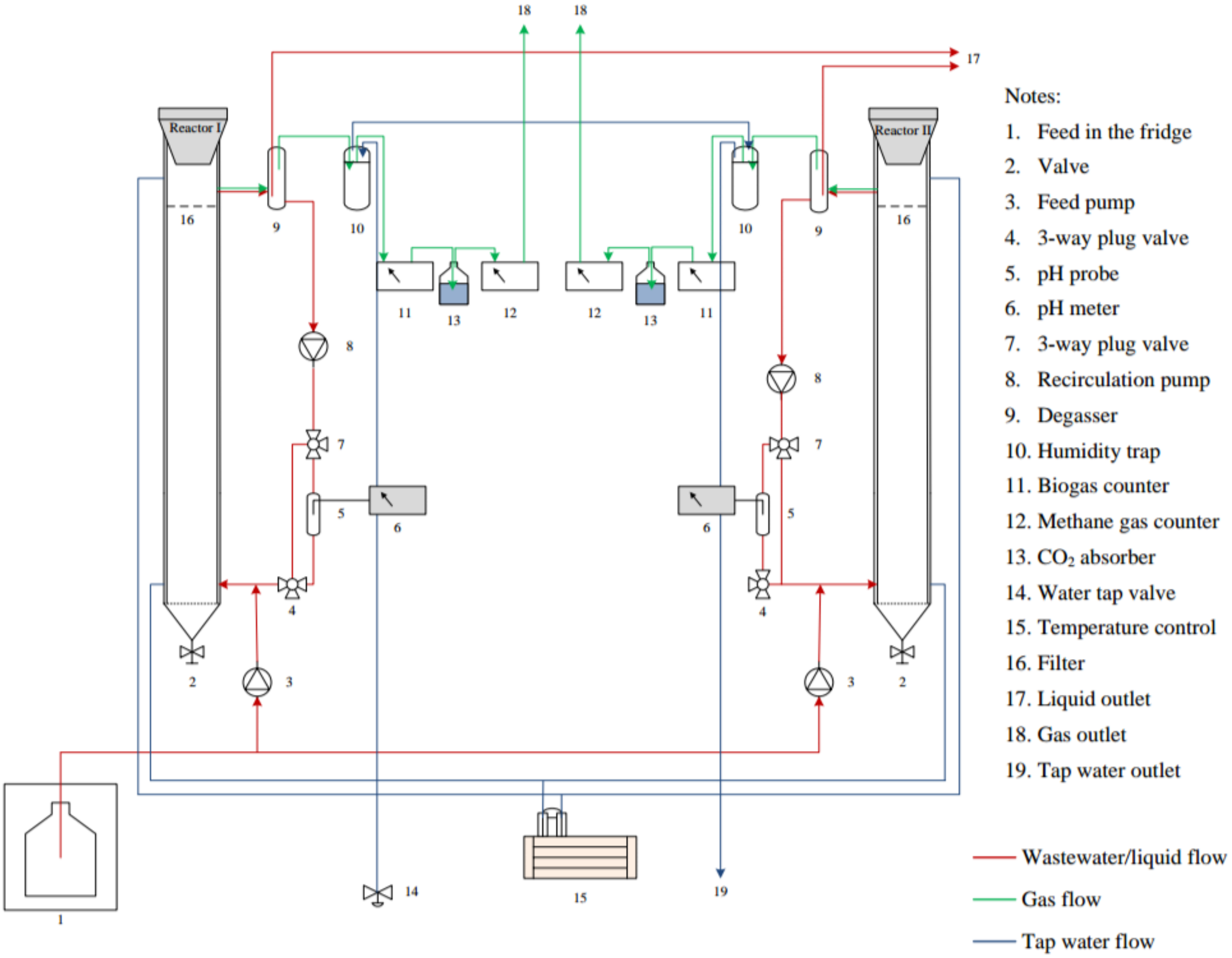


Figure 5.1. Laboratory scale UASB Reactor I and II.

The pH of the liquid inside the reactors is kept at around 7, for the optimum growth of the methanogens. The humidity traps, to stop the biogas to evaporate before it comes to the gas counters, and gas counters are also installed with the UASB reactors (Reactor I and Reactor II) for measuring the produced biogas volume. As biogas is the mixture of methane and CO_2, so a 3M NaOH solution was used as the CO_2 gas absorber. The CO_2 in the biogas produced is absorbed in this solution and the methane gas goes to the next gas counter which then measures the volume of methane produced. A total of four gas counters were used for the two UASB reactors.



Notes:

1. Feed in the fridge
2. Valve
3. Feed pump
4. 3-way plug valve
5. pH probe
6. pH meter
7. 3-way plug valve
8. Recirculation pump
9. Degasser
10. Humidity trap
11. Biogas counter
12. Methane gas counter
13. CO_2 absorber
14. Water tap valve
15. Temperature control
16. Filter
17. Liquid outlet
18. Gas outlet
19. Tap water outlet

Figure 5.2. Schematic diagram of lab scale UASB Reactors I and II.

Both the UASB reactors were also attached to a cooling system and circulation pipes. The cooling system allowed the liquid to be cooled inside the external layer of the reactors through the circulation pipes. This helped maintaining the psychrophilic condition in the reactors at average temperature of 10 °C. The temperature in Reactor I and Reactor II was changed from mesophilic to psychrophilic temperature (10 °C) two months before starting the experiments/measurements. Table 5.1. shows the equipment used for UASB reactors.

Table 5.1. Properties of equipment used for UASB reactors

Equipment	Manufacturer	Properties	
Feed and recirculation pump	Ismatec	Type	Peristaltic pump
		Model	Reglo ICC
		Flowrate	0 – 43 ml/min
Filters	Sefar	Type	Sefar® Flourtex
		Model	09-1000/45
		Pore size	1000 µm
Gas Counters	Ritter	Model	MGC-1 V3.3 PMMA
		Gas flowrate	1 ml/h – 1 l/h
		Max. pressure	100 mbar
		Min. pressure	5 mbar
		Measuring accuracy	Less than ±1 %
Heating circulator	Lauda Alpha	Model	RA 8 LCK 1907
		Max. flowrate	15 l/min
		Temperature range	-25 – 100 °C
		Bath volume	20 L

5.2 Start-up of UASB reactors

The UASB reactors were already in operation and the tubes were clogged with biomass produced so it was necessary to clean-up the system before taking new measurements. The whole system was turned-off on 15th Nov 2016 and the tubes, humidity traps and all the four gas counters (methane and biogas counters) were washed, cleaned and calibrated. The CO_2 gas absorber solution was also made using 3M NaOH. The gas counters were also washed with tap water, they were air dried and calibrated using 0.5 M sulphuric acid (H_2SO_4) solution. The system was in run/operation again on 18th Nov 2016. This thesis work was started on 13th Jan 2017, until then the system was running and it was supposed to be in steady-state condition.

Sometimes the liquid level in the degasser was too high or low during the reactor operation, which caused the gas to go out of the liquid outlet tube or the liquid water was sucked into the humidity trap and to the gas counters. This issue was solved by adjusting the pressure by increasing or decreasing the height of liquid outlet tube and the degasser until the liquid comes to its normal level. Both the reactors were adjusted to a fixed flowrate that was seen on the pump. The flowrate was also taken manually by counting the volume of effluent liquid into the measuring cylinder per unit time to make sure that the flowrate was maintained. The Reactor II was run at two different flowrates (explained in Results section) to investigate its effect on the COD removal efficiency and the methane production rate during the experiment period.

5.3 Analytical Methods

This sub-chapter explains how the analytical methods were performed in the laboratory to estimate the effectiveness of anaerobic treatment of wastewater. The following parameters were calculated/measured:

- pH and conductivity measurement
- COD measurement
- Total volatile fatty acid and alkalinity measurement

5.3.1 Conductivity and pH

Conductivity and pH was measured using WTW Multi340i instrument which was attached to conductivity probe (WTW Tetra Con® 325) and pH probe (WTW pH-Electrode SenTix 41). The pH probe was calibrated with the buffer standard solution. The unit of conductivity was also noted as µS/cm which was then converted to mS/m, a unit consistent with the TITRA 5 software programme for measuring the alkalinity and VFA concentration (explained in following section).

5.3.2 COD Measurement

The COD, both total and dissolved COD analysis was done for the influent wastewater sample and for Reactor I and II effluent samples. The COD analysis was taken twice a week. To measure the dissolved COD, an influent sample was filtered using glass microfiber filters (VWR European Ca. No. 516-0876). The filtrate was then used for the measurement of dissolved COD.

The COD test kits were used to analyze the COD of the water samples. The COD test kit is a small glass tube (also known as vial or reaction cell) which contain digestion and catalyst solutions that react with the samples to be measured. There is a unique barcode label on each COD kit which is read by the spectrophotometer to identify the appropriate measurement method for COD reading/measurement. The COD kits used in this study were Merck Spectroquant® with Product Number 109773 (100 and 1500 mg/l of COD range).

For measuring the COD, a 2 mL sample (influent and effluent) were taken into the COD vials. For dissolved COD 2 mL sample was taken from the filtrate. The vials were shaken and mixed sharply and were placed into the thermo reactor (Merck Spectroquant® TR 620) for digestion at 148 °C for 2 hours. After 2 hours, the vials were taken out of the thermo reactor and were cooled down in a test tube rack for about 30 minutes at room temperature. When the vials were cooled, they were placed into the spectrophotometer (Spectroquant Pharo 300) and the reading gave the COD measurement value.

5.3.3 Alkalinity and VFA Measurement

For the alkalinity and VFA measurement 40 mL of influent and effluent samples were taken and placed onto the magnetic stirrer at low rotation speed. The initial pH of the samples was noted and samples were titrated against 0.1 M HCl. Titration was done manually using a 50 mL burette and during the titration the samples were placed onto the magnetic stirrer. The samples were titrated to four different pH values (6.7, 5.9, 5.2 and 4.3) and the volume of acid used to reach to these pH values for each titration was recorded. The data recorded i.e. initial pH, four pH values, volume of acid added and conductivity was then put into the computer software TITRA 5. This software calculated the alkalinity concentration as mg/l $CaCO_3$ and the total VFA concentration as mg/l acetic acid.

6. Results

This chapter presents all the results obtained from the experiments. All the data and figures are summarized in this chapter while the raw data obtained is presented in the Appendixes.

6.1 COD Removal Efficiency

The UASB reactors performance was investigated by their COD removal efficiency and methane production rate. The flow rate in both UASB reactors (Reactor I and Reactor II) was not constant but varied from time to time when measured manually. The maximum COD removal obtained was 80 % in Reactor I at OLR of 1.8 g/l.d, flow rate of 2.0 ml/min and HRT of 8 h, while 79 % in Reactor II at OLR of 2.6 g/l.d, flow rate of 2.2 ml/min and HRT of 8 h. The flow rate in Reactor II was increased 20 % on 23rd Feb 2017. Figure 6.1 shows that as the flow rate increases the COD removal efficiency of UASB reactors increased.

Similarly, the effect of OLR on COD removal efficiency of UASB reactors was also measured (Figure 6.2), it shows that as the OLR increases the COD removal efficiency of UASB reactors increased. Figure 6.3 shows the relationship between COD removal efficiency and OLR when only the volume of sludge blanket and active biomass known as the effective treatment volume was considered which is about 30 – 40 % of total UASB reactor volume. So, in general with the increase in OLR the COD removal efficiency of UASB reactors increased (Figure 6.2 & 6.3). While, in Reactor I it shows that the COD removal efficiency increased with the increase in OLR but decreased again.

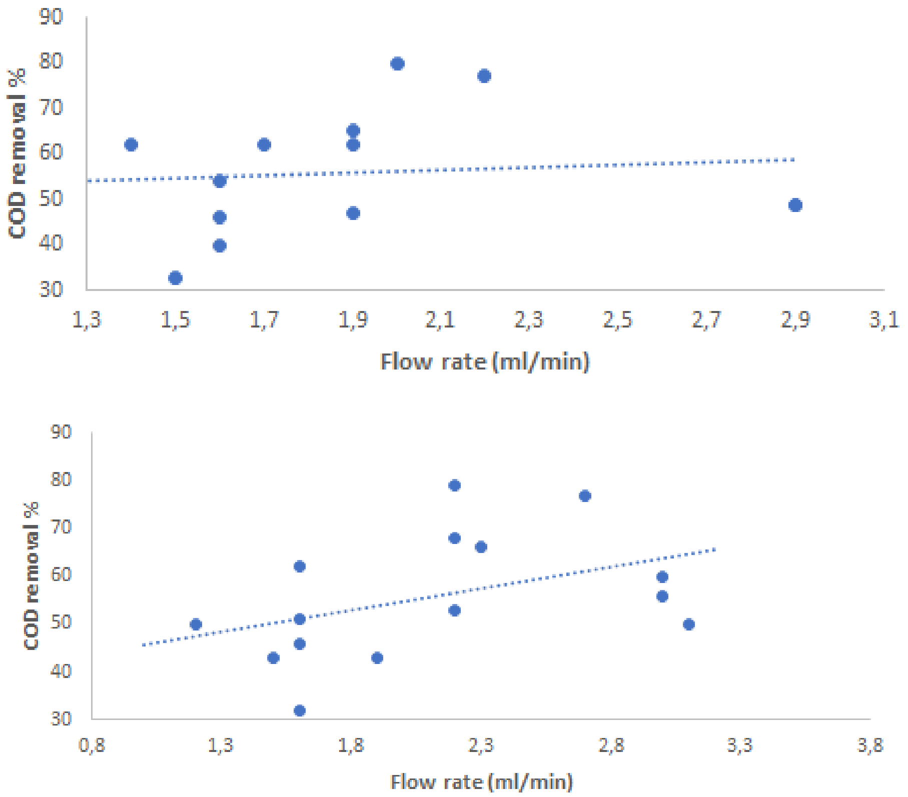


Figure 6.1 COD removal vs Flowrate of Reactor I (above) & Reactor II (below).

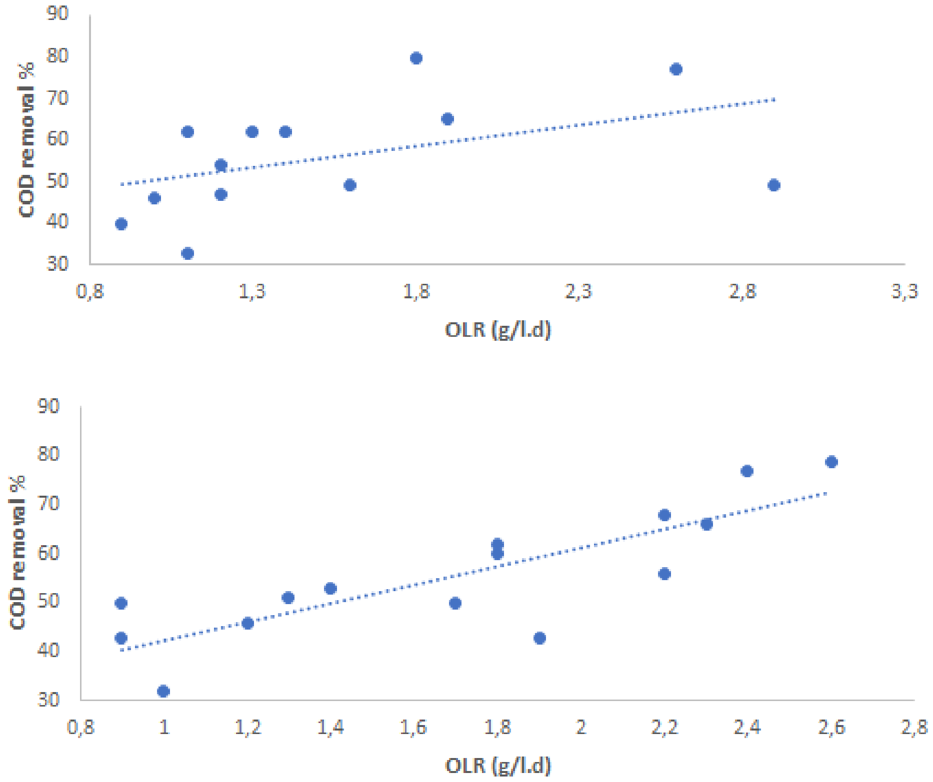


Figure 6.2 The relation between OLR and COD removal in Reactor I (above) & II (below).

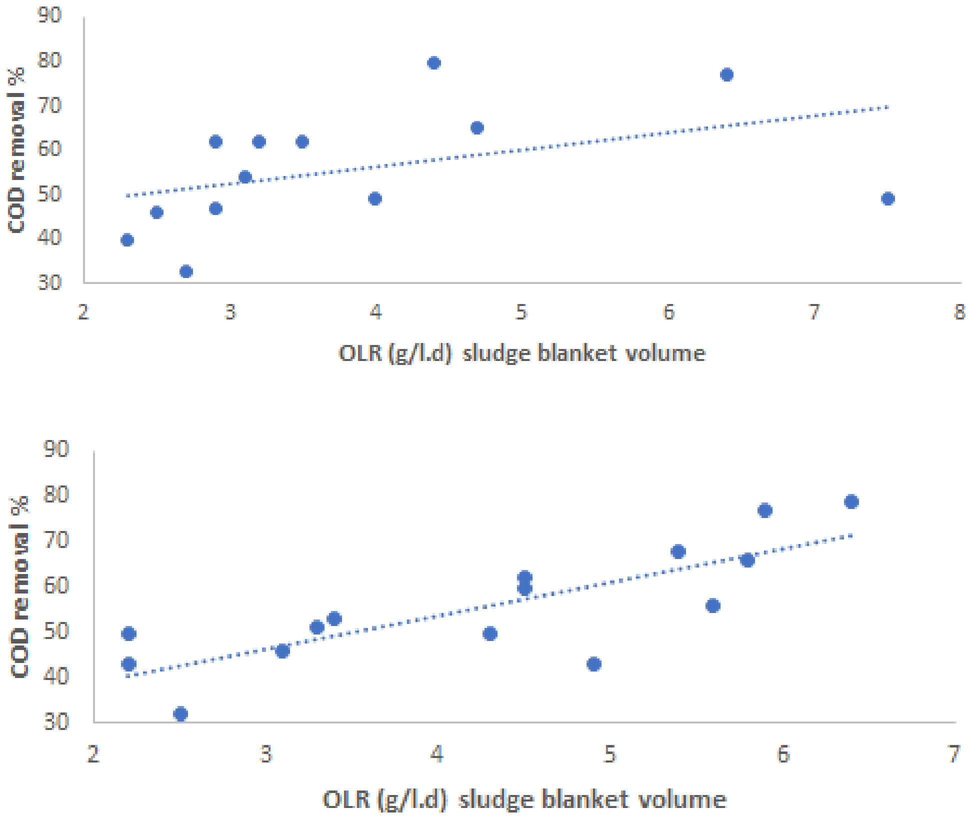


Figure 6.3 The relation between COD removal and OLR (with only sludge blanket volume) of Reactor I (up) & II (below).

6.2 pH, Alkalinity and VFA variability

During the experimental period the pH values were between 7.2 – 7.8 and 7.2 – 7.6 in Reactor I and Reactor II respectively, which is acceptable for the growth of methanogens. So, there was no need to add any buffer for the pH control. The accumulation of VFA was observed in both reactors. In Reactor I, the maximum VFA accumulation was observed to be 172.5 mg/l acetic acid while in Reactor II it was 168.4 mg/l acetic acid, with the influent VFA in Reactor I and Reactor II was 237.8 mg/l acetic acid and 226.5 mg/l acetic acid respectively. The VFA accumulation results in the decrease in alkalinity. In Reactor II, the alkalinity decreased from 769.2 mg/l $CaCO_3$ to 588.7 mg/l $CaCO_3$ on day 15 and 16 respectively, because of the VFA increase from 0 to 168.4 mg/l acetic acid. Figure 6.4a represents the relation between pH and VFA of influent wastewater and both reactors (Reactor I and Reactor II). It shows that the increase in VFA results in the decrease the pH. Figure 6.4b shows the relation between VFA and alkalinity. In Reactor I, the increase in VFA resulted in decreased pH but increased alkalinity. While in Reactor II, the increased VFA concentration resulted in the decrease in pH and alkalinity. There were variations in VFA accumulation from time to time in both reactors.

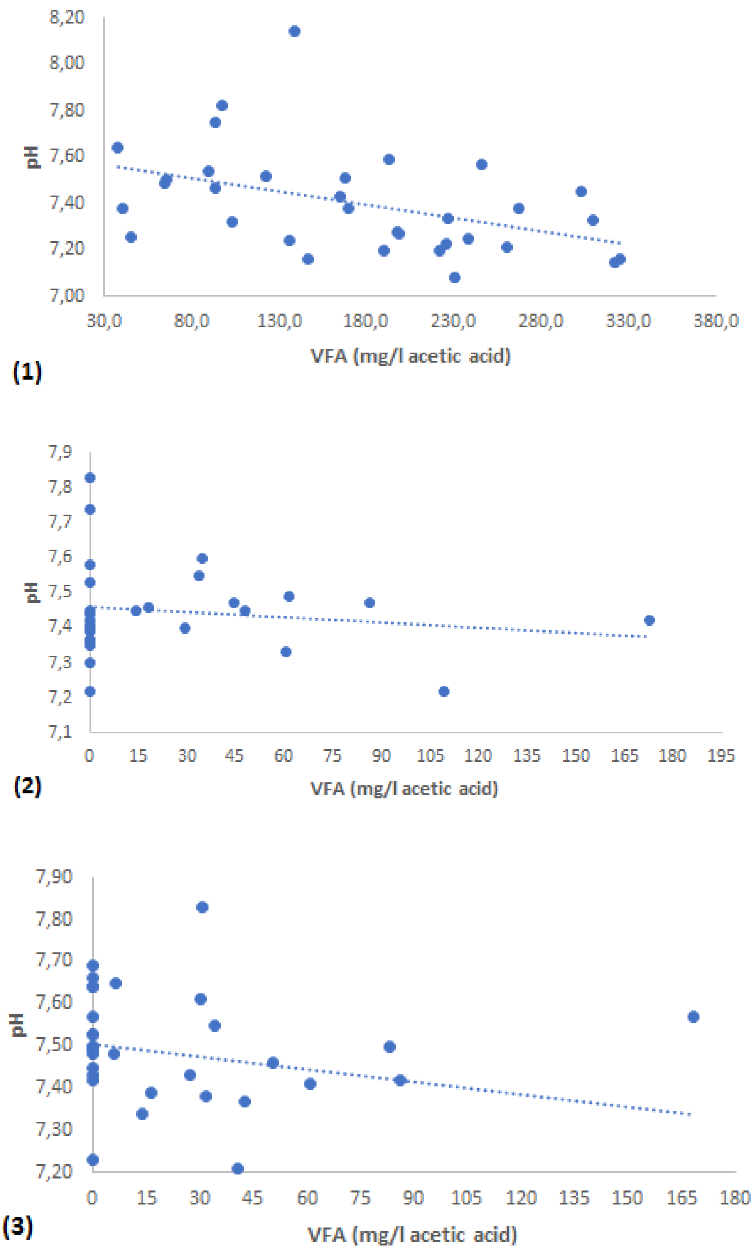


Figure 6.4a Relation between pH and VFA (1) Influent (2) Reactor I (3) Reactor II.

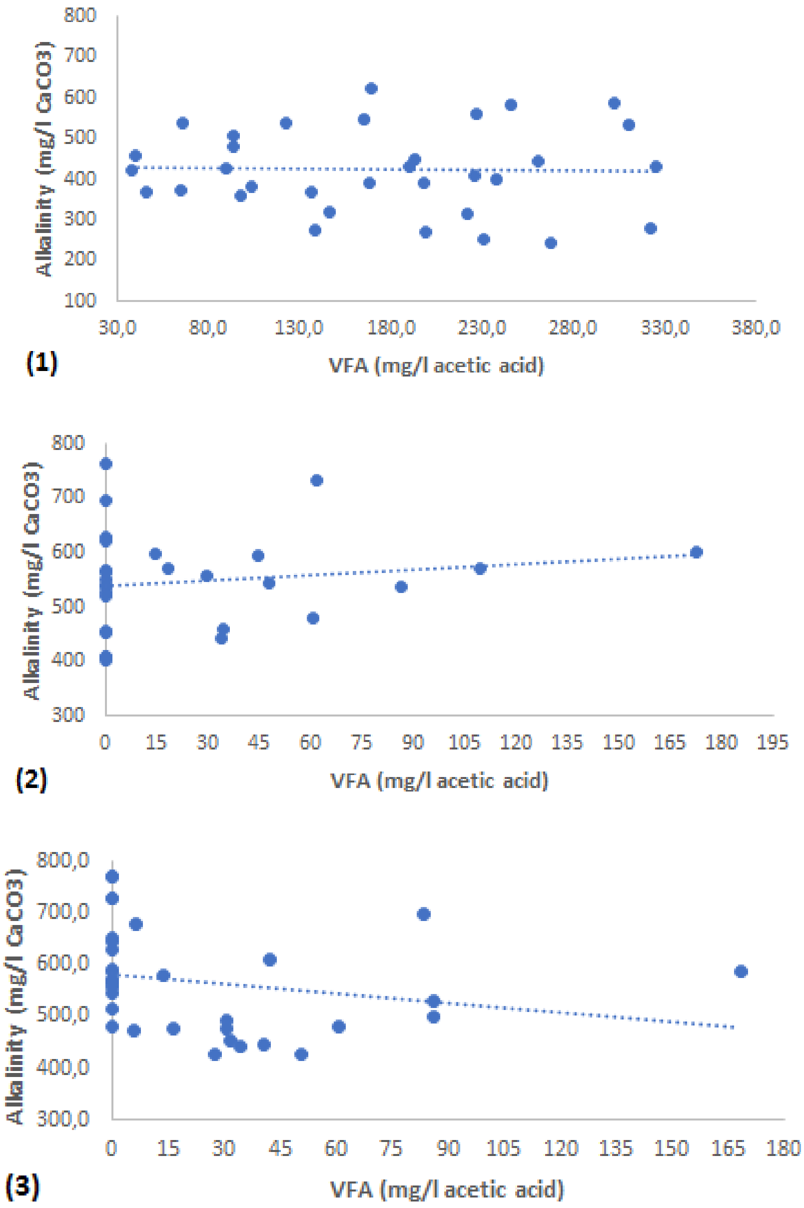


Figure 6.4b Relation between VFA and Alkalinity (1) Influent (2) Reactor I (3) Reactor II.

6.3 Methane Production

Biogas is the mixture of methane and CO_2 and according to data obtained in this study the biogas was mainly 65 – 90 % methane and 10 – 35 % CO_2. In Reactor II, more than 100 % of methane fraction was observed and this could be due to high solubility of CO_2 in water because the solubility of gaseous compounds increases with the decrease in temperature so, CO_2 was supposed to chemically bound to water phase forming carbonic acid. Figure 6.5 presents the methane production rate (l/l.d) in function of OLR (g/l.d). From Day 24 in Reactor I and Day

28 in Reactor II, the methane production rate values are not real measurements by gas meters but estimated values i.e. 65 % methane fraction in biogas was estimated for calculation purpose because the gas meters did not measure gas properly. The real data obtained gave methane production which is in accordance to theory (explained in Discussion part) while less than 0.1 l/l.d methane was obtained from estimated data which is not possible in practice because it will result in the process failure but this was not the case in current study as the system was operating by removing COD.

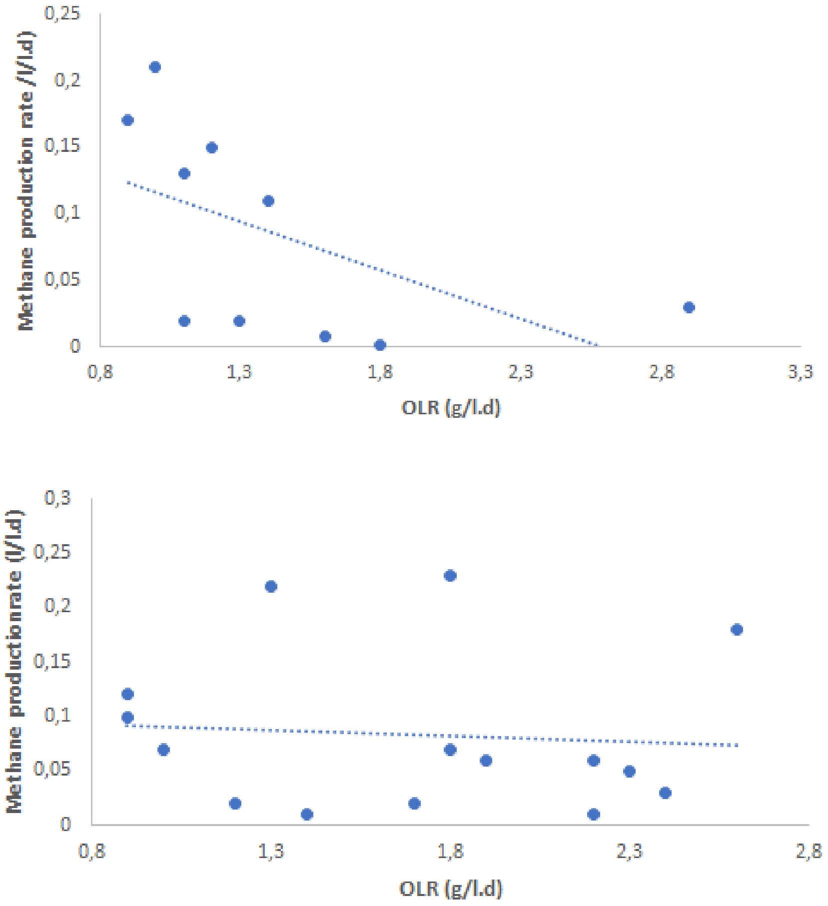


Figure 6.5 Methane rate against OLR profiles Reactor I (up) Reactor II (below).

Basically, the methane production rate increases with the increase in OLR due to the increase in substrate concentration. But the Figure 6.5 shows the opposite trend i.e. the methane production rate in both reactors decreased with the increase in OLR and this is due to analytical errors in measurements and that the gas meters were not measuring produced gas accurately towards the end of experimental study. The change in flow rate also affects the HRT i.e. the increase in flow rate decreases the HRT which in turn effects the production rate of methane gas because methanogenesis is the reaction in anaerobic digestion which is more prone to the changes in the environment. Figure 6.6 shows the methane production rate in the function of changing HRT, which shows that the methane production rate increased with the increase in HRT and decreased with the decrease in HRT. The 0 l/l.d of methane production shows the wrong measurement by gas meters or the decrease in HRT resulted in acidogenesis to occur while, methanogenesis was too slow causing the accumulation of VFAs and it might cause the accumulation of gas in the sludge.

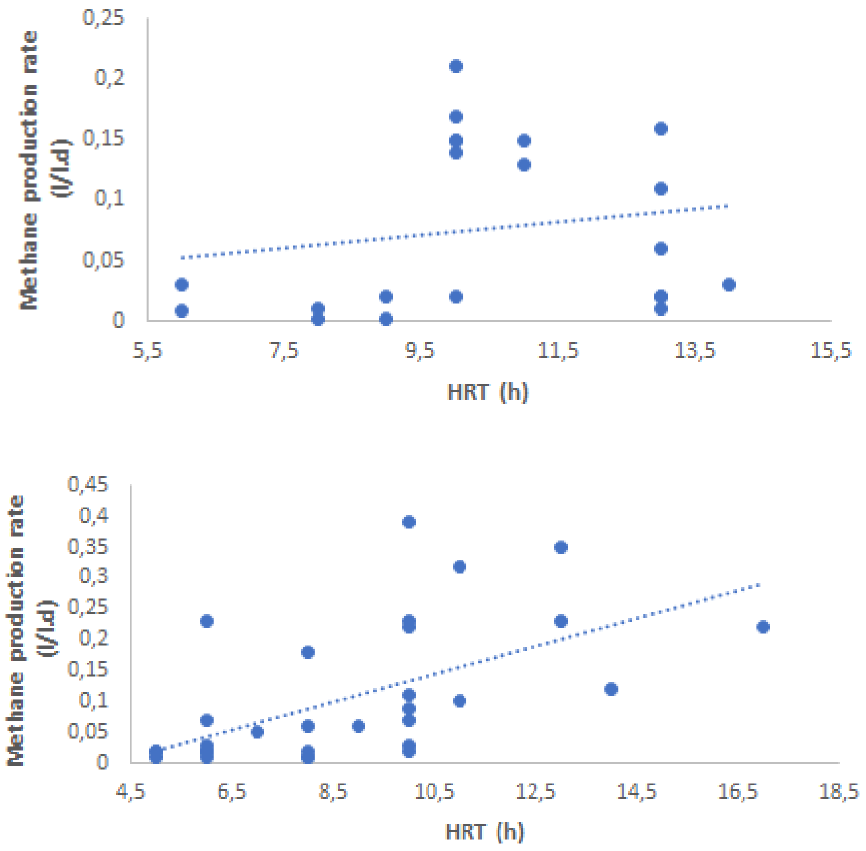


Figure 6.6 The methane production rate against HRT profiles Reactor (above) Reactor II (below).

6.4 COD and COD Balance

COD is an important parameter in anaerobic digestion process therefore, COD balance is used to estimate the proper functioning UASB reactor system. The COD balance was calculated by using influent total COD, effluent COD and methane production as mg COD/d by using a factor of 0.35 l CH_4/gCOD (at 10 °C). The COD of biomass was not included in the calculation of COD balance because it was not determined but it can be assumed i.e. the proportion of influent COD converted to biomass is assumed to be 10 % [24]. Figure 6.7 and Figure 6.8 shows the profiles of COD balance against the increasing flow rate.

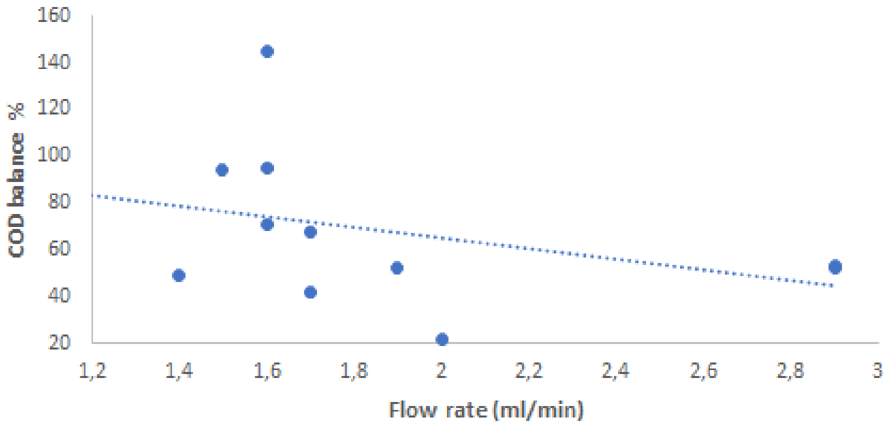


Figure 6.7 The COD balance vs Flow rate profile of Reactor I.

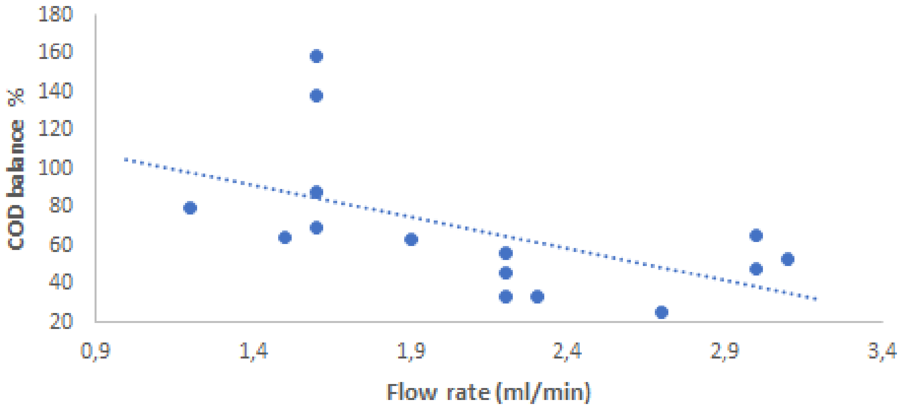


Figure 6.8 The COD balance vs Flow rate profile of Reactor II.

At the start of experimental period the COD balance % in both the reactors was calculated to be around 80 % which shows that the system was functioning properly with the COD removal efficiency in both UASB reactors was around 50 %. On the Day 18 of experimental period the COD balance % or COD recovery in Reactor II decreased from 159 % to 34 % due to a sudden increase in OLR and flow rate from 1.6 ml/min to 2.8 ml/min. The flow rate in Reactor II was increased purposely and COD recovery achieved was around 50 %. The increase in flow rate in Reactor II caused a decrease in COD recovery at 10 °C (Figure 6.8). COD balance % in Reactor I was around 100 % in the start of experimental period but the reactor was turned-off for some time (explained later in this chapter) and after the start-up again the and 49 % of COD was recovered. In general, the increase in flow rate caused a decrease in the recovery of COD (COD balance %).

The effect of OLR on COD balance % was also calculated as shown in Figure 6.9 and Figure 6.10, it shows that the increase in OLR caused a decrease in the COD balance % and hence the performance of the system at psychrophilic condition (10 °C).

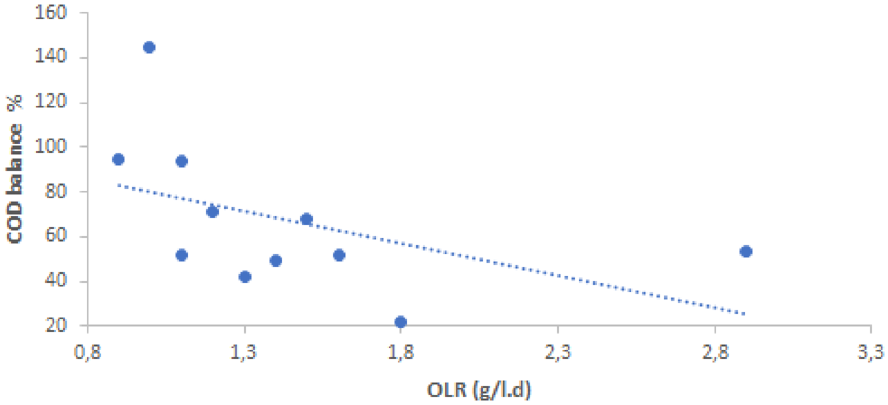


Figure 6.9 The COD balance and OLR profiles of Reactor I.

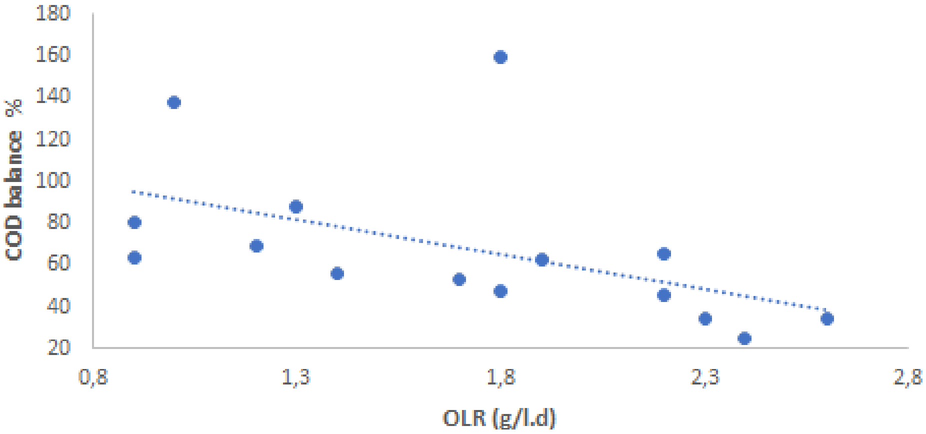


Figure 6.10 The COD balance and OLR profiles of Reactor II.

A gap in COD balance % is correlated with the instability or improper functioning of the system. The reason for gap in COD recovery is due to the entrapment of TSS in influent wastewater being not fully biodegraded but accumulated in the sludge bed. On Day 10 of experimental period the degasser attached to Reactor I was full of effluent water which caused the water to pass through the tube to the gas meter because of the insufficient pressure required to keep the water level low in degasser (Figure 6.11). The digital part of the gas meter that measure methane gas value was destroyed. Because of this the Reactor I was turned-off for 3 Days. It was in operation again on 6th Feb 2017. Because one of the gas meters was destroyed so there was only one gas meter left which was attached to the Reactor I for the biogas measurement and to the 3M NaOH solution for keeping enough pressure for the system run. But the water level in the degasser fluctuated more often because there was not enough back pressure, which was adjusted by changing the position of the effluent pipes and the degasser. As there was only one gas meter for measuring the biogas, the methane gas was calculated based on the biogas measurement by assuming 65 % of methane present in biogas. From Day 24 of experiment period all the calculations are based on the assumed methane gas values in Reactor I.

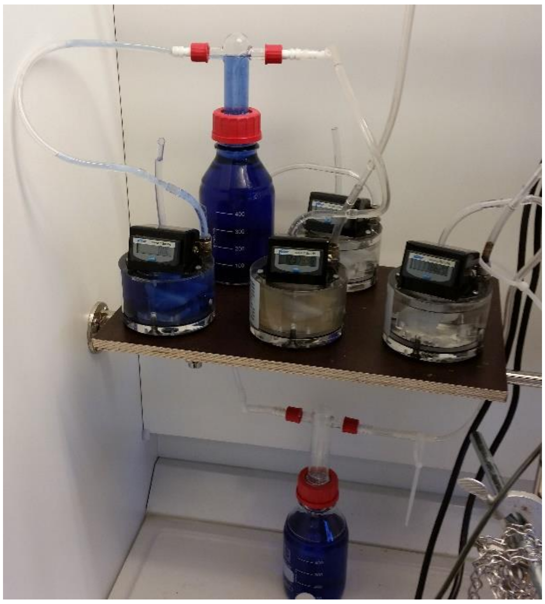
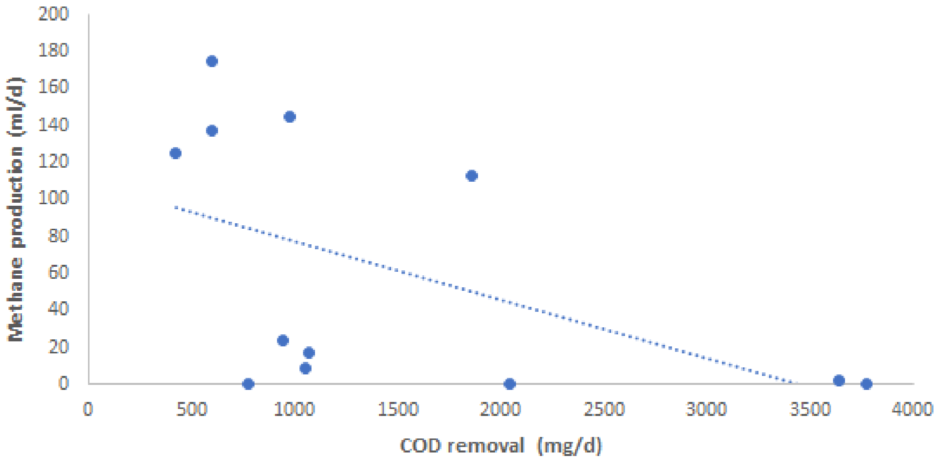


Figure 6.11 Gas meters filled with effluent water and NaOH solution.

On the other hand, Reactor II also showed COD balance of more than 100 % some days (Figure 6.10), but it was more stabilized and kept on running. From the Day 28 the methane production was too low around 10 % or no production at all, the reason for this decrease in methane production could be because the flow rate of Reactor II was increased by 20 % which caused a decrease in HRT which means there was not enough time for methanogenesis to occur at low HRT and at psychrophilic condition (10 °C). The other reason is that the gas meters stopped measuring gas properly leading to analytical error. Therefore, for the calculation of COD balance %, COD removal % and OLR, the methane production values were assumed to be 65 % of methane present in biogas.

Figure 6.12a shows the relation between COD removal (mg/d) and methane production rate (ml/d) in Reactor I and Reactor II. The increase in COD removal results in the increase in methane production, but in Reactor I the opposite trend is seen which is explained by the wrong gas measurements by the gas meters resulting in a huge gap in COD recovery. While, Figure 6.12b shows the same relation between COD removal and methane production with real data for both Reactor I and Reactor II (estimated values not included) which shows an expected trend i.e. increase methane production rate with the increase in COD removal. This indicates that the deviation in results was due to wrong gas measurements by gas meters resulting in COD balance % different from 100 %.



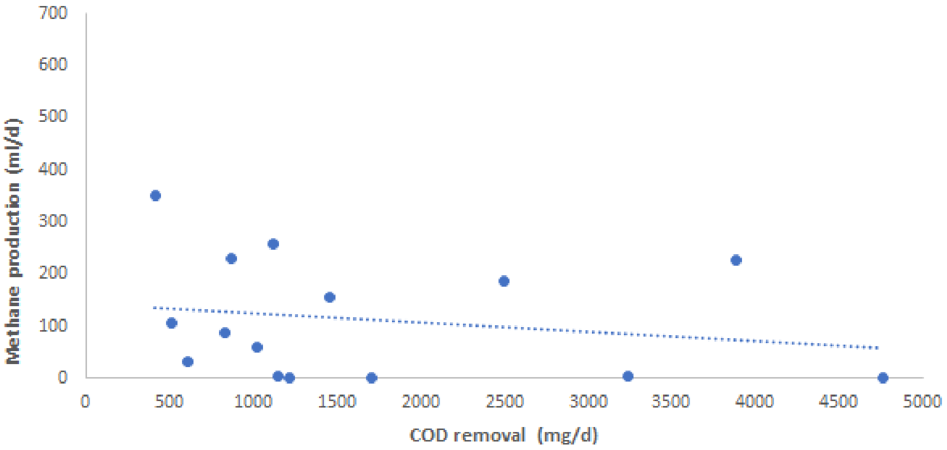


Figure 6.12a Relation between COD removal and methane production in Reactor I (above) and Reactor II (below) including all data.

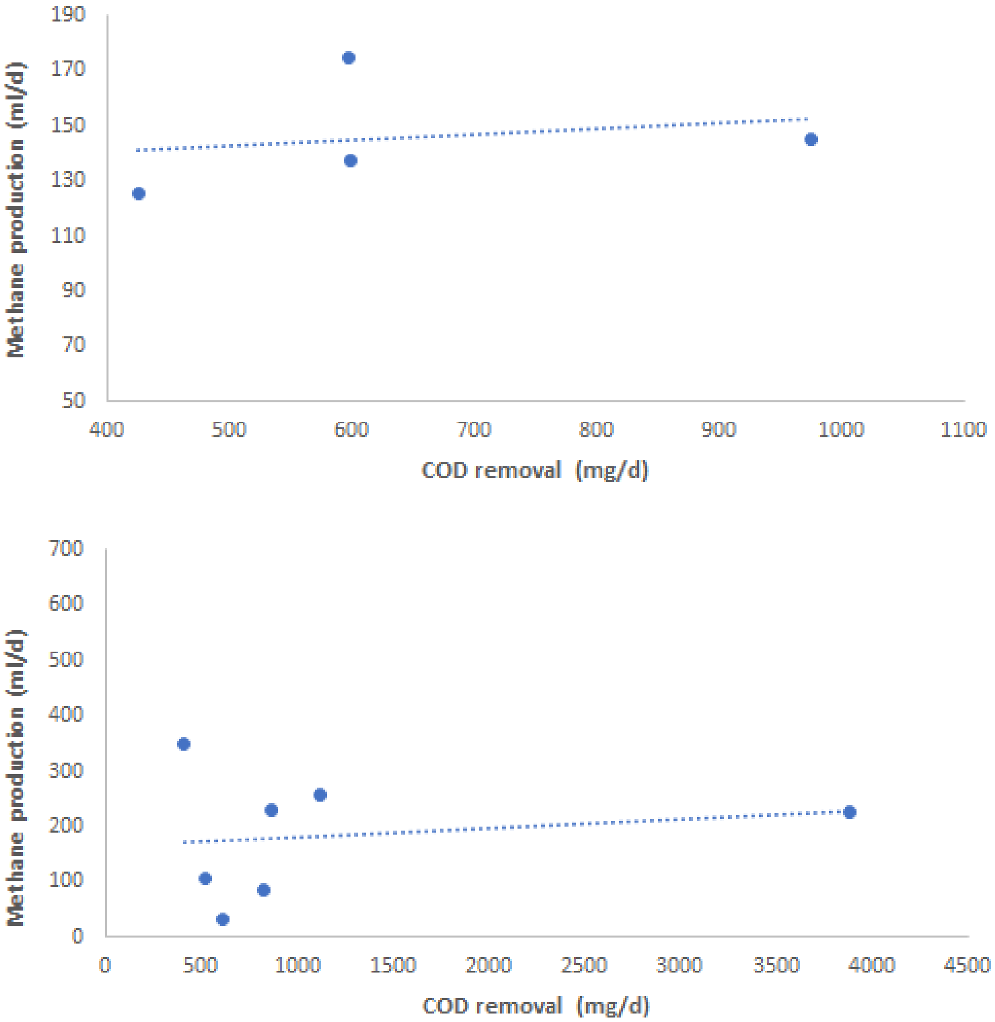


Figure 6.12b Relation between COD removal and methane production in Reactor I (above) and Reactor II (below), with real data.

Total suspended solids (TSS) were calculated based on the total and dissolved COD (influent), shown in Figure 6.13.

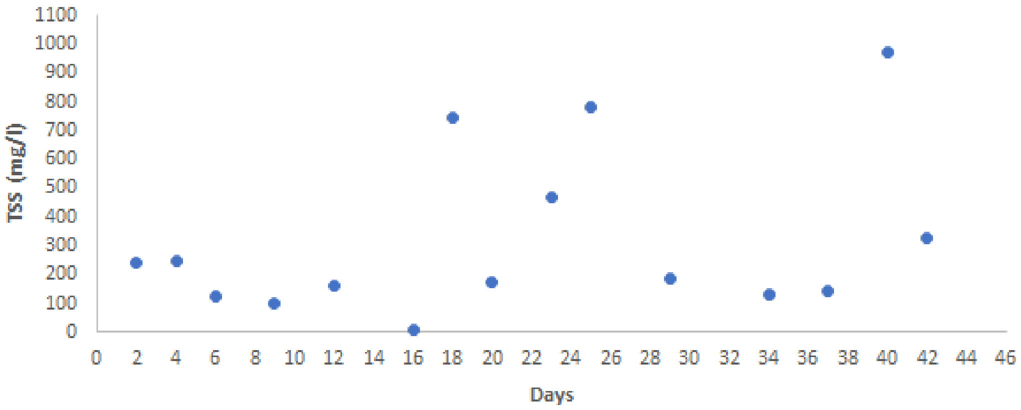


Figure 6.13 Graph showing total suspended solids (TSS) influent.

On the Day 16 the TSS concentration in feed wastewater was low i.e. 6 mg/l, because the wastewater was diluted due to rainy days and it was reddish in color probably because Grødaland WWTP receives wastewater from slaughterhouse industry which means it contained a large amount of blood and nitrogen in form of NH_3/NH_4^+. While on Day 18 the TSS concentration increased from 6 mg/l to 745 mg/l and the feed wastewater was much concentrated in comparison to the previous days as it was also seen from COD measurements. The effluent TSS was not calculated but it was assumed from the color of effluent samples from both reactors as they were very clear and were assumed to contain low amount of TSS which shows that the granular sludge in UASB reactors has good settling properties. Figure 6.14 shows the conductivity measurements of influent and effluent. It shows that conductivity of effluent samples was higher than of influent feed. The increase in conductivity could be due to the release of ions during the degradation of organic matter.

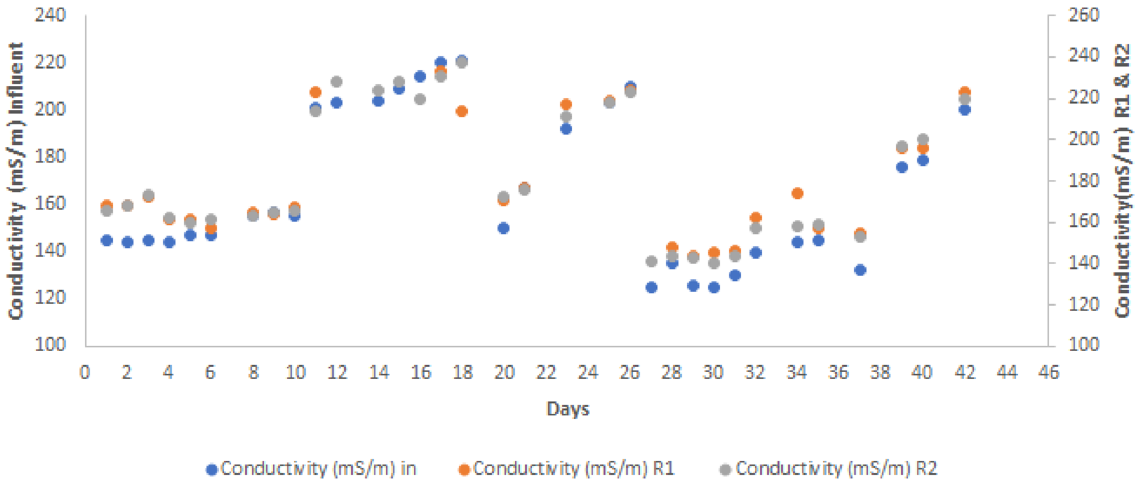


Figure 6.14 Conductivity measurements of Reactor I & II and influent feed.

6.5 Experimental Period

The experimental study period was from 16th Jan 2017 to 22nd Mar 2017. During this period, there were days when no measurements were taken because the effluent pipes, tubes, degasser and gas meters were washed and cleaned as they were clogged with the sludge particles so the whole system was turned-off during cleaning and was started agin. And also, due to lack of wastewater sample sometimes and waiting for the system to stabilize after cleaning and after some variation such as change in flow rate etc.

7. Discussion

The discussion chapter is divided into two sub-chapters: (1) the UASB reactors performance based on COD removal efficiency, COD balance and methane production rate and; (2) the effect of environmental variations such as flow rate, HRT, temperature, pH, alkalinity and VFA variability on UASB reactors performance.

7.1 UASB Reactors Performance

In this sub-chapter, the reactors performance is discussed based on the two parameters, COD removal efficiency and methane production rate under psychrophilic condition (10 °C). These two parameters are of great importance in investigating the effectiveness of anaerobic treatment of industrial wastewater.

7.1.1 COD Removal Efficiency

Figure 6.1 and 6.2 shows the COD removal efficiency in a function of flow rate (ml/min) and OLR (g/l.d). In average 56 % of COD removal was achieved in both reactors, Reactor I and Reactor II which is in accordance to theory (see Table 2.3). The COD removal efficiency of both UASB reactors (Reactor I & II) increased with the increase in flow rate and same trend was seen against OLR as the COD removal efficiency increased with the increasing OLR during operation period. The increase in flow rate resulted in decrease in the HRT and it probably caused the agitation and mixing in the system which increased the contact between wastewater and biomass and so increased the COD removal. The increase in OLR increased the substrate concentrations in the reactors which were taken up by the microorganisms (acetogens, methanogens) and converted to biogas and in turn caused an increased in COD removal. The graphs represent that COD removal efficiency in Reactor I and Reactor II first increased with the increase in flow rate but after some days it started to decrease because of the decrease in HRT which decreased the contact time between substrate and biomass.

The same trend was seen for OLR as the methanogenesis activity is slower under psychrophilic temperatures because the maximum specific growth rate of methanogens is 0.03 1/d at psychrophilic temperatures (see Figure 2.4), so they are more prone to environmental variations than the other anaerobic reactions. The increase in OLR results in high loading on reactor which caused the increase in COD removal. In Reactor I, the increase in OLR caused an increase in

COD removal in the start but decreased over time at higher OLR. The OLR was variable throughout the experimental period and a sudden increase or decrease in OLR caused accumulation of VFA in both reactors and overall the accumulation of VFA caused a decrease in COD removal efficiency. The accumulation of VFA shows that the acidogenesis is less affected by the higher OLR and low temperature than methanogenesis. This was seen in Reactor II where the increase in flow rate caused the accumulation of VFA but the amount of accumulated VFA decreased over time. The OLR was also calculated using only the volume of sludge blanket which is 30 – 40 % of the total volume (1L) of the reactors. The sludge blanket volume is where the actual anaerobic activity takes place. Figure 6.3 shows the COD removal % against OLR considering sludge blanket volume and it shows that with the increase in OLR the COD removal efficiency increased in both Reactor I and Reactor II. The COD removal % can be related to a study done at different operational temperatures, that shows a better COD removal efficiency can be achieved by UASB reactor at psychrophilic temperature even when the methane production is low [38]. This happened due to the accumulation of suspended solids (SS) in the sludge (reactor), resulted in better COD removal.

7.1.2 COD Balance

COD is an important parameter in anaerobic digestion process because it gives vital information about the functioning of the system. The experimental data of this study shows that in average 68 % of influent COD was recovered in the effluent and biogas production in Reactor I and Reactor II. COD balance % was calculated using Equation 8, where Q is flow rate and COD_{gas} is COD of methane gas produced. The particulate fraction of influent COD consists of the biodegradable substrates and a small amount of inert COD. While, the particulate COD in the effluent is mainly the biomass lost from the reactor.

$$COD_{in} \times Q = COD_{out} \times Q + COD_{gas} \tag{8}$$

The COD of biomass growth was excluded in calculations for COD balance % because it was not determined but the proportion of influent (particulate) COD converted to biomass was assumed to be 10 %. By assuming, 10 % biomass COD the COD recovery in both Reactor I and Reactor II would increase to 78 % at psychrophilic temperature (10 °C).

Figure 6.7 and 6.8 shows the profiles of COD balance % against the flow rate (ml/min). It shows that the increase in flow rate caused a decrease in COD balance % (COD recovery). On Day 18, the OLR in Reactor II suddenly increased which decreased the COD recovery. The flow rate in Reactor II was increased purposely of about 20 % to investigate its effect on anaerobic process. The increase in flow rate decreased the HRT which first increased the COD recovery but decreased afterwards because the contact between biomass and wastewater increased for a while but when the system was stabilized it decreased the COD recovery as well as methane production rate. Figure 6.9 and 6.10 shows the COD balance % profiles against the OLR. It represents that as the OLR was increased it caused a decrease in COD recovery. The reason for the decrease in COD recovery is due to the fact that the methanogenesis activity is slower at psychrophilic temperatures while acidogenesis is favourable at 10 °C. Therefore, the VFA accumulation occurred when OLR was increased and the OLR was variable throughout the experimental period.

As 78 % of COD is recovered in Reactor I and Reactor II, of which 56 % COD is removed, 10 % COD is assumed to be converted to biomass growth and the rest 12 % is assumed to be accumulated in the sludge due to capture of TSS which is not fully degraded at this low temperature. This kind of observation was made in a study done with UASB reactor at 15 °C [37]. The gap in COD recovery in both the reactors is due to low methane production rates and the wrong measurements of biogas and methane gas by gas meters. The VFA accumulation in UASB reactor is not considered a problem if the VFA to alkalinity ratio is less than 0.3 mg/mg and it was less than 0.3 mg/mg throughout the experimental period in this study. The wastewater from Grødaland WWTP used for this study was from dairy and animal slaughtering industry, consisting of high fat containing substrates and according to theory the fat or LCFA-containing substrates results in very high COD removal efficiencies but low methane production rates which results in a huge gap in COD recovery [29]. One reason for this gap in the COD recovery which is quite often is mostly caused by the loss of electrons when they are channelled to oxidized anions like SO_4^{2-} and NO_3^-. Therefore, to remove COD recovery gap either all reduced gases should be taken into account or the concentration of electron acceptors needs to be measured [3]. Another reason for this gap is explained by the entrapment or accumulation of COD in the sludge blanket [29], the analytical measurement errors and that the gas meters, not measuring the gas properly. The bubble formation, assumed to be the gas bubbles, were observed in both the reactors during analysis period but gas meters did not measure gas properly which results in equipment and analysis error in COD recovery calculations.

7.1.3 Methane Production

Figure 6.5 presents the methane production rate against the OLR. It shows that the increase in OLR caused a decrease in the methane production rate in both UASB reactors even when the COD removal efficiency was increased. In Reactor I, the maximum methane production of 0.21 l CH_4/l.d, which is 0.37 l CH_4/g COD was obtained at OLR of 1.0 g/l.d while in Reactor II, the maximum methane production obtained was 0.23 l CH_4/l.d which is 0.29 l CH_4/g COD at OLR of 1.8 g/l.d. These two methane production values are in accordance to the theoretical value of 0.35 l CH_4/g COD at 10 °C [30]. According to theory, the increase in OLR increase the methane production rate because of the increase in substrates and biomass but the results obtained in this study contrasted with theory. This is explained by the fact that the particles surround the active biomass which can lead to substrate transfer limitations [31].

Another reason is the analysis/measurement errors and the unexpected gas leakage as the liquid level in the degasser was supposed to be low but sometimes the water level was too high therefor, some pressure adjustments needed to be done by changing the positions of effluent pipes. The gas meters did not read the gas values accurately because of the instabilities in equipment resulting in pressure adjustment problems and there might be accumulation of gas in the sludge bed which came out as a flush due to which no methane production was observed some days. There was sludge washout, in very small amounts during operational period causing methane loss. The Reactor I was stopped for a while due to the effluent and NaOH solution went into gas meters (explained in Results section) which destroyed the gas meters so the methane gas values were not directly measured by the gas meters, therefore, from Day 24 the biogas and methane gas values were assumed for the purpose of calculations. From Day 28 the methane gas values were assumed (65 % methane) in Reactor II as well because the methane gas measured by the gas meter was too low. The Results are based on the estimated values of methane gas such as from 0.002 – 0.11 l/l.d methane in Reactor I and from 0.003 – 0.10 l/l.d methane in Reactor II, not the real values. So, the lower methane production values are associated with gas leakage and equipment/gas meters error. In reality, from the real data obtained it is assumed that UASB reactor, if operated with proper gas measuring device/meters, is an attractive option to treat wastewater anaerobically at psychrophilic temperature producing, economically useful, methane gas around 0.37 l CH_4/g COD at laboratory scale.

So, the lower methane production values (<0.1 l/l.d) in Figure 6.5 and 6.6 are estimated values not the real measured values and these values do not mean the system was stopped operating due to no methane production but it was analytical/ equipment error for this deviation and another reason could be the slow methanogenesis activity at 10 °C. In fact, the UASB reactors were functioning well as from the COD removal % and COD recovery data obtained in this study it was observed that the UASB system has the potential to recover quickly from the environmental variation such as a sudden change in OLR and flow rate and in turn HRT because when the flow rate changes the HRT also changes (Eq. 9).

$$HRT = \frac{Volume\ of\ reactor\ [l]}{Flow\ rate\ [\frac{l}{h}]} \qquad (9)$$

The methane fraction was also calculated in both the UASB reactors to investigate the biogas quality. In Reactor I, before turning off the reactor, the methane fraction % in the biogas was calculated to be more than 90 % and this is accepted as the same amount of methane fraction was obtained last year with the same UASB reactor [22]. From Day 24, the methane fraction was estimated to be 65 % in biogas. In Reactor II the obtained methane fraction was more than 100 % which is not possible in practice. From Day 20, the methane fraction % in Reactor II started to decrease which might be due to decrease in HRT and increase in OLR. From Day 28, the methane gas meter was not measuring gas which might be due to the low methane production rate or the pressure instability in the flask containing CO_2-absorber (3M NaOH) solution, so it was estimated to be 65 % in biogas.

The unexpected huge fraction of methane in biogas in Reactor II is explained: firstly, that the gas meters were not functioning/measuring gas accurately (analytical error) and secondly the higher solubility of CO_2 in water at 10 °C as the gases are more soluble in water at lower temperatures. It is possible that CO_2-absorber (NaOH solution) absorb CO_2 due to inadequate back pressure or equipment uncertainty (Figure 6.12a & Figure 6.12b). The occurrence of particulate matter in the wastewater can lead to biogas of poor quality, with the low amount of recoverable methane gas [32]. It is possible that some part of the influent COD might be non-biodegradable which resulted in low methane production rates [34].

Figure 6.6 shows the methane production rate against HRT. It explains that with the increase in HRT the methane production rate also increased. The increase in flow rate in Reactor II decreased the HRT and in turn the methane production decreased because the contact time of wastewater with the granules was decreased as well. The methanogenesis is slower at psychrophilic temperature so it requires more time for the methanogens to take up fatty acids and convert them into methane gas. There was probably not enough agitation in the system due to the low amount of methane produced.

7.2 pH, Alkalinity and VFA variability

The other environmental factors affecting the performance of UASB reactors were also measured such as pH, alkalinity and VFAs. The pH range for the proper functioning of anaerobic reactor system is between 6.6 and 7.4. In this experimental study, the pH for Rector I was between 7.2 – 7.8 while in Reactor II it was between 7.2 – 7.6, so the pH values were within the range throughout the experimental period. There was sometimes the accumulation of VFA inside both reactors as the maximum VFA measured in Reactor I was 172.5 mg/l acetic acid while in Reactor II it was 168.4 mg/l, with the influent VFA in Reactor I and Reactor II of 237.8 mg/l acetic acid and 226.5 mg/l acetic acid respectively. With the increase in flow rate in Reactor II the accumulation of VFA was more consistent. Despite the VFA accumulation, the pH in the system did not drop because the alkalinity was high to buffer the acids and the VFA to alkalinity ratio was less than 0.3 mg/mg throughout the experimental period in this study. The lower methane production at increasing flow rate could be associated with the VFA accumulation. It shows that the acidogens can tolerate psychrophilic temperature (10 °C) but methanogens are most affected by this change in temperature. There was no need to add buffer to the reactors for pH control because the influent wastewater had high enough alkalinity of 423.1 mg $CaCO_3$/l in average and also the alkalinity was provided to the system by recirculation and by the carbonate system i.e. the CO_2 reacted with water to form carbonic acid, H_2CO_3, which is a diprotic acid and dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) [33].

The nutrient availability is also important for the anaerobic process. A reddish colour and reddish filamentous precipitates were observed in both UASB reactors which might be due to the ferric ion (Fe^{3+}) present in the influent feed as the Grødaland wastewater is from the animal slaughterhouse industry containing high concentration of iron (Fe) present in blood. If the influent wastewater contains sulphate (SO_4^{2-}) it is converted to toxic sulphide under anaerobic conditions. Sulphide is considered much more toxic to methanogenic bacteria than sulphate [35]. Inhibition by sulphide is caused by the undissociated acids (H_2S), so pH has a strong influence on how much sulphide concentration is allowed in anaerobic reactors [35]. However, reactors with high sludge retention times such as UASB and anaerobic filters are relatively insensitive to inhibition by the sulphide [36]. In these systems, an attractive loading rate can be achieved even with the methanogenic activity of the sludge are low. A COD removal efficiency of 60 – 80 % can still be reached under these conditions. A pH range of 7.5 – 8.0 is the primary strategy to control the inhibition by sulphide [35]. The toxicity of sulphide is related to the concentration of free hydrogen sulphide which means that the low pH (<6.5) increases the toxicity. The presence of iron reduces the sulphide toxicity due to precipitation of ferrous-sulphide [34].

8. Conclusion

The performance of two laboratory scale UASB reactors (Reactor I and Reactor II) was investigated by their efficiency of removing COD and producing methane gas at psychrophilic temperature of 10 °C. Reactor I and Reactor II were proved to be efficient and attractive to treat industrial wastewater from Grødaland WWTP. In this study, 30 – 80 % and 30 – 79 % of COD removal efficiency was achieved in Reactor I and Reactor II respectively. The highest COD removal achieved was 80 % in Reactor I at OLR of 1.8 g/l.d and HRT of 8 hour while, 79 % in Reactor II at OLR of 2.6 g/l.d and HRT of 8 hour. On Day 9, the COD removal in Reactor I was lowest of 33 % at HRT of 11 hour after which the Reactor I stopped working because the effluent wastewater went into the gas meters through degasser and pipes probably due to inadequate pressure. It was started again and 77 % of COD removal was achieved after some days of operation. The COD removed was converted into methane (biogas) and according to results obtained the biogas produced during anaerobic treatment consisted of 65 – 90 % of CH_4 and 10 – 35 % of CO_2. The methane fraction in Reactor II was more than 100 % some days which is due to the inaccuracy of gas meters or due to high solubility of CO_2 in water. In Reactor I, the maximum methane production obtained was 0.21 l CH_4/l.d, which is 0.37 l CH_4/g COD at OLR of 1.0 g/l.d while in Reactor II, the maximum methane production obtained was of 0.23 l CH_4/l.d which is 0.29 l CH_4/g COD at OLR of 1.8 g/l.d. The average COD recovery achieved in both the reactors was 78 % with average COD removal efficiency of 56 % at 10 °C. The pH in both reactors was within the range throughout the experimental period i.e. 7.2 – 7.8 and 7.2 – 7.6 in Reactor I and Reactor II respectively. Some VFA accumulation was observed in both reactors but it did not cause any disturbance in rector performance as the alkalinity concentration was high enough to buffer the acids and maintained pH within the range for anaerobic processes.

Anaerobic treatment of wastewater under psychrophilic conditions is an important research because mostly the effluents of municipal wastewater treatment are discharged at ambient or low temperature (< 20 °C) in temperate climate regions. From the results obtained in this study it is concluded that the UASB reactor technology of treating municipal and industrial wastewater in cold climate like Norway is an attractive and realistic alternative treatment method at psychrophilic conditions at 10 °C. For IVAR Grødaland WWTP using UASB treatment to remove dissolved COD prior to SBR could achieve at least 50 % of COD removal and thus save energy for aeration in the SBR and recover energy as biogas (methane).

9. Recommendations

The results show that the UASB reactor technology to treat the industrial and municipal wastewater is attractive and a good alternative to the aerobic treatment. All the experiments were done at psychrophilic temperature (10 °C), and the results indicate that in average 56 % of COD removal efficiency was achieved by both Reactor I and Reactor II. Towards the end of experimental period the methane production in both reactors was too low or even close to zero despite high COD removal efficiency. This was assumed to be due to wrong gas measurements by the gas meters or gas leakage and an increase in flow rate. In future, it will be interesting to carry out more measurements at higher flow rate, shorter HRT and with accurate gas meters to see if it was an analytical error or the methanogenesis activity is slower and get disturbed by the sudden change in environment.

To get economically valuable product of anaerobic digestion i.e. methane gas at 10 °C it is recommended to use low OLR, higher HRT and higher SRT because at low temperature it needs more time for system stabilization from environmental variations. For the laboratory scale UASB reactor the average HRT of 10 hr and average OLR of 1.2 g/l.d is required for getting methane production rate around 0.20 l CH_4/ g COD. The higher HRT means the low flow rate around 1.6 ml/min is required for the UASB system to perform efficiently at 10 °C. But low flow rate and high HRT will not give enough mixing and contact between wastewater and biomass so it will be a good idea to improve or increase the effluent recirculation that could be done by re-design UASB reactor with more feed inlets which will help to get better distribution of water. To increase the upflow velocity could be a better idea to increase contact between biomass and wastewater and this could be done by increasing the height of the reactor.

To get a better picture of UASB performance at psychrophilic temperature it is recommended to continue taking the measurements for a longer period because methanogenesis is more prone to the variations in anaerobic system so the methanogens might require longer time to convert accumulated VFA to methane gas because of low temperature. The sludge washout with the effluent would also be a good idea to do on daily basis or more often for keeping the system running properly because inert particulates sometimes attach to the biomass and limits its availability for microorganisms.

The new pilot-scale UASB plant is in operation at Grødaland WWTP to treat effluent from DAF unit and to remove dissolved COD prior to SBR. By applying the same operational conditions what were applied at the laboratory scale UASB reactor it will be interesting to investigate if same amount of COD removal efficiency and methane production is achieved at pilot-scale plant at psychrophilic temperature.

10. References

[1] Tchobanoglous, G., Burton, F. L., Stensel, H. D., Metcalf, & Eddy. (2003). Wastewater engineering: treatment and reuse (4th ed. revised by George Tchobanoglous, Franklin L. Burton, H. David Stensel. ed.). Boston: McGraw-Hill

[2] Biological Wastewater Treatment by Arun Mittal, Water Today – 2011.

[3] van Lier, J. B., Mahmoud, N., and Zeeman, G., 2008, Anaerobic Wastewater Treatment.

[4] Carlos Augusto de Lemos Chernicharo, *Anaerobic Reactors*, 2007, ISBN: 1 84339 164 3. Published by IWA Publishing, London, UK.

[5] Speece, R.E., and McCarty, P.L., Nutrient requirements and biological solids accumulation in anaerobic digestion. 1964, Adv. Water Pollut. Res., vol. 2, Pergamon Press, London, p. 305.

[6] Chih-Ju G.Jou*, Guo-Chiang Huang, “A pilot study for oil refinery wastewater treatment using a fixed-film bioreactor”. 2003, Advances in Environmental Research 7, p. 463 – 469.

[7] de Man, A., Grin, P., Roersma, R., Grolle, K., & Lettinga, G., Anaerobic treatment of municipal wastewater at low temperatures 1986.

[8] Seghezzo, L., Zeeman, G., van Lier, J. B., Hamelers, H. V. M., & Lettinga, G. A review: The anaerobic treatment of sewage in UASB and EGSB reactors. 1998, Bioresource Technology, 65(3), 175-190. doi:10.1016/s0960-8524(98)00046-7.

[9] Seung J. Lim, Comparisons Between the UASB and the EGSB Reactor.

[10] Lettinga, G., Rebac, S., and Zeeman, G., “Challenge of psychrophilic anaerobic wastewater treatment”. September 2001, TRENDS in Biotechnology Vol.19 No.9.

[11] Zeeman, G., and Lettinga, G., The role of anaerobic digestion of domestic sewage in closing the water and nutrient cycle at community level. 1999, Water Sci. Technol. 39, p. 187–194

[12] Hills, D.J. and Nakano, K., “Effect of particle size on anaerobic digestion of tomato solid waste”. 1984, Agric. Wastes 10, p. 285–295.

[13] Henze, M., van Loosdrecht, Mark CM., Ekama, George A., Brdjanovic, D., Biological wastewater treatment: principles, modelling and design. 2008, London: IWA Publ.

[14] Metcalf & Eddy, Wastewater Engineering, Treatment, Disposal, Reuse. McGraw-Hill Book Company, New York, USA, Inc., 1991, p. 1334.

[15] Leitao, R. C., van Haandel, A. C., Zeeman, G., Lettinga, G., “The effect of operational and environmental variations on anaerobic wastewater systems: A review”, 2006, Bioresource Technology 97 p. 1105 – 1118.

[16] Speece, R. E., Environmental requirements for anaerobic digestion of biomass. 1983, *In Advances in Solar Energy – An Annual Review of Research and Development.*

[17] Lettinga, G., Hulshoff, Pol, L.W., and Zeeman, G., "Biological wastewater treatment" Part 1: Anaerobic Wastewater Treatment. Lecture Notes. Wageningen Agricultural University, edn January 1996.

[18] Cohen, A., Breure, A.M., van Andel, J.G., van Deursen, A., Influence of phase separation on the anaerobic digestion of glucose – II: stability and kinetic response to shock loadings, 1982, Water Research 16, p. 449 – 455,

[19] van Lier, J.B., Tilche, A., Ahring, B.K., Macarie, H., Moletta, R., Dohanyos, M., Hulshoff Pol, L.W., Lens, P., Verstraete, W., "New perspectives in anaerobic digestion" Water Science and Technology 43 (1), p. 1 – 18, 2001.

[20] Leito, R.C., Robustness of UASB reactor treating sewage under tropical conditions, Ph.D. Thesis, Wageningen University 2004.

[21] McHugh, S., O'reilly, C., Mahony, T., Colleran, E., O'flaherty, V., Anaerobic granular sludge bioreactor technology. Reviews in Environmental Science and Biotechnology, 2003. 2(2-4): p. 225-245.

[22] Master Thesis report by Anissa Sukma Safitri, University of Stavanger.

[23] A document about Description from Grødaland Wastewater Treatment Plant by Leif Ydstebø, 2017.

[24] Lopez-Lopez, A., Leon-Becerril, E., Rosales-Contreras, M. E., Villegas-Garcia, E., Influence of alkalinity and VFAs on the performance of an UASB reactor with recirculation for the treatment of Tequila vinasses. Environmental Technology, 2015. 36(19): p. 2468-2476.

[25] Hashimoto, A. G., Methane production and effluent quality from fermentation beef cattle manure and molasses. Biotechnology and Bioengineering Symp., #11, p. 481- 492, 1981.

[26] Baker-Blocker, A., Donahue, T. M., and Mancy, K. H., Methane flux from wetlands areas. 1977, Tellus, 29, p. 245-50.

[27] Chen, Y. R., Varel, V. H., and Hashimoto, A. G., Effect of Temperature on Methane Fermentation kinetics of beef-cattle manure. 1980, Biotechnology and Bioengineering Symp. # 10, p. 325-339.

[28] Yi-Chun Lin, "Maximum Specific Growth Rates of Mixed Anaerobic Populations at Psychrophilic Temperatures", Oregon State University, August 1995.

[29] Henze, M., van Loosdrecht, Mark CM., Ekama, George A., Brdjanovic, D., Biological wastewater treatment: principles, modelling and design. 2008, London: IWA Publ.

[30] Grady, C.P.L., Daigger, G.T., Love, N.G., Filipe, C.D.M., Biological wastewater treatment. 3rd ed. ed. 2011, London: IWA Publishing.

[31] S. E. Aiyuk and W. Verstraete, Sedimentological evolution in an UASB treating SYNTHES, a new representative synthetic sewage, at low loading rates. 2004, Biores. Technol. Vol. 93, pp. 269-278.

[32] Lettinga, G., A. W. A. de Man, A. R. M. Van der Last, W. Wiegant, K. Knippenberg, J. Frijns and J.C.L. Van Buuren, Anaerobic treatment of domestic sewage and wastewater. 1993, Water Sci. Technol. Vol. 27, pp. 67-73.

[33] Brezonik, P., Arnold, W., Water Chemistry: An Introduction to the Chemistry of Natural and Engineered Aquatic Systems. Water Chemistry. 2011, Oxford: Oxford University Press, USA.

[34] Henze M., P. Harremoes, “Anaerobic Treatment of Wastewater in Fixed Film Reactors – A Literature Review”, Wat. Sci. Tech. Vol. 15, Copenhagen, IAWPRC/Pergamon Press Ltd. 1983.

[35] Lettinga, G., Hulshoff POL, LW., Koster, I. W., Wiegant, W. M., De Zeeuw, W. J., Rinzema, A., Grin, P. C., Roersma, R. E., Hobma, S. W., “High Rate Anaerobic Wastewater Treatment Using the UASB Reactor under a Wide Range of Temperature Conditions” Department of Water Pollution Control, Agriculture University, The Netherlands.

[36] Parkin, G. F., Speece, R. E., Attached versus suspended growth anaerobic reactors: response to toxic substances. 1983, Water Science and Technology 25 (8/9), p. 261 – 290.

[37] Akila, G., Chandra, T. S., “Performance of an UASB reactor treating synthetic wastewater at low-temperature using cold-adapted seed slurry”. 2007, Process Biochemistry 42 p. 466 – 471.

[38] Lew, B., Tarre, S., Belavski, M., and Green, M., UASB reactor for domestic wastewater treatment at low temperature: a comparison between a classical UASB and a hybrid UASB-filter reactor, Water Science and Technology, Vol. 49 NO 11 – 12, p. 295 – 301, IWA Publishing 2004.

11. Appendixes

Appendix 1: Daily measurements of Influent Wastewater

Appendix 2: Daily measurements of Reactor I

Appendix 3: Daily measurements of Reactor II

11.1 Appendix 1

Influent wastewater (feed)

Table A1 shows daily measurements and calculated data for influent wastewater samples.

Table A1. Daily measurements of influent wastewater.

Day	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l $CaCO_3$)	VFA (mg/l acetic acid)	TCOD (mg/l)	dCOD (mg/l)	TSS (mg/l)
1	145	7.47	481.5	93.3			
2	144	7.64	420.3	37.9	784	540	244
3	145	8.14	275.7	138.8			
4	144	7.75	505.8	93.4	652	406	246
5	147	7.49	373.7	65.0			
6	147	7.51	392.4	168.3	561	435	126
7							
8	155	7.54	426.3	89.7			
9	157	7.52	538.2	122.2	599	498	101
10	155	7.50	538.2	66.2			
11	201	7.43	547.9	164.9			
12	203	7.38	621.1	169.7	744	580	164
13							
14	204	7.21	444.3	260.3			
15	209	7.57	582.9	246.0			
16	214	7.34	562.4	226.5	791	785	6
17	220	7.33	532.5	309.8			
18	221	7.45	587.4	302.4	1553	808	745
19							
20	150	7.28	391.1	197.7	609	434	175
21	167	7.38	457.6	40.5			
22							
23	192	7.23	406.6	225.7	1154	686	468
24							
25	203	7.16	433.1	324.7	1483	704	779
26	210	7.25	398.4	237.8			
27	125	7.08	254.1	230.9			
28	135	7.15	277.9	322.2			
29	126	7.20	315.7	221.7	706	519	187
30	125	7.27	270.8	198.9			
31	130	7.38	244.4	267.5			
32	140	7.82	357.5	97.6			
33							
34	144	7.26	370.7	45.8	516	386	130
35	145	7.32	382.1	103.2			

Day	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l $CaCO_3$)	VFA (mg/l acetic acid)	TCOD (mg/l)	dCOD (mg/l)	TSS (mg/l)
36							
37	132	7.24	367.8	136.2	560	418	142
38							
39	176	7.16	321.6	146.5			
40	179	7.20	431.6	190.2	1589	615	974
41							
42	200	7.59	451.0	192.8	1048	718	330

11.2 Appendix 2

Table A2 shows daily measurements and calculated data for Reactor I.

Table A2. Daily measurements of Reactor I.

Day	Flowrate (ml/min)	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l CaCO3)	VFA (mg/l acetic acid)	COD (mg/l)	COD removal (%)	Methane rate (l/l.d)	Methane fraction (%)	HRT (h)	OLR (g/l.d)	OLR (g/l.d) with 40% reactor volume	COD balance (%)
1	1,6	168	7,44	550,5	0				82	10			
2	1,6	168	7,6	460,9	34,6	361	54	0,15	95	10	1,2	3,1	71
3	1,6	172	7,55	441,6	33,8			0,15	91	10			
4	1,6	161	7,22	572,3	109,4	393	40	0,17	92	10	0,9	2,3	95
5	1,4	161	7,33	478,6	60,7			0,16	92	13			
6	1,6	157	7,22	536,3	0	301	46	0,21	92	10	1,0	2,5	145
7								0,16	92				
8	1,6	165	7,58	565,8	0			0,14	91	10			
9	1,5	164	7,53	629,3	0	402	33	0,13	92	11	1,1	2,7	94
10	1,5	167	7,45	545,4	47,9			0,15	91	11			
11 1)	1,5	223	7,49	734,5	61,6					11			
12 1)													
13 1)													
14 1)													
15 1)													
16 1)													
17 2)	2,3	233	7,44	764,8	0					7			
18 2)	2,2	214	7,83	568,6	0	360	77			8	2,6	6,4	

Day	Flowrate (ml/min)	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l CaCO3)	VFA (mg/l acetic acid)	COD (mg/l)	COD removal (%)	Methane rate (l/l.d)	Methane fraction (%)	HRT (h)	OLR (g/l.d)	OLR (g/l.d) with 40% reactor volume	COD balance (%)
19$^{2)}$													
20$^{2)}$	1,9	171	7,47	537,0	86,2	325	47			9	1,2	2,9	
21$^{2)}$	1,4	177	7,39	621,1	0					13			
22$^{2)}$													
23$^{2)}$	1,9	217	7,40	559,1	29,6	408	65			9	1,9	4,7	
24$^{3)}$								0,01	65				
25$^{3)}$	1,4	219	7,47	595,6	44,6	559	62	0,11	65	13	1,4	3,5	49
26$^{3)}$	1,4	224	7,42	600,0	172,5			0,06	65	13			
27$^{3)}$								0,06	65				
28$^{3)}$	1,4	148	7,30	527,4	0			0,02	65	13			
29$^{3)}$	1,7	144	7,36	403,4	0	268	62	0,02	65	10	1,3	3,2	42
30$^{3)}$	1,2	145	7,35	452,4	0			0,03	65	14			
31$^{3)}$	1,4	146	7,42	408,5	0			0,02	65	13			
32$^{3)}$	1,3	162	7,45	408,3	0			0,01	65	13			
33$^{3)}$								0,01	65				
34$^{3)}$	2,9	174	7,45	598,6	14,4	264	49	0,01	65	6	1,6	4,0	52
35$^{3)}$	1,9	157	7,40	455,0	0			0,002	65	9			
36$^{3)}$								0,02	65				
37$^{3)}$	1,9	155	7,46	572,5	18,1	214	62	0,02	65	9	1,1	2,9	52
38$^{3)}$								0,16	65				
39$^{3)}$	2,0	196	7,41	519,9	0			0,01	65	8			
40$^{3)}$	2,0	196	7,37	540,5	0	324	80	0,002	65	8	1,8	4,4	22
41$^{3)}$								0,00	65				

Day	**Flowrate (ml/min)**	**Conductivity (mS/m)**	**Initial pH**	**Alkalinity (mg/l CaCO3)**	**VFA (mg/l acetic acid)**	**COD (mg/l)**	**COD removal (%)**	**Methane rate (l/l.d)**	**Methane fraction (%)**	**HRT (h)**	**OLR (g/l.d)**	**OLR (g/l.d) with 40% reactor volume**	**COD balance (%)**
42 3)	2,9	223	7,74	696,7	0	532	49	0,03	65	6	2,9	7,5	53

1) Reactor I was turned-off due to effluent water went into gas meters.
2) Gas meters (measuring biogas and methane gas) were not working.
3) Estimated methane fraction % and methane production rate (l/l.d) values.

11.3 Appendix 3

Table A3 shows daily measurements and calculated data for Reactor II.

Table A2. Daily measurements of Reactor II.

Day	Flow Rate (ml/min)	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l CaCO3)	VFA (mg/l acetic acid)	COD (mg/l)	COD removal (%)	Methane rate (l/l.d)	Methane fraction (%)	HRT (h)	OLR (g/l.d)	OLR (g/l.d) with 40 % reactor volume	COD balance (%)
1	1,6	166	7,42	516,0	0			0,09	168	10			
2	1,6	168	7,83	489,9	30,6	424	46	0,02	97	10	1,2	3,1	69
3	1,6	173	7,61	475,1	30,5			0,03	65	10			
4	1,5	162	7,21	446,2	40,7	371	43	0,10	72	11	0,9	2,2	64
5	1,4	160	6,95	498,8	85,9			0,35	95	13			
6	1,6	161	7,23	570,0	0	382	32	0,07	100	10	1,0	2,5	138
7								0,10	86				
8	1,6	163	7,43	555,9	0			0,11	100	10			
9	1,2	165	7,57	651,4	0	300	50	0,12	99	14	0,9	2,2	80
10	1,5	166	7,64	564,1	0			0,64	99	11			
11	1,4	214	7,50	699,0	83,3			0,23	104	13			
12	1,6	228	7,53	728,2	0	367	51	0,22	102	10	1,3	3,3	88
13								0,20	106				
14	1,0	224	7,66	726,4	0			0,22	101	17			
15	1,6	228	7,50	769,2	0			0,39	105	10			
16	1,6	220	7,57	588,7	168,4	303	62	0,23	102	10	1,8	4,5	159
17	2,8	231	7,49	770,9	0			0,23	103	6			
18	2,2	237	7,64	591,1	0	327	79	0,18	102	8	2,6	6,4	34

Day	Flow Rate (ml/min)	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l CaCO3)	VFA (mg/l acetic acid)	COD (mg/l)	COD removal (%)	Methane rate (l/l.d)	Methane fraction (%)	HRT (h)	OLR (g/l.d)	OLR (g/l.d) with 40 % reactor volume	COD balance (%)
19								0,06	104				
20	2,2	172	7,42	530,5	86,2	287	53	0,01	55	8	1,4	3,4	56
21	2,2	176	7,48	545,4	0			0,02	6	8			
22								0,19	13				
23	2,2	211	7,45	569,3	0	366	68	0,06	63	8	2,2	5,4	46
24								0,003	63				
25	2,3	218	7,37	608,9	42,5	505	66	0,05	10	7	2,3	5,8	34
26	2,2	223	7,50	630,0	0			0,01	60	8			
27	3,2	141	7,41	478,8	61,0			0,01	22	5			
28 1)	2,8	144	7,39	477,9	16,4			0,02	65	6			
29 1)	3,0	143	7,46	428,3	50,6	310	56	0,01	65	6	2,2	5,6	65
30 1)	2,9	140	7,38	452,0	31,7			0,02	65	6			
31 1)	3,0	144	7,43	427,1	27,3			0,02	65	6			
32 1)	3,0	157	7,50	642,5	0			0,06	65				
33 1)								0,02	65				
34 1)	3,1	158	7,55	441,7	34,4	258	50	0,02	65	5	1,7	4,3	53
35 1)	3,1	159	7,69	479,4	0			0,02	65	5			
36 1)								0,02	65				
37 1)	3,0	153	7,53	585,8	0	223	60	0,07	65	6	1,8	4,5	48
38 1)								0,10	65				
39 1)	2,8	197	7,48	472,6	6,0			0,02	65	6			
40 1)	2,7	200	7,34	581,1	13,7	365	77	0,03	65	6	2,4	5,9	25
41 1)								0,03	65				

Day	Flow Rate (ml/min)	Conductivity (mS/m)	Initial pH	Alkalinity (mg/l CaCO3)	VFA (mg/l acetic acid)	COD (mg/l)	COD removal (%)	Methane rate (l/l.d)	Methane fraction (%)	HRT (h)	OLR (g/l.d)	OLR (g/l.d) with 40 % reactor volume	COD balance (%)
42[1)]	1,9	220	7,65	679,1	6,5	602	43	0,06	65	9	1,9	4,9	63

1) The methane fraction % and methane production rate (l/l.d) values are estimated not real measurements due to gas meters not working.