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THE OPERATIONAL CHALLENGES OF UASB REACTOR DIGESTING INACTIVE AND ACTIVE GRANULES: UTILIZATION OF THE SUCCESSFUL ROUTE

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Abstract

This study aims to investigate the challenges coupled with an anaerobic treatment of municipal wastewater using UASB reactor to convert organics into biogas. A laboratory scaled reactor is set up to treat synthetic wastewater with COD below 1200 mg/l under psychrophilic conditions. Reactor I is carried with inactive granules, while Reactor II is carried with fresh active ones. Hydraulic retention time (HRT) applied was 84 hours 31-17 hours for Reactors I and II, respectively.

The idea of running two experiments under the same conditions is to investigate the COD removal efficiency differences and methane production between both reactors. The removal efficiencies were very low in Reactor I (around 20%), while the accumulation of VFAs was very high as expected. In Reactor II, the removal efficiency reached 84% of the inlet COD, the desired value. The optimum biogas production was hard to determine due to a mechanical failure in the gas detector, but the bubbles flowing up from the inlet to the top of the Reactor were obvious. Nutrient removal was another drawback for both reactors where the values in the effluent were high. Some challenges led to having those values while running the Reactor, which are appropriately discussed in the discussion section.

For Reactor I, it was impossible to save the performance of the inactivated granules for optimizing a stable reactor with constant pressure and pH. We proposed to transform the aim of running a reactor of similar conditions to utilize VFAs production instead of biogas production. The VFAs have a significant market value and could be beneficial economically and environmentally.

A suggestion of pre-treatment and post-treatment techniques is also presented for Reactor II in order to have the optimal removal efficiency, and to stay within the margins before disposing the effluent.

Keywords: *anaerobic treatment, VFA, UASB reactor, COD removal efficiency, methane production.*

Acknowledgement

I want to start with a memorial tribute for my 23 years old cousin, Oussama Al Aryan, who has passed away three months ago after suffering from the COVID-19 virus for more than two months. I hope your soul rests in peace, angel.

Despite all the circumstances that is occurring due to this pandemic, I wouldn't have been able to fulfil my task without the help of:

First of all, a special thanks to my advisor, Assoc. Prof. GopalKrishnan Kumar, for my research's continuous support and guidance, and for his vast knowledge, despite the technical and personal challenges.

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Last but not least, tremendous respect for my Fiance and future wife, Nour Bakkar, my family, and my friends for their continuous support and presence in my life.

Stavanger, August 2021

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Abbreviations

AMB	Acetolactic Methanogenic Bacteria
AMD1	Anaerobic digestion Model design number 1
AP	Anaerobic Process
ATP	Adenosine Triphosphate
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
F:M	Food Mass Ratio
HAc	Acetic Acid
HMB	Hydrogenotrophic Methanogenic Bacteria
HRT	Hydraulic Retention Time
LCFA	Long Chain Fatty Acid
OLR	Organic Loading Rate
SRB	Sulphate Reducing Bacteria
STP	Standard Temperature and Pressure
TN	Total Nitrogen
TP	Total Phosphorous
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
WWTP	Wastewater Treatment Plant

1. Introduction

Wastewater originates from the initial water supply to the society after being consumed in different applications. It carries nutrients and toxins that characterize it as inadequate for further use before treatment. The accumulation of untreated wastewater will enhance the decomposition of organic matters, which creates inconvenient conditions, including the release of smelly gases. In addition, the wastewater carries various pathogens that inhabit the human respiratory system. It also carries nutrients, which can affect the growth of aquatic life, and may have compounds that might potentially become carcinogenic or already existing toxic compounds. For the mentioned reasons, wastewater must be adequately driven out of its source, which has to be treated, then either disposed of or reused, to protect the public health of the environment.

The evolution of the wastewater treatment industry is driven by the rise in environmental and health concerns, especially in urban areas. The increase in wastewater production caused by the population and lifestyle evolution urged the engineers to develop the techniques to keep the situation under control.

First, the treatment objectives were limited by removing floatable materials, treating organics, and eliminating pathogens. The obligatory degree of treatment has risen significantly during the last fifty years, which introduced further goals and objectives.

The wastewater sent to the treatment facility is collected from the following sources: (1) Domestic Wastewater (residential areas), (2) Stormwater (water runoffs and melted snow), (3) Industrial wastewater, and (4) Infiltration (water that gets into the collection system via direct and indirect means) [35].

The pressure exerted by the human species on the environment by generating more and more waste and the extraction of natural resources is globally recognized. The sustainable circular economy is being developed as a potential key for the efficient use of resources. It can be expressed as a technology-based concept that can increase the economic gains while releasing the pressure on the environment by considering the waste as resources for energy generation or reusing rather than discharging unethically [48].

Several technologies have been developed, including anaerobic treatment, aerobic treatment, and a combination of both conditions to reach a perfect CBE. Anaerobic treatment became the universal

most commonly used method since it preserves the resources and protects the environment. Originally, anaerobic treatment is used widely in the food industry. Then, it was utilized to deal with Potable water [31]. Its COD concentration measures the wastewater strength. Average municipal wastewater has a COD between 800 and 1200 mg COD/L. A high power (concentrated) is due to low infiltration and water consumption (condensed).

Around fifty years ago, Lettinga and his colleagues offered an efficient alternative for treating potable wastewater while working with an Up-flow anaerobic sludge blanket (UASB) reactor. It has been proven a feasible technique for pre-treating wastewater from different origins, such as industrial and urban areas that might carry toxic compounds.

In addition to the low cost of operation, and the simplicity of setting up the reactor that won't need nutrients and chemicals, the main goal of the UASB reactor is the biogas production and high removal of COD even at low temperatures. The biogas produced carries out a good amount of methane which can be an alternative for fossil fuels. It is essential to know that the effluent needs to be sent to other facilities to be treated since it will carry the rest of the pathogens and nutrients which were not removed [8].

A successful anaerobic treatment plant requires the retention of biomass in the mentioned system. It makes the usage of this process efficient economically and environmentally. A gradual increase in the installed Anaerobic treatment plants has been occurring in recent years.

Temperature is the main driving force for the efficiency of Anaerobic digestion. The optimal temperature for a mesophilic digester range between 35 and 37°C. The temperature of some wastewater fractions might be either warmer or cooler. In this case, the cost of treatment will be higher due to the addition of cooling or heating systems (both operational and cost of installation).

Many researchers have been testing the efficiency of anaerobic digestion under psychrophilic conditions (below 25°C), and positive results are starting to appear. Therefore, anaerobic digestion of wastewater under low temperatures can be feasible by using a granular sludge reactor system on a laboratory scale [43]. The best feature of this condition is that the retention of active biomass within the reactor by which the high organic removal can be achieved [25].

1.1. Scope of Work

This study is conducted without any cooperation with the operating company in the region. Due to the current situation regarding COVID 19 rules, it was hard to have a continuous supply of wastewater for more than 100 days, so we used synthetic sewage produced in the lab. The synthetic wastewater was made similar to the *wastewater in* the Grødaland treatment plant, by which the operating condition fits the local ones, using the following chemical in a 25L beaker [5,28,41]:

Table 1.1 synthetic wastewater composition

Ingredients	Concentration(mg/l)	Mass Added (Mg)
Peptone	17.4	435
Yeast Extract	52.2	1305
K_2HPO_4	250	6250
KH_2PO_4	100	2500
KCl	40	1000
$MgCl_2$	50	1250
$CoCl_2$	0.4	10
$FeCl_2$	3.56	89
$(NH_4)_4Mo_7O_{24}$	70	1750
$NiCl_2$	0.81	20.25
$ZnCl_2$	0.6	15
$CuCl_2$	0.3	7.5
EDTA	0.1	2.5
Starch	122	3050
NH_4CH_3COO	70	1750
$NaHCO_3$	400	40 000
Glucose	1000	4500

Note that we increased the amount of Sodium Hydroxide and Glucose to fit the experimental conditions.

1.2 Objectives

The main objective of this study is to spot the light on the challenges that face students and researchers with limited time and resources to make an efficient digestion for long-stored granules vs. how it goes with fresh ones. It will include an investigation of the different aspects that a successful reactor has, such as methane production, COD and nutrient removal, alkalinity, and volatile fatty acids accumulation under specific operating conditions. The study is handled in a laboratory-scale UASB reactor.

1.3 Thesis Outline

This master thesis is entitled: " **THE OPERATIONAL CHALLENGES OF UASB REACTOR DIGESTING INACTIVE AND ACTIVE GRANULES: UTILIZATION OF THE SUCCESSFUL ROUTE** "

And it is divided into nine chapters.

1. Introduction.
2. Literature Review and Theoretical Background.
3. Materials and Methods.
4. Results.
5. Discussions.
6. Challenges
7. Conclusion
8. Recommendations
9. Proposal

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

2. Literature Review and Theoretical Background

This chapter describes, analyses, and investigates the detailed intellectual explanation of AP that occurs in the reactor and defines the stoichiometry of the process. The progress of anaerobic treatment for synthetic wastewater in recent studies is also interpreted. Furthermore, the factors influencing AP of UASB reactor are also presented

2.1. Anaerobic Treatment

AD is a biological treatment of waste by which microorganisms break down biodegradable organics in a medium deprived of oxygen and with a low redox potential [9]. The metabolic operations followed in the breakage of the carbon and energy source for anaerobic and aerobic processes have only two fundamental differences: (a) the terminal outcome of the produced electrons in the oxidation reactions; and (b) the quantity of ATP forms generated by oxidative phosphorylation. The amount of ATP formed when a couple of electrons passes over the electron transport system is driven by the differences in redox potential between the electron acceptor and donor. Therefore, ATP generation efficiency will usually be higher when the respiration is aerobic [9].

Figure 2.1 represents the carbon and energy outcome in both aerobic and anaerobic wastewater treatment, assuming that the oxidation of 1 kg COD requires 1 kWh of aeration energy. Aerobic treatment is generally characterized by high operational costs (power);. At the same time, a significant fraction of the influent COD is converted to sludge, about 50% (or more) new sludge from the COD converted. The carbon/energy flow principles of aerobic and anaerobic conversion primarily affect the corresponding wastewater treatment system [13].

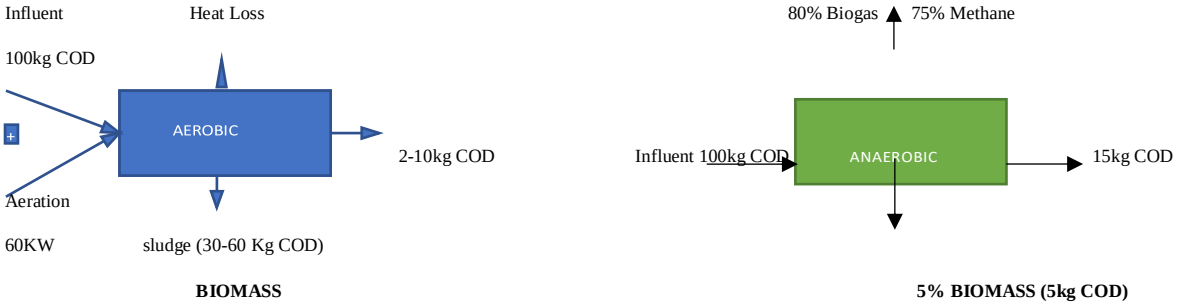


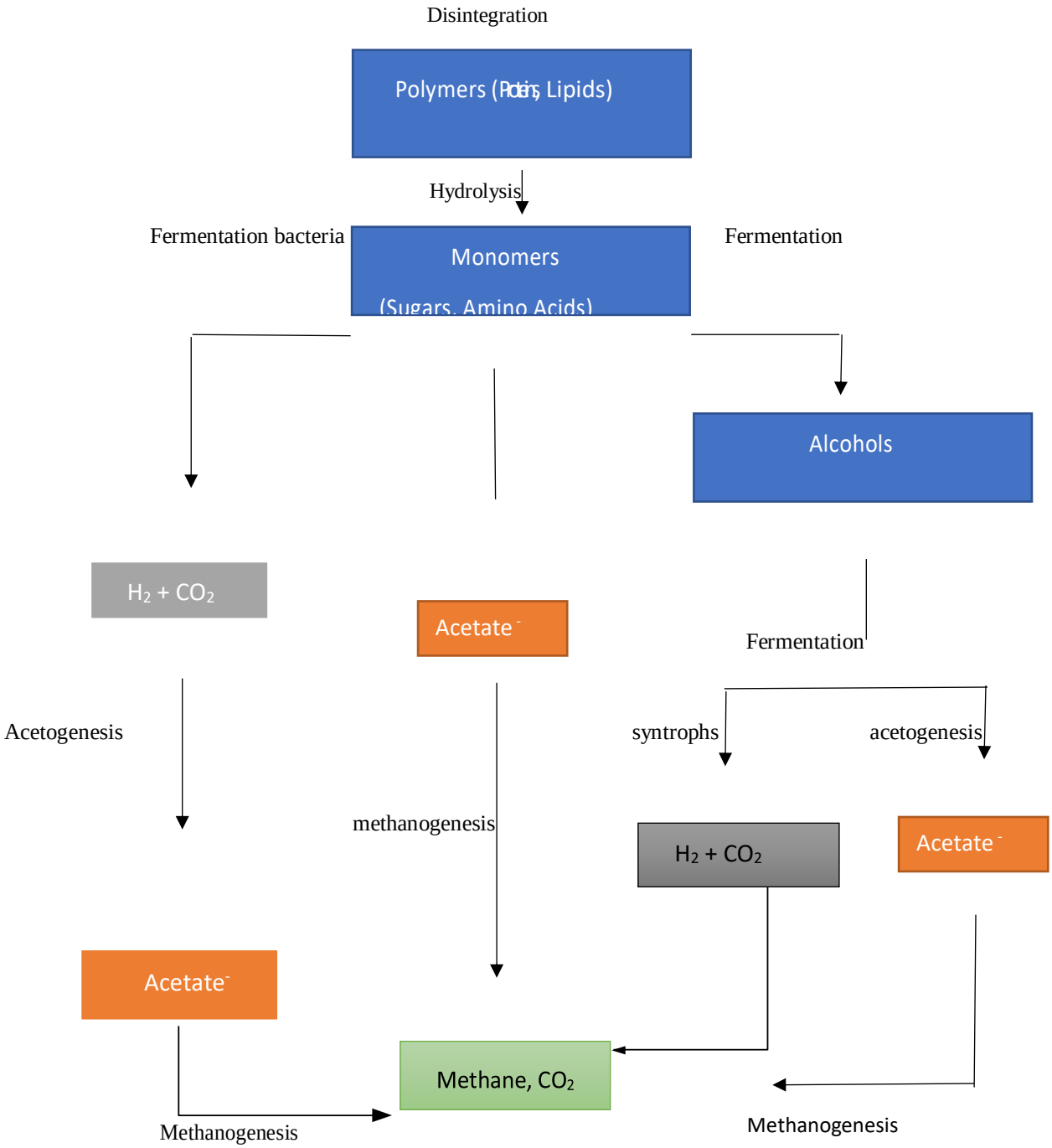
Figure 2.1 The fate of carbon and energy in aerobic and anaerobic wastewater treatment [13]

Based on Figure 2.1, the significant advantages of the anaerobic process compared to the aerobic process are:

1. Less sludge is produced per unit of the substrate.
2. It has a market value when granular anaerobic sludge production occurs in the bioreactor.
3. It has more organic loading potential since the process does not restrain oxygen transfer effectiveness at high oxygen utilization rates.

Regarding the cons, it requires elevated temperatures to maintain an efficient speed for the microbial activity, and the utilization of organic matters will not be completed [13, 23].

Figure 2.2 anaerobic process steps *[33]*



AP contains a complex group of microorganisms, and this multistep type of anaerobic operation is expressed in Figure 2.2. The system consists of three bacteria groups, and they are a) Acidogens, b) Acetogens, and c) methanogens, where the cumulative activities of these groups of bacteria formulate the process stability and continuity as shown in figure 2.3. The general process of biochemical reactions occurred by these bacterial groups is characterized by four basic processes: (1) Disintegration and Hydrolysis; (2) Acidogenesis; (3) Acetogenesis; and (4) Methanogenesis. Will detail the four main processes in the following sections.

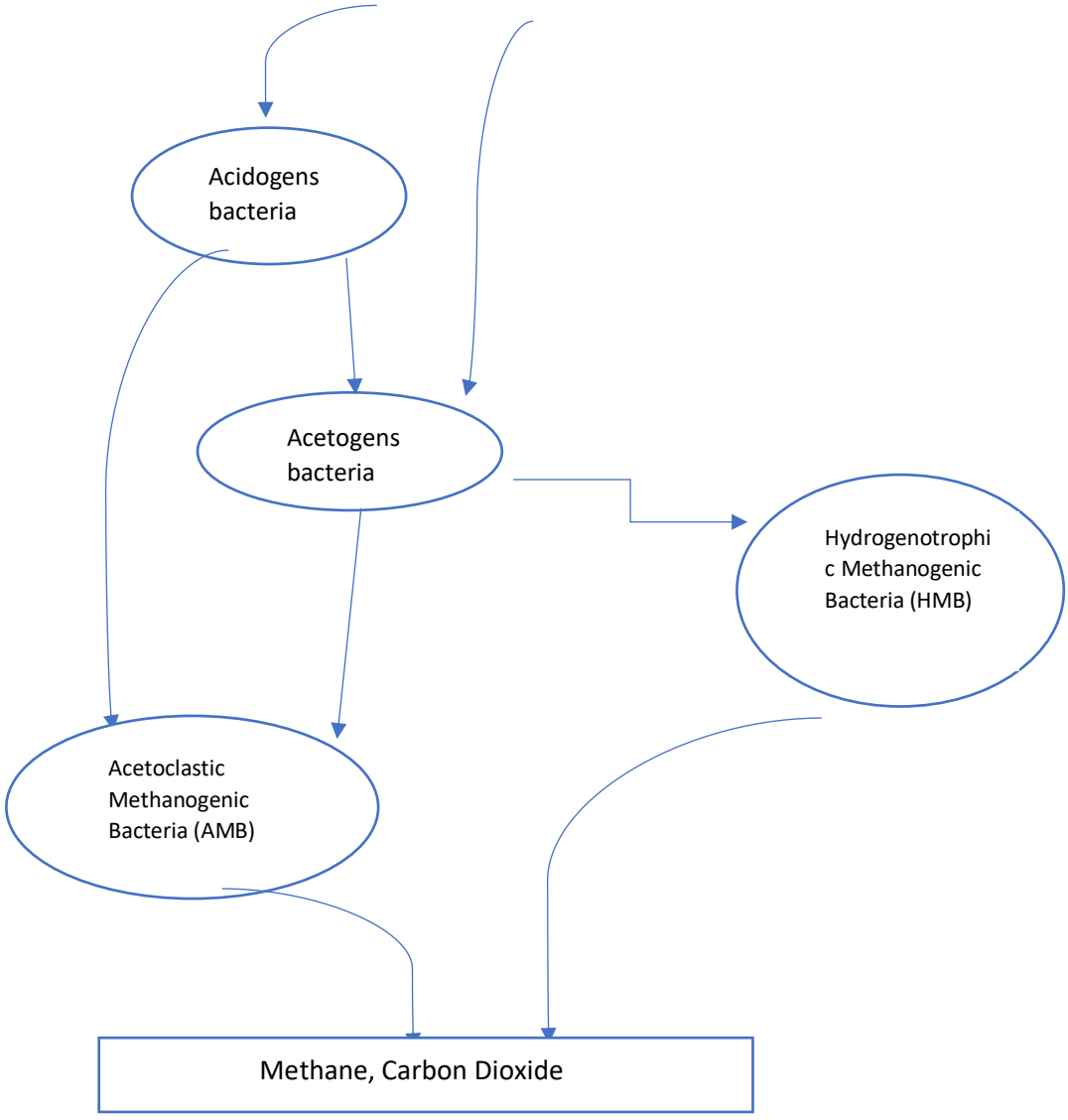


Figure 2.3 Groups of microorganisms in anaerobic processes [51]

2.1.1. Disintegration and Hydrolysis

The breakdown and solubilization of complex organic material happen through an extracellular biological and non-biological process known as disintegration and hydrolysis. A specific bacteria control this stage of the methane production process named a hydrolysing bacterium; an enzyme derived from the hydrolase's enzymes. The substrate consists of composite particulates, particulate carbohydrates, lipids, and proteins. The disintegration of composite particulates forms the three particulate substrates. Hydrolysis, by definition, is the degradation of a chemical compound by breaking one or more chemical bonds (depolymerization). To digest the long polymeric chain, materials such as carbohydrates, proteins, and lipids, cannot be directly degraded by microorganisms and therefore must be reduced to smaller products to allow their passage across the cell membrane of the organisms. The enzyme-producing bacteria benefit directly from the soluble products in the process, hence accelerating the process [9]. Lytic enzymes will also break down during hydrolysis, and they are divided into three main groups 1) proteases (work on proteins); 2) cellulases, amylases, gluconates (work on polysaccharides); and 3) lipases (work on fats and oil; lipids) [9,33,53].

The degradation of Carbohydrates, proteins, and lipids that occurs during this process produce monosaccharides, long-chain fatty acids (LCFA), and proteins, respectively. These products generated from the hydrolysis step are used as substrates for the acidogenic organisms in the next step. An amount of energy is needed to boost the reactions, which the catabolism of other smaller molecules can cover. Few lipopolysaccharides are degraded to

monosaccharides and LCFA, although polymers are hydrolysed to dissolved rapidly biodegradable substrates of their monomeric composition. [9].

2.1.2. Acidogenesis

Acidogenesis, which can also be named fermentation, is an acid-producing microbial operation carried out without oxygen. The organics serve as electron acceptors and donors (no need for additional electrons). The process involves the degradation of amino acids and sugars to hydrogen, Volatile fatty acids, and other intermediates such as propionate and ethanol. The speed of fermentation is relatively high, and Acidogens carry it out. The overall energy yield is generally higher. Hence these reactions are most often carried out at higher concentrations of hydrogen or formate and higher biomass yields. [3].

The fermentation production's efficiency is controlled by various factors such as inlet's composition, environmental conditions (pH, temperature, etc.) and operating factors (loading rate, retention time, etc.) in the reactor [3]. The absence of electron acceptors alters the electrons from the substrate to get captured in reduced organic compounds, which exerted from the cell as products. The growth yield of the final product is hugely affected by the consumed energy fraction related to the power exerted by the fermentation products. The stoichiometric balance of product formation using glucose as substrate is shown in Table 2.1. few compounds can be fermentable. Moreover, Energy rich organic intermediates are formed which can generate high Adenosine triphosphate (ATP) by phosphorylation [26].

LCFA and the fermented alcohols are oxidized anaerobically to VFA, H_2, and CO_2 by β-oxidation. The whole process starts by the conversion of lipids are converted by lipase into glycerol and fatty acids. The glycerol is converted to acetate by acidogenesis using H^+ as electron acceptor [3].

Table 2.1 *Stoichiometry of product formation using glucose as substrate* [42].

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

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

2.1.3. Acetogenesis

Acetate, Carbon Dioxide, and hydrogen are produced by the conversion of The VFAs, excluding acetate, produced from the previous step using the acetogenic bacteria. Table 2.2 shows the product formation stoichiometries of acetogenesis. The key intermediates in the anaerobic digestion process are propionate and butyrate. Hydrogen and Acetic acid are treated directly by the methanogens while the other products are converted into acetic acid and H_2 in this step. Acetogenesis is mandatory for VFAs that were formed during lipase activity. The formic acid and hydrogen produced during this process must be conserved in low concentrations so that the formation reaction is favored thermodynamically with $\Delta G^o < 0$. The low concentration is managed by the hydrogen utilizing methanogens. [2].

Table 2.2 *Stoichiometry showing the product formation of the different substrates* [42]

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

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

Interspecies hydrogen transfer is the interaction between the consumption and generation of hydrogen, and ΔG' refers to the different hydrogen concentrations for the anaerobic oxidation of VFA's. The hydrogen concentration must be within the window, where the partial pressure is in the range of 10^{-4} to 10^{-6}, otherwise acetogenesis and autotrophic methanogenesis will be hindered [2].

2.1.4. Methanogenesis

The outcome of the last stage of the anaerobic process is Methane generation. The methanogenic bacteria transform the by-products from the previous stage into methane. This methane generation is carried out in two primary approaches by two different groups of methanogenic bacteria, as shown in Figure 2.3. The first pathway is by fermenting the primary product of the 3rd stage to methane and Carbon dioxide. Acetolactic Methanogenic Bacteria (AMB) feeds on acetic acid as a substrate. The overall reaction is presented in Equation 2-1. [17].

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

In the second pathway, H_2 is utilized by Hydrogenotrophic Methanogenic Bacteria (HMB) as an electron donor ton increase the conversion rate of methane with respect to CO_2. The overall reaction in Equation 2-2 [43].

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

In methanogens, substrate level phosphorylation does not influence the energy generation; instead, electron transport and ATPase do the work [17]. The methanogens require specific environmental conditions, and their activity is widely affected if they changed, unlikely for the Acidogens. Accordingly, methane production is the limiting phase in anaerobic processes [43]. The maximum growth rate (μ_{max}) of methanogenesis ranges between 0.12-0.71 d^{-1}, and long retention time is necessary for the methane producing processes [39]. The growth rate is also low (0.05 – 0.1 gVSS/gCOD), since most of the energy in the substrate has to be converted into methane [39,53].

2.2 Anaerobic Stoichiometry

Organic compounds present in the wastewater are usually evaluated and determined by the oxygen consumption per volume. The upcoming sections discuss the stoichiometry of the anaerobic process.

2.2.1. Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD), a universal parameter of testing pollutant's strength, is a measure ofthe capacity of water to deplete oxygen during the degradation of organic matter

and the oxidation of inorganics such as ammonia [1]. In order to determine the COD, oxidation of organics has to be done at a specific temperature and determined time. Oxygen equivalent is the number of electrons provided by a strong oxidant and in gO_2/m^3 (or mgO_2/l). It can be determined while knowing that 1 mol of O_2 weight 32 g and has 4 electron equivalents. Therefore, one electron equivalent (eeq) counts to 8 grams COD, as shown in Equation 2-3 [23].

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

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

The ThOD (theoretical COD) of any organic compound in the form of $C_nH_aO_b$ can easily be determined based on the oxidation reaction, assuming that the oxidation is complete, shown in Equation 2-4 [6].

$$C_nH_aO_b + ¼\,(4n+a-2b)\,O_2 \rightarrow n\,CO_2 + ½a\,H_2O \qquad 2\text{-}4$$

Equation 2-4 shows that 1 mol of an organic compound requires *¼(4n+a-2b)* mol or grams Oxygen. When the conversion equation includes compounds that have ammonia, Equation 2-4 needsto be updated for the number of electrons that will stick with N and the total mass of N in the compound, presented in Equation 2-5 [23].

$$C_nH_aO_bN_d + ¼\,(4n+a-2b-3d)\,O_2 \rightarrow n\,CO_2 + ½(a-3d)\,H_2O + d\,NH_3 \qquad 2\text{-}5$$

ThOD can be calculated by the oxidation balance of glucose, as shown in Equation 2-6. Referring to Equation 2-6, glucose oxidation needs 6 moles of oxygen per mole of glucose. Therefore, 1 gram glucose counts for 1.067 gCOD (192/180). (180 and 192 are the molecular weights for glucose and oxygen respectively)

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

180 g 192 g

ThOD per unit mass varies for each chemical compound depending on the molecular formula. For a strongly reduced compound such as methane, using Equation 2-4, the theoretical COD of methane is high as shown in Equation 2-7.

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

The carbon oxidation state in methane is -4, which is the most reduced state. The lower the carbon oxidation state in the compound is, the more oxygen can bound by the compound; Hence, the higher COD value. Buswell Equation (Equation 2-8) can be used when the compound ($C_nH_aO_bN_d$) is completely biodegradable and will be fully converted by the anaerobic organisms (no sludge yield) into CH_4, CO_2 and NH_3. The theoretical numbers of methane gas (and CO_2) produced can be calculated from them mention equation [].

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

2.1.1. COD Fraction

The COD in wastewater is divided into various fractions based on its biodegradability, presented in Figure 2.4. Furthermore, the proportions of biodegradable and non-biodegradable COD can be divided into particulate and dissolved COD. Dissolved biodegradable COD is readily degraded by microorganisms, while particulate biodegradable COD must be hydrolysed into smaller molecules to grow microorganisms. Particulate non-biodegradable COD will adsorb to biomass; it will be accumulated into the sludge since it cannot be used by microorganisms. Similarly for dissolved non-biodegradable COD will accumulate in the sludge and pass through the effluent [23].

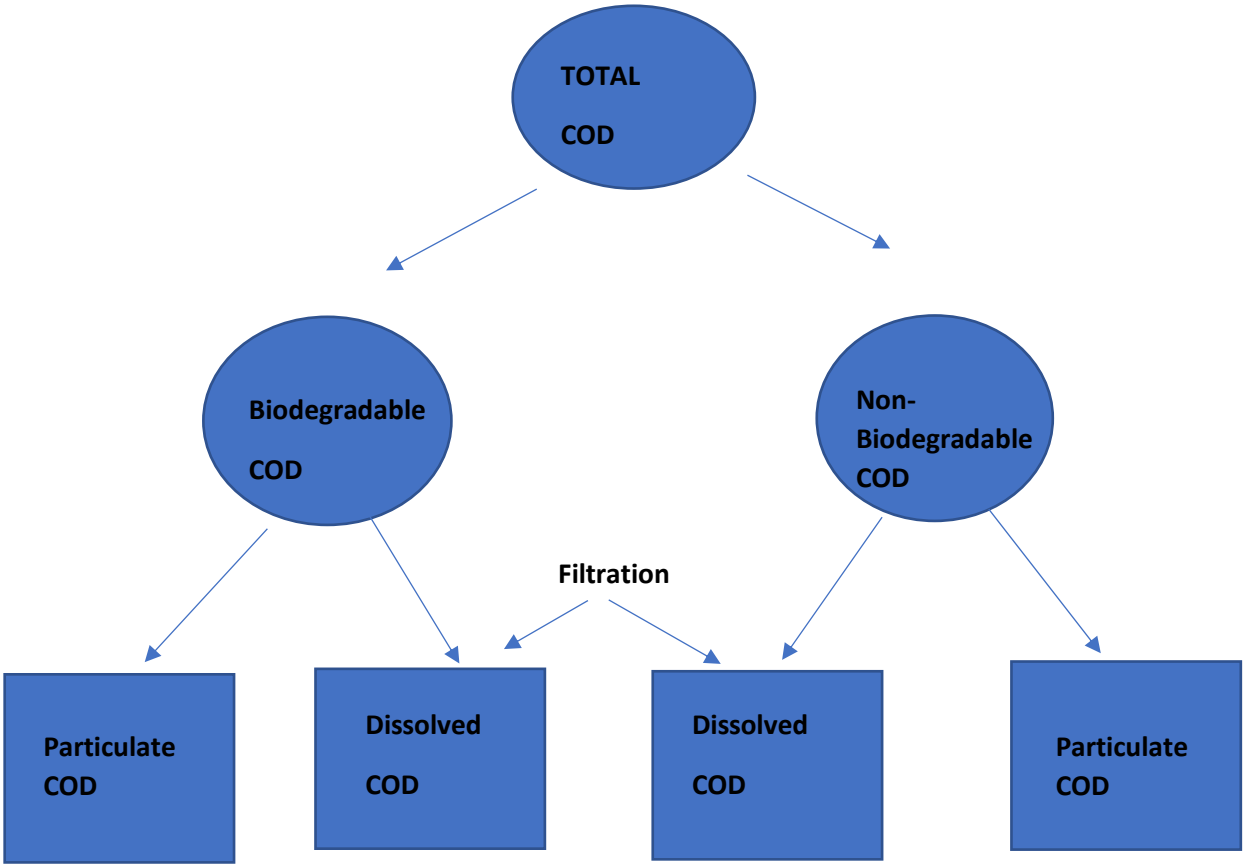


Figure 2.4 *COD fraction in wastewater*

Distinguishing between total input COD and available degradable substrate is crucial since noticeable portion of the input COD can be anaerobically non-biodegradable. An influent with 100% biodegradable COD is rarely found. The COD flowchart presented in Figure 2.5 shows the COD pathway through intermediates until reaching the methane. When organic material conversion is complete, inert soluble and inert particulate only count for 10 % of total COD converted [9]. The common non-biodegradable fractions for total inlet COD for raw and settled (primary effluent) wastewaters are presented in Table 2.3.

Table 2.3 *Non-biodegradable fraction of total COD for raw and settled (primary effluent)wastewater [23]*

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

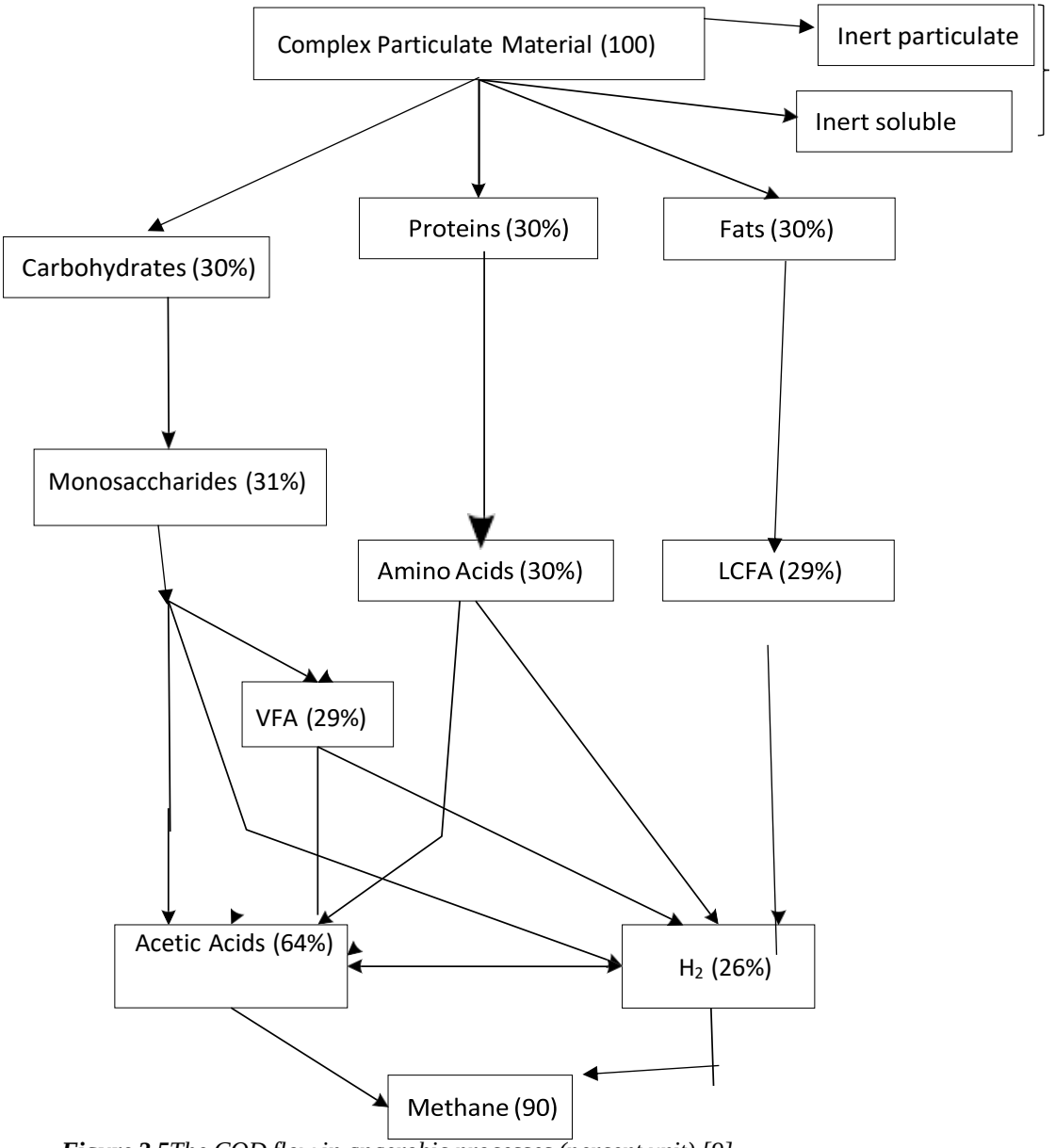


Figure 2.5*The COD flow in anaerobic processes (percent unit) [9]*

2.1.1. Methane Production

The total amount of methane produced during the anaerobic process is based on the amount of organic matter available in the sample as CH_4 is related to a certain number of COD. Generally, as discussed previously, fraction of the organic matter is non-biodegradable and some of the organic substrate will be consumed for cell synthesis [23]. Based on the main influent features such as the flow rate, COD concentrations, and the given-on biodegradability of the COD, an estimation can be drawn for the expected methane production using Equation 2-9.

$$CH_4 + 2O_2 \rightarrow CO_2 + H_2O \qquad 2\text{-}9$$

One mole of methane requires two moles of oxygen to get oxidized into carbon dioxide and water. Therefore, the COD equivalent of methane is 4 kgCOD/kg CH_4. At STP (standard temperature and pressure) of 0 °C and one atmospheric pressure, 0.35m^3 of methane can be produced per one kilogram of COD. While raising the temperature to 35 °C and within constant pressure, 0.40m^3 of methane can produced per one kilogram of COD.

Figure 2.6 show that methane production is strictly controlled by temperature. The total biogas production is determined by reading the record on the gas counter. For determining the conversion rate of COD into biogas, the effluent and inlet COD must be calculated. The carbon dioxide counts up to 50% and usually ranges between 30 and 50% of the total gas produced in the process [21,23].

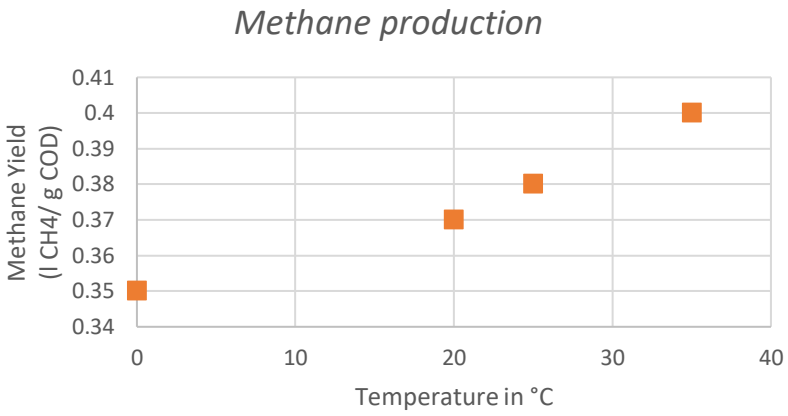


Fig 2.6 *Methane production of 100% COD conversion in function of temperature*

2.1.2. COD Balance

To keep the operation under control, several parameters have to be measured and analysed for the anaerobic process which is considered a biological system. The fate of the COD in the inlet is divided into two parts i) The produced methane, ii) the COD that is integrated in the biomass, as it appears in **Figure 2.7**. The mass balance in the system is only calculated for COD parameter (Equation 2-10). Hence, the COD is the unit that controls the anaerobic digestion process.

$$COD_{in} = COD_{out} \qquad 2\text{-}10$$

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

Complete identification of the solid and liquid effluents must be performed to identify the amount of biogas produced. The estimated characteristic of the influent biomass is referred to the following molecular formula: C5H7O2N. The theoretical COD of the biomass can be calculated and gives a value of 1.42 kgCOD/kgVSS. The final products and newly grown bacteria are noted as COD so that the balance is set and requires a measurement. [21, 23].

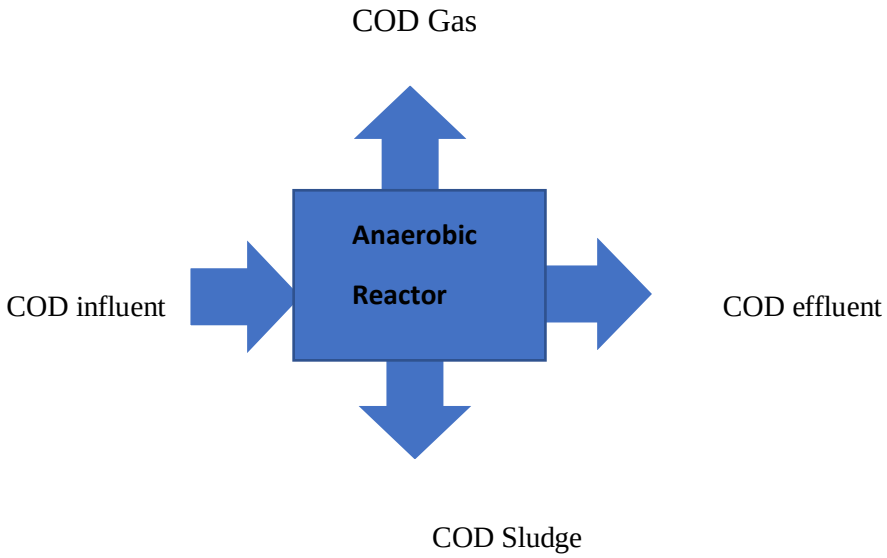


Figure 2.7 COD balance [13]

2.3. Factors Affecting the Anaerobic Processes

For a practical design for any biological wastewater treatment process, it is essential to have a basic knowledge of factors influencing the processes, such as the nutritional demand of microorganisms, operating conditions, and the environmental factors in the region that alter the microbial growth. In this subchapter, a short description of the factors affecting the anaerobic process will be presented.

2.3.1. Sludge Retention Time (SRT)

Sludge retention time (SRT) exerts a significant influence on the ability and performance of any biochemical operation. It controls the activity of microorganisms, so that it determines the species that can grow in the reactor, also affects the effluent's quality. The chosen SRT must be higher than the minimum SRT that is related to the microorganisms that controls the biochemical transformation. A low SRT value is set when the substrate needs to be consumed for growth, which leads to a lower storage yield. The average SRT values for all the anaerobic conversion processes steps at 35°C are as the following (Lower temperature requires Longer SRT values). First, hydrolysis of carbohydrates and proteins takes around

three days to synthesize monosaccharides and amino acids which is considered a fast one. In comparison, the hydrolysis of lipids is regarded as a longer step and requires around six days to produce LCFA. Moving toward the Acidogenesis step, the fermentation of Amino acids and sugars to form hydrogen and carbon dioxide occurs in a day. Unlikely for LCFA (including Butyric acid) and VFA, the conversion takes six and twelve days respectively to produce Aceticlastic. Finally, the methanogenesis of Hydrogen and Acetilclastic occurs in two and up to fifteen days respectively to produce methane [21].

In general, anaerobic treatment of wastewater consists of carbohydrates and protein to produce methane can be achieved with an average SRT value of eight days. Slight methane production can occur at low SRT values around five to six days, but notable load of propionic acid accumulates since the SRT value is low, which allows the growth of bacteria that oxidizes propionic acid to form acetic acid and hydrogen. Generally, a minimum of ten days is specified for the SRT to assure complete degradation of lipids in bioreactors [21].

2.3.2. Organic Loading Rate (OLR)

Organic loading rate (OLR) is main parameter which affects the conversion rate of the substrate. It is related to the SRT by the active biomass concentration that is injected in the bioreactor. An efficient design of the wastewater treatment process requires an effective OLR. Optimum range of OLR rely on the source of wastewater to be treated, and the nature of the organic substrates to be added [38]. Treating a sludge that has high nitrogen concentrations for example, will lead to an increase in the ammonia concentrations within the system, thus will generate toxicity issues. High OLR creates a mis proportion in the system where more VFA will be formed during the acidogenesis process, while the methanogens will not be able to convert the whole VFA quantity to methane since the growth rate is low. The rest of VFA will accumulate in the system first and reduce the pH which directly influence the activity of methane forming bacteria [21]. Fluidized bed reactors (FBR), and Fixed film reactors can combat higher OLR [23].

The OLR is expressed in kgCOD/m^3. d or gCOD/l. d as represented in Equation 2-11.

$$OLR = \frac{QQ.\,CCCn}{V} \qquad 2\text{-}11$$

Where Q is flow rate (l/d).

C_{in} is feed concentration (gCOD/l).

V is reactor volume (l).

The OLR can also be expressed as a function of hydraulic retention time (HRT) and the inlet concentration (C_{in}), as shown in Equation 2-12.

$$OLR = \frac{CCCn}{HRT} \qquad 2\text{-}12$$

When setting a reactor with no reflux, the loading is only affected by SRT only because the SRT and HRT are equivalent. For a reactor with reflux, the SRT is separated from HRT. SRT and OLR are inversely proportional as shown in Equation 2-13 . SRT is also inversely proportional to the volume (V) and volume associates with the SRT through biomass concentration (X) in the bioreactor. A boost in the OLR occurs as the biomass concentration is more significant, allowing the bioreactor to be more miniature [21].

$$SRT = \frac{XX.V}{Y.QQ.CCCn} = \frac{XX}{Y.OLR} \qquad 2\text{-}13$$

2.3.3. Temperature

The temperature is the most crucial condition for all the growth processes involved in the anaerobic digestion, in addition to and the rates and activities of these reactions. The microbial growth rate is also controlled by the temperature along with the total amount yielded. The maximum growth rate can be reached within a determined increase in the temperature. A further increase for the temperature will not affect the growth rate anymore, but it decreases the growth rate as the heat-sensitive enzymes are denatured [10]. The temperature effect is expressed in Equation 2-14.

$$\mu_{m\,(20)} = \mu_{m(T)} . \theta\theta^{(T-20)} \qquad 2\text{-}14$$

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

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

and θ is temperature coefficient.

Microorganisms are categorized into temperature classes based on the optimal temperature range by which the species can grow and metabolize, presented in Figure 2.8. There is no boundary between the three classic groups of psychrophilic, mesophilic, and thermophilic microorganisms, as Figure. 2.8 shows an overlapping region for growth at different temperatures [30].

The degradation of organics requires more energy to initiate at low temperatures. Therefore, the chemical and biological reaction rate within psychrophilic conditions is lower than that under mesophilic conditions, which is also lower than that under thermophilic conditions, as shown in figure 2.8. The term optimal temperature is when the utilization of the substrate is maximum while changing the temperature. Generally speaking, decreasing the operational temperature is proportional to the growth rate and utilization of a substrate. However, it can also cause an increase in the net biomass yield (g biomass per g substrate converted) of the methanogenic population or acidogenic sludge [31, 41].

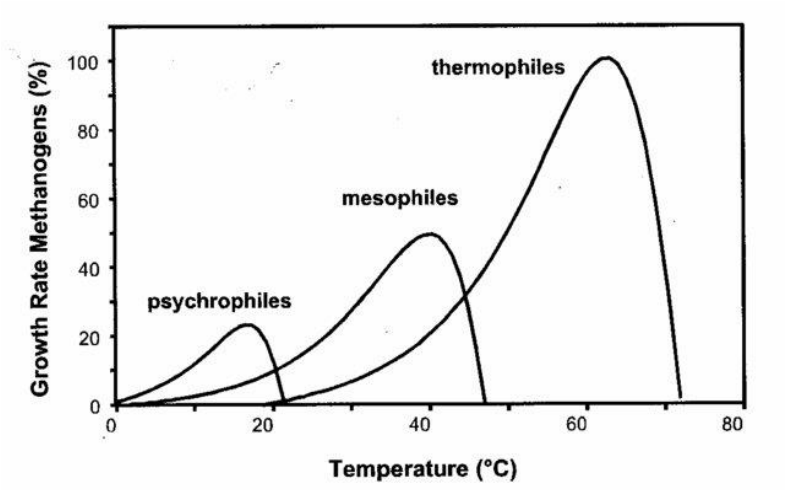


Figure 2.8 Relative growth rates of methanogens with different temperature [18]

The majority large scale treatment processes of wastewater are limited by temperature over 18 °C. On the other hand, methane production can occur under low temperatures, but

restricted to a longer SRTs to counter the low growth rate. Moreover, under moderate climate conditions, many dilute wastewaters, including domestic and industrial wastewaters, are discharged at low ambient temperatures. Furthermore, many diluted wastewaters are being treated at low temperatures, with COD below 1500 mgCOD/L [5,41].

2.3.4. pH

The growth rate for the bacteria is strictly affected by the pH factor. The pH usually ranges between 4 to 9 depending on the origin of the bacteria. Regarding the methanogenic microorganisms, the ideal pH is approximately 7.0. Their activity decreases when the pH is not in the range of 6.0 – 8.0 which leads to no further production of methane. The free energies for both The AMB and HMB rely on proton motive force by the electron flow in the membrane since they have low free energies. The main factor that controls the enzyme activity is the hydrogen-ion concentration [23]. Enough buffer capacity amount must be available in the system in order to hold the productivity of CO2 and VFA that will be dissolved within the pressure of the system. Higher alkalinity is necessary to control the accumulation of VFA. In a pH controlled medium, anaerobic processes operates normally within a wide range of VFA concentrations (from less than 100 mg/l to over 5000 mg/l) [15]. A stable pH value leads to a stable system. Sodium bicarbonate ($NaHCO_3$) is the commonly used buffer over the other chemicals since it provides the appropriate equilibrium value, while conserving the continuity of chemical and physical balance. The sodium bicarbonate also maintains the pH to stay on the desired value (7) which is suitable for methanogen bacteria [50].

2.3.5. Nutrients

Microorganisms require nutrients for the creation of cytoplasmic material, considered as energy source for cell growth and development, and because they serve as electron acceptors

[10]. Activated sludge treatment normally requires the COD:N:P ratio to be 100:5:1 ,while maximal methane generation requires a C:N:P ratio to be 100:2.5:0.5 [15].

Industrial wastewater treatment process carried out anaerobically, requires several inorganic nutrients in low concentrations. The absence of these nutrients could yield into a lower performance for the process and harms the growth rate. Nickel and Cobalt promote the methanogenesis. Micro and macronutrients needed for the process completion is determined based on the COD biodegradable in the wastewater, nutrient concentration in the cells, and the cell yield. Normally, the inlet nutrient concentration should be modified to the double of the minimal nutrient concentration needed to initiate the process, so that there is no limiting nutrient in the process [51].

2.3.6. Inhibitory Substances

The maximum specific growth rate (μmax) can be achieved by increasing the concentration to a specified value. Any further increase in this concentration will have no positive influence, and controversially, the maximum growth rate will decrease since it has already reached the threshold. Once toxicity is indicated, any further addition of concentrations is considered toxic—the toxicity is proportional to the concentration when the concentrations cross the threshold values. The inhibitory substances are adequately described in the following sub-chapters. [21]

2.3.6.1 Volatile Fatty Acids (VFAs)

Acid accumulation in the anaerobic reactors alters the medium and makes it acidic since it decreases the pH. When the medium is neutral with pH ranges around 7, both acetic and butyric acids have no significant toxic consequences on the hydrogen-utilizing methanogenic bacteria within concentrations that are equal to or less than 10000 mg/l. However, Propionic acid is considered a toxic substance to the methanogenic bacteria when

its concentration is 1000 mg/l or above, at neutral pH [49]. Their toxicity is yet to be determined upon this pH range [23]. Referring to the previously mentioned research outcomes, inhibition by VFA slightly affects the anaerobic operations.

A reduced methanogen activity directly influences anaerobic treatment methods since their bacteria are sensitive to pH. Any drop in hydrogen consumption by the HMB leads to a decrease in pH as the available hydrogen concentration is high in the system. Thus, it will affect the AMB activity as well. This leads to a decrease in the acetic acid production, then an accumulation for VFA or acetic acid will occur. pH has a minor influence on acidogenic bacteria. Figure 2.9 illustrates the acidification of anaerobic processes when VFA production surpasses the maximal capacity of methanogenic consuming hydrogen and acetic acid.

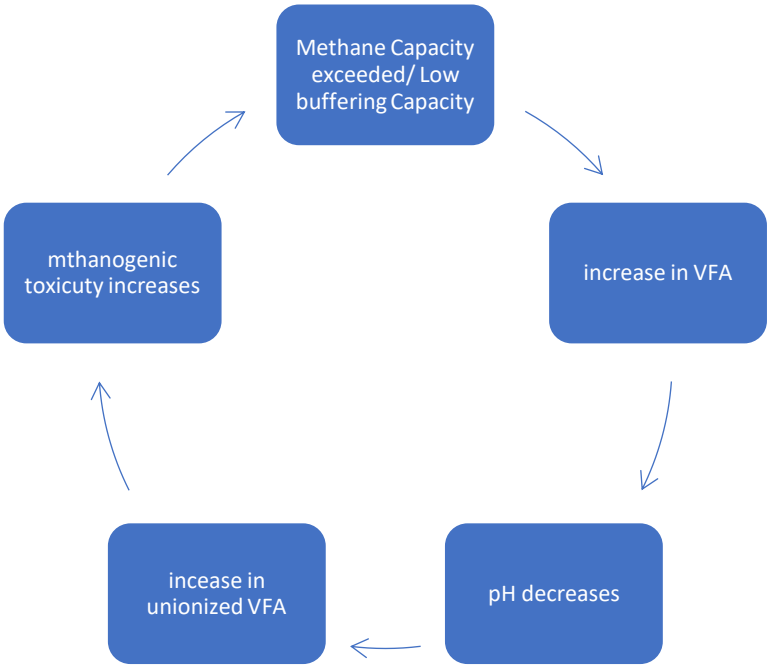


***Figure* 2.9** Reactor pH drop because of methanogenic overloading and VFAs accumulating [23]

2.3.6.2. Ammonia

Ammonia is usually found in the proteins, so the wastewater that carries high protein content has higher ammonia quantities. The break-down of the protein will release the nitrogen presented there as ammonia in different forms as the following: ammonium ion, NH_4^+, dissolved ammonia, or NH_3 which is controlled by the pH in the system. Anaerobic metabolism is inhibited by ammonia at high concentrations. Bacteria can hold a certain concentration of ammonia, but concentration variance within the inlet can stop the process [23]. Ammonia is a weak base and dissolves in water, represented in Equation 2-15.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad 2\text{-}15$$

Free ammonia is more toxic than ammonium ion is more available at high pH. A concentration of 150 mg/l or more of free ammonia is considered as severe toxicity, whereas the ammonium ion concentration should be twenty times higher to have the same influence. Similarly, to the high pH, a high concentration of ammonia will lead to a process failure, but it can be countered by adding acetic acid. One of the results of ammonia is an increase in VFA, thus it is more toxic in the methanogenic bacteria rather than the non-methanogenic one.

2.3.6.3. Sulphides

A diverse microbial community is presented in the anaerobic processes. Other bacteria, rather than the methanogenic, more often compete for food and energy resources. Specific bacteria can use various electron acceptors, including sulphate or sulphide by sulphate-reducing bacteria (SRB), which converts sulphate into hydrogen sulphide (H_2S). The three bacteria groups are SRB, methanogens, and obligate hydrogen-producing bacteria (OHPB), will be responsible for the degradation process since they can perform within similar environmental conditions, but they might compete for resources. A faster growth rate will dominate; the comparison is between the SRB and methanogens due to the complexity of the competition [23].

A high concentration of sulphate found in the Wastewater will lead to a greater influence of the sulphide toxicity. The cell growth of the methanogenic population will be hindered if the sulphide concentration surpasses 200 mg/L. It is possible to tolerate concentrations between 100 and 200 mg/l if acclimatization has occurred. At neutral pH, hydrogen sulphide exists in equilibrium with the hydrogen sulphide ion, as shown in Equation 2-16 [21].

$$H_2S \leftrightarrow HS^- + H^+ \qquad HS^- \leftrightarrow S^{2-} + H^+ \qquad \text{2-16}$$

Because it's only mildly soluble in water, hydrogen sulphide will get evenly distributed between the liquid and gas phase. In the process, there is a reduced overall energy balance, and this results in a lower methane output per unit of degraded organic waste. Some of the biogas sulphide ends up as H2S in the biogas, which lowers the quality of the biogas [23]. The sulphide has an odor. The produced biogas containing sulphide increases the corrosivity of it, causing the generation of sulphur oxides when the gas is burned. [21].

2.3.6.4. Heavy Metals

Bacteria require heavy metals for enhancing enzyme activity and formulating the needed structure but can also be poisonous and inhibiting in more significant amounts. According to Table 2.5, modest quantities of heavy metals significantly affect anaerobic processes, inhibiting their operation at 50% inhibition. While these chemicals are poisonous, their concentrations can be lowered to non-hazardous levels by precipitation with sulphides generated during the reaction. Sulphur is sometimes added in instances where low sulphide is formed. Since sulphides can also be inhibitory to methane-forming bacteria, this must be done with extreme caution.

One milligram of heavy metal requires around 0.5 milligrams of sulphide to precipitate. The perfect chemical to supply additional sulphide to the system is ferrous sulphide. Ferrous iron appears to be substantially less inhibiting than other heavy metals, as shown in Table 2.4. be substantially less inhibiting than other heavy metals as seen in Table 2.5. Furthermore, the more inhibiting heavy metals (e.g., Pb, Hg, As, etc.) precipitated by the sulphide makes

it less soluble than the ferrous sulphide. Therefore, the additional sulphide will keep the concentration of these heavy metals at trace levels. Whenever the pH is more than 6.4 (optimal conditions), excess iron precipitate as iron carbonate, which is incapable of inhibiting the enzyme [21].

Table 2.4 Concentration of soluble heavy metals exhibiting 50% inhibition of anaerobic processes [42].

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

2.3.6.5. Light metal cations

Maintaining a neutral pH requires the addition of a base. Caution is advised while conducting this since the light metal cations usually found in base solutions may also pose harmful effects on the entire microbial community. Despite their widespread usage, the toxicity of Calcium, sodium, potassium, and magnesium demonstrates a complicated interaction within the community, so they are considered as a particular concern. Those nutrients are needed for microbial growth, and, as a result, they can affect the growth rate of all other nutrients [21]. For varying concentrations of cations, some generalizations can be established; they are listed in Table 2.5.

Table 2.5 *Stimulatory and inhibitory concentration of light metal cations in anaerobic processes* [42]

Cations	Concentration (mg/l)		
	Stimulatory	**Moderate Inhibitory**	**Strongly Inhibitory**
Sodium	100-200	3500-5500	8000
Potassium	200-400	2500-4500	12000
Calcium	100-200	2500-4500	8000
Magnesium	75-150	1000-1500	3000

The concentrations laying under the stimulatory sections are those which lead to maximal reaction rates. They will provide optimal metabolic activity of the bacteria under standard conditions. Regarding the Moderate inhibitory, these concentrations can be tolerated after a stage of adaption but need to be done quickly; However, the productivity of the reactor can be stopped for few days when increasing the concentrations. Finally, the strong inhibitory concentrations can directly affect the bacterial growth within the system, which requires longer SRT to save the reactor's performance.

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

There are two sections branched out of this sub-chapter. The material presented in the first section illustrates the general idea of a typical UASB reactor’s operating environment for practical application, while the use of a UASB reactor to treat municipal wastewater is explained in the second section.

2.4.1. General Concept

An Up-flow Anaerobic Sludge Blanket is wastewater treatment system that uses bacteria in absence of air and oxygen resources. It is designed to eliminate the organic pollutants in sludge, slurry, and wastewater. Anaerobic microorganisms turn organic pollutants into "biomethane" that contains methane and carbon dioxide. Besides, the liquid effluent is rich in Volatile fatty acids and contains a small portion of dissolved biomethane.

$$WasteWater + Granules \rightarrow \ Liquid\ effluent\ +\ Biogas \quad \text{eq-2.17}$$

The wastewater is added to the reactor from the bottom, which makes them in contact with the granules, making the water flows upward with speed equals to that of biomass settling to maintain the formation of the sludge blanket. For the gas created to escape, a specific zone is required to ensure that the sludge particles will not follow the gas. Compact granules, including mixed cultures of bacteria, are found in the reactor's biomass. In the UASB reactors, a large portion of the biomass is retained, and as a result, the reactors are well-suited for treating wastewater with low substrate concentrations, which is why it is ideal for treating municipal wastewater [21].

Designing a proper UASB reactor is done by the retention of active biomass in high levels. This allows treating high OLRs, while choosing an efficient usage of the provided space and cost of treatment and installation [9]. A standard UASB reactor is presented in Figure 2.10. The wastewater flows to the reactor from the bottom of it, using a pump, to pass through the granular sludge. At this stage exactly the treatment happens, where biogas and VFA are formed. The ascending flow of the biogas maintain the gas bubbles to be partially fluidized. At the top of the reactor, the gas-liquid separator ensures the rising of the gas into the collecting part in the upper part, while the liquid carrying the VFA will be in the effluent [21]. The treatment efficiency is set to be lower within psychrophilic conditions since the internal mixing was far away from optimal, and thus leads to a dead space in the reactor [14].

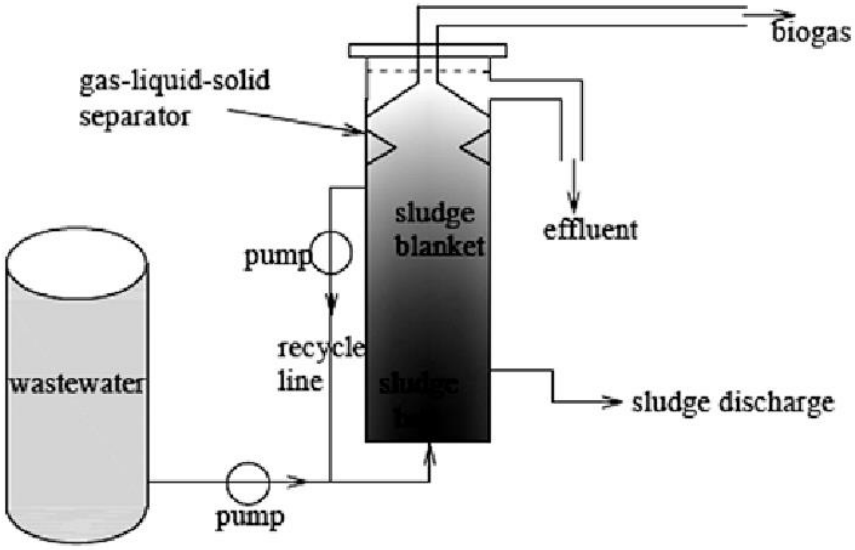


Figure 2.10 Schematic diagram UASB bioreactor [36].

2.4.2. History of Municipal Wastewater Treatment Application

Anaerobic processes are the main techniques used to treat the sludge due to their influence on removing unwanted chemicals. The process is not limited to low-strength wastewater, but it can also perform high-strength ones and effectively Treat it [30]. Municipal wastewater has been treated using different anaerobic techniques in the last three decades, providing efficient results within specific conditions (low HRT and tropical weather) [4].

The anaerobic treatment was first only limited to mesophilic conditions in the tropical regions. In Cali, Columbia, recorded the first 64 m^3 UASB reactor testing in 1982 to treat municipal wastewater. The outcome resulted from this process showed a feasibility of operating a UASB reactor to treat sludge under the previously determined conditions. Following Columbia, many countries introduced full scale UASB reactors to their facilities [23].

Adapting for the local conditions, and to make the process economically feasible, many researchers have been studying the performance of UASB under psychrophilic during the last 35 years [5,41]. Seghezzo [44] claims that his 6 m^3 UASB reactor provided with sewage sludge which operates under HRT of 14 – 17 h. COD reduction reached 55-70%

and 65-85% at 13-17 ℃ and 20 °C respectively. Therefore, he has proven the viability of treating municipal sludge under low temperatures.

The UASB reactor requires a post treatment facility to get rid of the leftovers in the effluent, and to obey the regulations of discharging. Many techniques can be used such as sand filtration, physio-chemical treatment etc. [23].

2.5. Knowledge Gaps (Specific Objectives)

This study aims to trickle down all the potential impediments that faces researchers while performing on a UASB reactor. Furthermore, the goal is to investigate the effectiveness of methane production by UASB from organic matter. To run this process, a laboratory designed UASB reactor systems, is built to treat up for treating of high strength synthetic wastewater treatment (below 1200 mgCOD/l) under psychrophilic condition (4 – 5 °C). The study focuses on identifying the critical factors affecting the performance of UASB reactor so that by controlling the operating conditions. A comparison is done between the long-stored granules (almost 5 years) and fresh granules. Therefore, the OLR capacity, up-flow velocity, pH variability, VFA production, alkalinity and nutrient availability, granular sludge behaviours, and what can be the cause behind negative results is discussed.

3 Materials and Methods

This chapter shows the laboratory-scale apparatus used in the experiment of anaerobic treatment of synthetic wastewater for energy generation in the form of methane in the UASB reactor. One UASB reactor is used in this study to investigate the performance of i) inactive granular sludge (long-stored sludge, Reactor I), and ii) Active granular sludge (Reactor II). Also, it discusses the operational, maintenance, and control steps and the analytical theories used in the study. All lab jobs for this project were held at the University of Stavanger Laboratories.

3.1. Anaerobic Granular Sludge Reactor Configuration

Building a UASB reactor in the laboratory needs crucial equipment, devices and other materials which are connected to ensure a great performance of the anaerobic treatment. Experiment II was held out it in the same reactor using the same apparatus.

3.1.1. The Configuration of the UASB Reactor

Figure 3.1 shows the UASB reactor that was used for conducting the performance of inactive granular sludge. In the same reactor, the active granules were added after finishing the first experiment and washing out the rest of the old granules. The granules for experiment I were brought from (a) pulp and paper company treating cellulose and lignin containing (Norske Skog, Moss); (b) agriculture pilot plant treating swine and cow manure supernatant (farm in Skien, Norway); and (c) hydrocarbon oil containing wastewater at Bamble Industrial Park, Telemark and have been stored since the beginning of 2016 (almost 5 years). While the granules for experiment II are a mixture of Potato and Vegetables industries waste (provided by Avecom company, Belgium). The configuration of Reactors I and II are shown in Figure 3.2. The reactor is built of polyethylene and fabricated by Ytre Vanntank (ID 350x8) by 3 L of volume. The wastewater feed is pumped from a container stored in a small fridge into the reactor by a diaphragm pump with controlled flow rate. The gas flows out by the gas trap to reach the gas counter due to liquid height, pressure and suction force provided

by the recirculation pump. Then, the biogas produced passes through CO_2 absorber using NaOH 3M solution (Product No. 106498, Merck) and methane is recorded in the following gas counter. Table 3.1 shows the equipment used in setting-up of the UASB Reactor. Gas counter is used to measure the produced volume of biogas.

Figure 3.1 *Photo of the laboratory scale UASB Reactor*

Figure 3.2 *a wider photo of the UASB apparatus.*

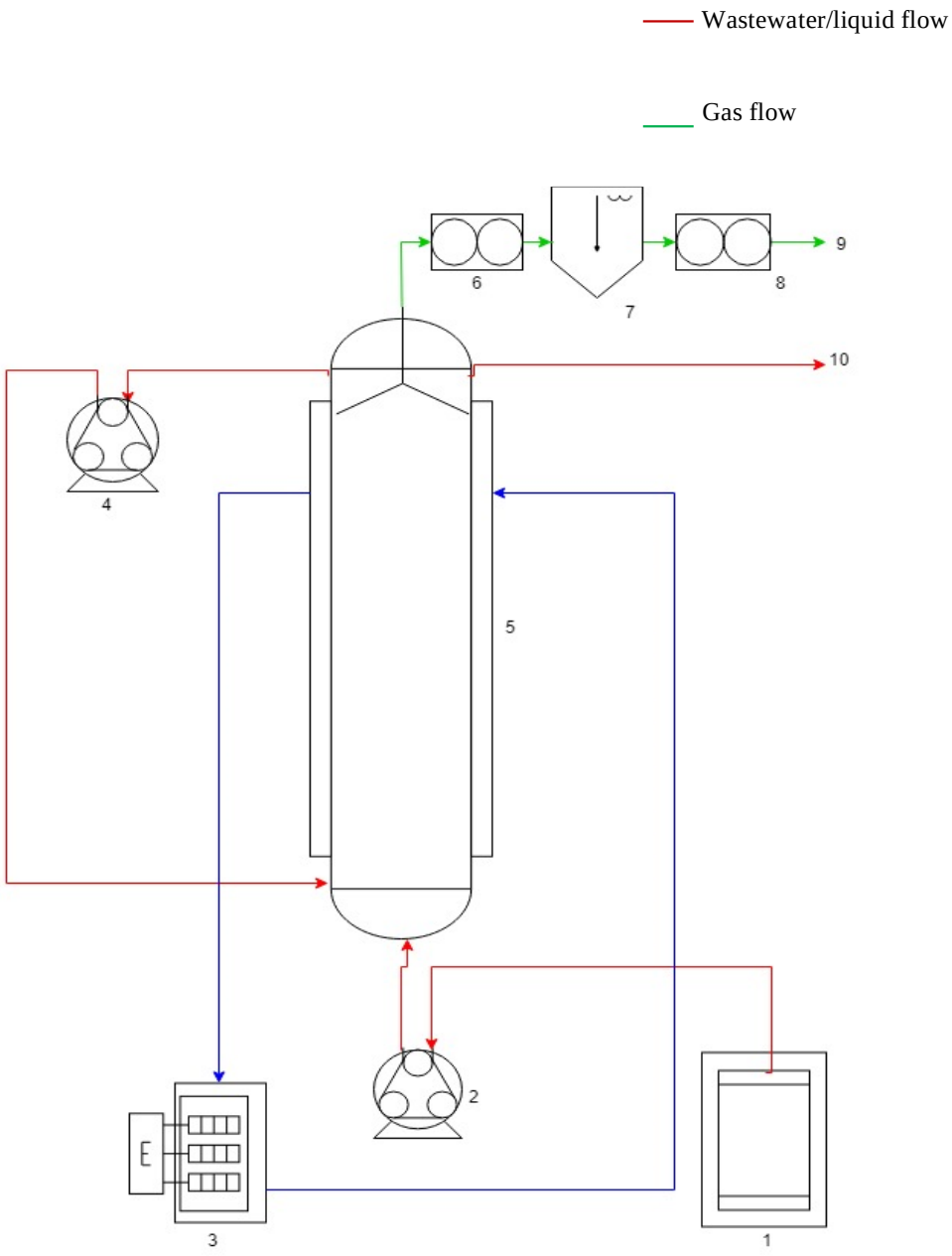


Figure 3.3 *The Flow diagram of the laboratory scale UASB Reactor I and Reactor II*

1. Refrigerated feed, 2. Peristaltic feed pump, 3. Thermostatic cooler, 4. Peristaltic recirculating pump, 5. Jacketed UASB reactor, 6. Biogas counter, 7. CO_2 stripper, 8. CH_4 counter, 9. Gas effluent, 10. Liquid effluent

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

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

Figure 3.2 shows the UASB reactor which is connected to a container of 30L laying inside the fridge to maintain the stabilization of the required conditions.

The reactor has a heating system (heater bath and circulation tubes) which allows the liquid to be warm enough so that the bacterium inside the reactor is adopted to the optimal conditions. The heat control set up helps in maintaining a condition which is almost mesophilic with a temperature of 16˚C. The warm water circulates along the reactor to maintain the needed temperature. Gas counter was used to measure thevolume of produced biogas. Table 3.1 describes the equipment used in setting- up of the UASB Reactor I and Reactor II.

3.2. Starting-up the Reactor and Operational Conditions

The first stage of starting up the reactors was to be in steady state condition and the reactors worked hydraulically well. A steady state was achieved in the reactors when the parameters, e.g., the effluent COD and the daily gas production remained constant at the same OLR. Figure 3.3 shows the general operation flowchart of the UASB reactors. This laboratory scale reactors were initially tested with tap water. The goal was to make sure that all necessary devices, instruments, and materials were set and installed correctly. Key parameters such as OLR, flow rate, pressure and pH were controlled to ensure that favourable conditions for anaerobic bacteria were maintained during the anaerobic treatment processes. This section describes the starting-up process and operation conditions of all three reactors used in this study.

A sludge volume of 20 – 30% v/v with respect to the UASB reactors was used. The UASB reactor was started-up at low OLR of 1.0 gCOD/l. d equivalent to 30% of the total COD concentration of wastewater. OLR was increasing gradually until 15.0 gCOD/l. d equivalent to 100% of the COD concentration with HRT 1.4 hour, changes in OLR were made when the reactors were assumed in steady state condition. Dilution was done usingtap water that was stripped by N_2 to dissolved oxygen (DO) below 1.00 mg/l (using DO meter WTW Oxi3315) to assure anaerobic treatment process. For an optimum growth of methanogens, pH of the liquid inside the reactors was controlled and maintained at about 7. Buffer, sodium hydrogen bicarbonate ($NaHCO_3$), was added into the reactor to bring the pH close to 7.

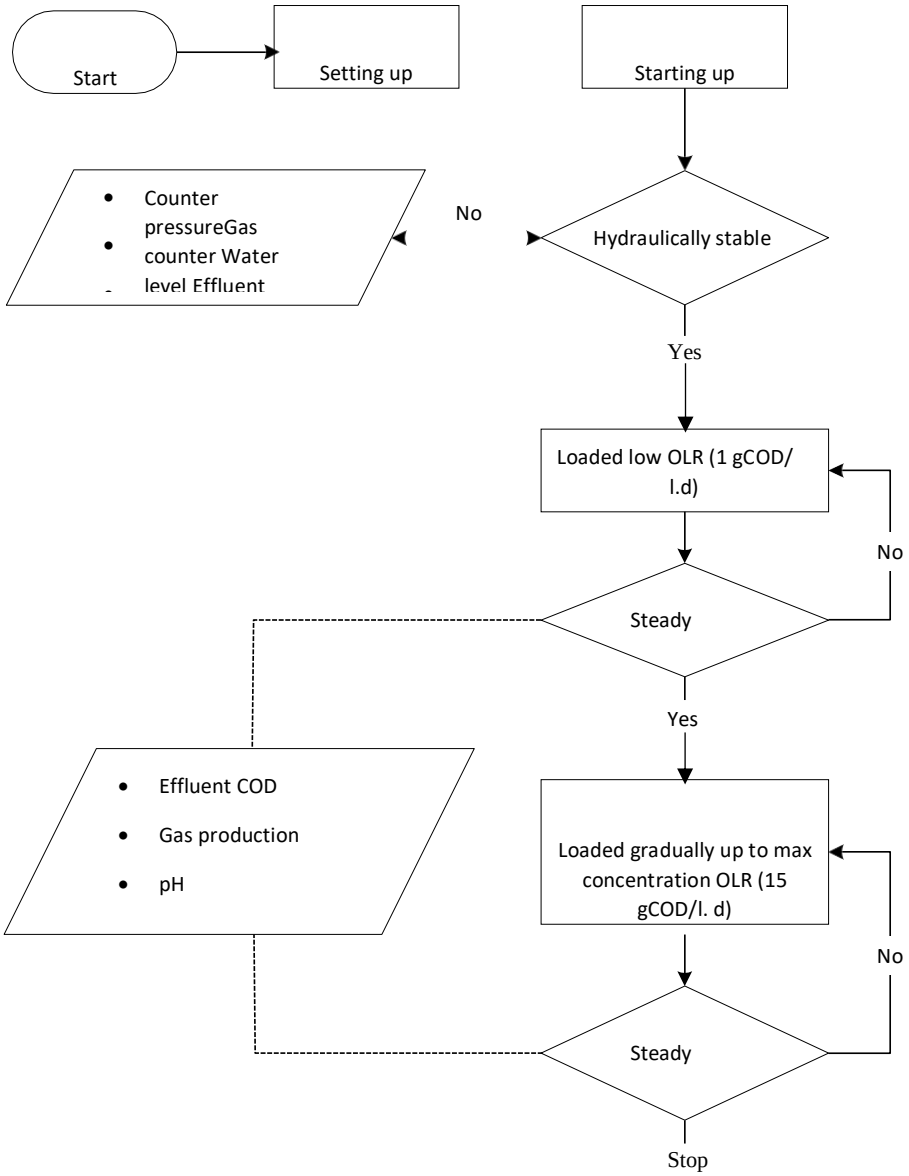


Figure 3.4 *The general operation flowchart*

3.2.1 Starting-up UASB Reactors I and II and Operation Conditions

Two different experiments were conducted in Reactors I and II. The first one was to test the activity of inactive granular sludge (from 9th March to 30 May 2021). It aimed to know the suitable conditions that would restore the activity of those granules using trial and error method to check the up-flow velocity, OLR, and HRT. The first week was left for observation and no tests were conducted to stimulate the activity of bacteria in the new medium. The compositions of nutrient added to the synthetic wastewater is shown in **table 1.1**. After monitoring for 67 days, the experiment was ended since the results did not reach the desired ones in the means of biogas production and COD removal.

The same conditions were set for Reactor II, except for the granules. We used a mixture of Vegetable and Potato sludge industry originated from Belgium. We ran the experiment for one month (it could have been more, but due to the time limit). The same purpose was to this reactor as well. The performance of both reactors will be analysed and discussed in the upcoming chapters. Temperature inside both reactors was fixed and maintained at 16 °C using a circulating laboratory heating bath. It is important to keep the tank of the heating bath full of distilled water since it regularly evaporates and may lead to an instability within the system. The flow rate in the inlet tube is subjected to fluctuations. We aimed to measure the flow rate manually on a daily basis to make sure that the pump is working properly, and we are achieving our demanded OLR and HRT. Also, the volume in effluent was measured for the same reason.

3.3. Analytical Methods

It is important to be aware of shaking the sample properly before doing any measurement to make sure that the sample is homogenized. Some tests required filtration before testing. Also, in some days the COD tended to be higher than the capacity of the testing kit. It was important to dilute it with distilled water before digesting the test. The methods used are described in the following sub-chapters.

3.3.1. pH and Conductivity Measurement

Conductivity can be measured using a WTW Multi340i. The probe first needs to be clean and dry, then it is immersed into the beaker carrying the sample until the value becomes constant. The conductivity is measured since it is used in the TITRA 5 program that measures the VFA and Alkalinity after being titrated using a TitroLine® 5000 Auto-Titrator. This allows to measure the pH as well. The tests are exactly the same for Reactors I and II.

3.3.2. COD Measurement

COD testing were carried out for the influent and the effluent of the reactor, where we only measured the dissolved COD. Measurements were conducted on an average of 5 times a week. The collected wastewater from the inlet and effluent must go through a filtration apparatus using 1.5 µm microfiber filter VWR European Ca. No. 516-0876) in order to continue the measurement.

COD test kit used in this study is Merck Spectroquant® which were Product Number 109773 (100 and 1500 mg/l of COD concentration range) to carry out the wastewater analysis. The kit usually contains digestion and catalyst solutions that, under high temperature, reacts with wastewater samples to achieve the measurement. It consists of a small glass tube (that can handle high temperatures) and has a barcode straight under the lid that can be automatically read by the spectrophotometer and does not need any further installations.

2ml of the filtered sample is brought to the COD testing kit. Then it is inserted in a in thermo reactor (Model TR 620), at 148 °C for 2 hours. After the digestion is completed, the glass is taken out of the heater to cool down for 30 minutes to be approximately like the room temperature. It is important to keep it under the hood for safety reasons. Then, the kits are brought to the spectrometer (Spectroquant Pharo 300), while making sure that it is properly placed (on the orientation mark). Finally, read the value recorded (COD in mg/L).

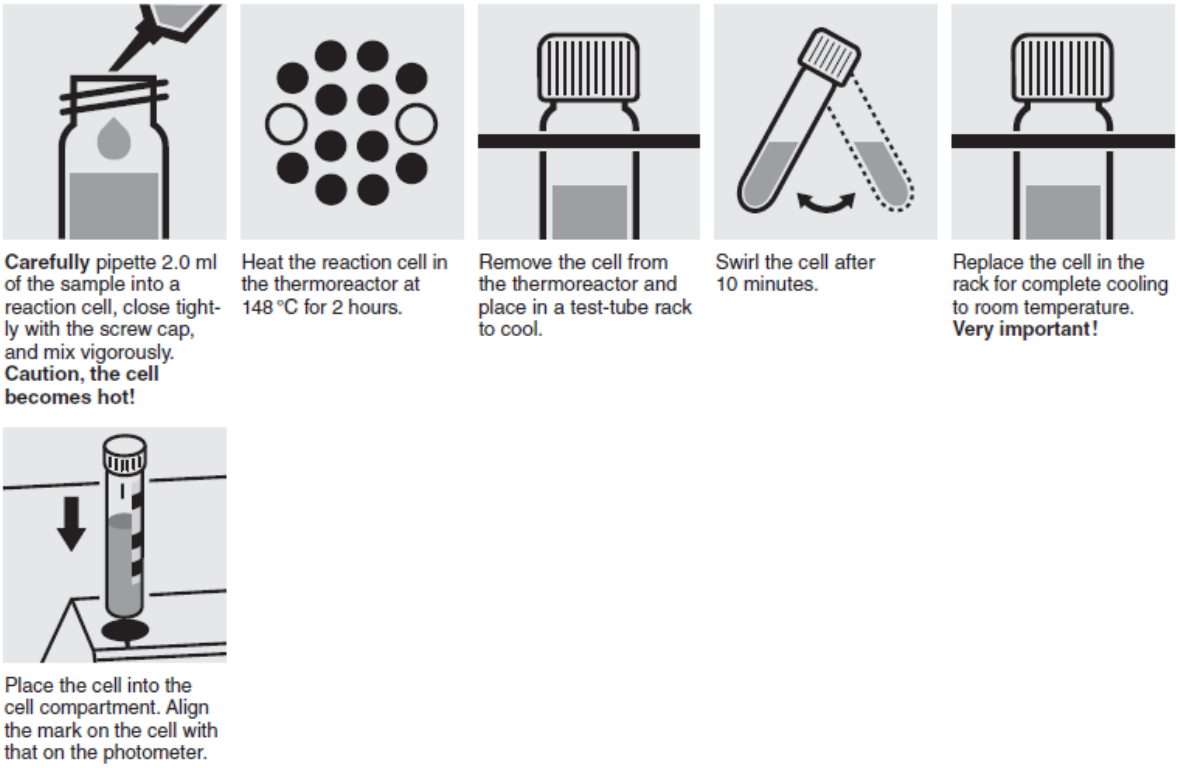


***Figure 3.5*:** Graphical presentation of the Spectro quant COD method 09773 Cell test.[42]

3.3.3. Total Volatile Fatty Acid Alkalinity Measurement

As mentioned before, the VFA and Alkalinity measurements were conducted in a TitroLine® 5000 Auto-titration (Instrument-teknikk AS, Oslo). The machine consists of the six components: valve-cover lid and display; probe; dosing unit; titration tip unit; stirrer; and acid bottle. The analysis is based on five pH point titration procedures starting from the initial pH, until it reaches pH of 4.3. Mainly, the testing was done for the effluent (except some few times for the inlet). To run the test, the sample is placed under the probe that measures and can do the titration. A magnetic stirrer is important factor to make sure that it is mixed properly. The dilution is done using a 0.1M HCl (Product No. 100317, Merck) to four different selected pH. A typical pH values are as the following: (6.7, 5.9, 5.2 and 4.3), but sometimes the pH in the effluent was less than 6.7, so we had to choose a mode that start from 5.9 and ends in 4.3. The volume added on each specific pH must be recorded and sometimes it becomes tricky to follow up due to the titration process. Then the collected values of pH and added volume are inserted to the TITRA 5

program, which gives us the measurements of alkalinity and VFA in mg $CaCO_3$/L and mg Acetic acid/l respectively.

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

For wastewater treatment, it is important to check the values of Total phosphorous (TP) and total nitrogen (TN) to make sure that there are enough resources for the bacterial growth during anaerobic digestion. This is performed to limit the search when the reactor's performance is dragged down. Similar to the COD testing, TP and TN measurement also uses spectroquant test kits. The TP tests kit is Merck Spectroquant® and a product number 114729 (0.50 and 5.00 mg/l PO_4^{3-}). For TN measurement, Merck Spectroquant® Product Number 100613 (0.50 and 15.0 mg/l N). TP and TN are also measured by a spectrometer (Spectroquant Pharo 300).

The TP measurement procedure starts by digesting a 5ml sample of filtered wastewater with a dose of reagent P-1K in a thermo reactor for 30 minutes with a temperature of 120 °C. Then, the tube is cooled in the same way as it is done for the COD tube. Afterwards, addition of 5 drops of reagent P-2K and 1 dose of reagent P-3K to the test vials; The kits must be mixed vigorously after each adding each reagent. Rest for 10 minutes until the reaction occurs, then place properly in the spectrometer and read the value in mg/l.

Digesting the TN occurs by the following process: 9ml of distilled water and 1ml of wastewater are added to an empty cell, 1 level of reagent N-1K, and 6 drops of reagent N-2K. Then it is placed in thermo reactor at 120 °C for 1 hour. Then, left for 30 minutes to cool down. Afterwards, a sample of 1.0 ml from digested sample and 1 dose of N-3K were added to a new test vial; it is crucial to mix the vial vigorously after adding the reagent. The reaction needs 10 minutes to be completed. At the end, measure the concentration after putting it in the spectrometer.

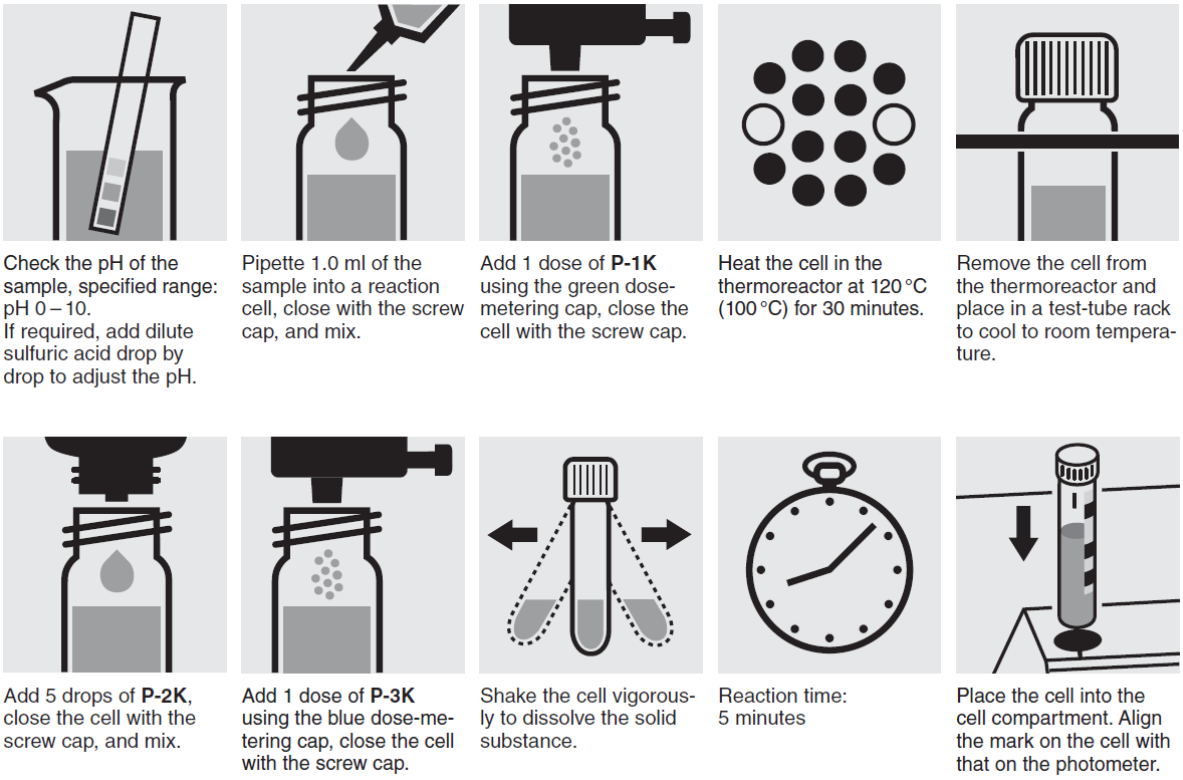


Figure 3.6: Graphical method for sample preparation and analysis procedure for the determination of Total P in wastewater (Spectroquant method 14729). Ortho phosphate is determined by omitting steps 3 -5. [42]

3.3.5. Total Solid of Granules (Sludge Blanket)

Total solid of granules measurement was conducted only for the granules in Reactor I to determine the characteristics of the granules. The instrument used in this study was moisture analyser (VWR International No. Product 611-2318), with maximum mass capacity is 160 grams (0.001 grams of accuracy). The standard method (SM 2540 B) is used to determine the total suspended solids. The sludge is dried for 1 hour at 105 °C and then evaporated residual would be measured automatically by this instrument. The desired temperature and time can be set by the control pad of the equipment.

4. Results

The collected data during the one hundred and seven days of running the experiment are divided as the following:

4.1 Reactors Performance

The performance of reactors I and II will be discussed in the following sub-chapter. The performance is based on the ability of the reactor to remove the highest COD and measure the amount of methane gas produced.

4.1.1 COD Removal Efficiency

Reactor I was operated for sixty-seven days, while Reactor II was operated for a month. Figures 4.2 and 4.3 show the COD inlet and outlet concentrations of Reactors I and II, and Figure 4.4 shows the COD removal efficiency with respect to time for both reactors. The HRT in Reactor I was meant to be high with a value of 83 hours, while Reactor II was initiated from 32 h to 17 h. The HRT values are relatively high and the purpose behind these values is to avoid the washout of the microorganisms in the reactor [24]. The OLR value varies between 0.4 and 1.4 gCOD/L. D in Reactor I, while in Reactor II it varies from 0,5 to 2 gCOD/ L.D as shown in figure 4.4. The COD removal efficiency for Reactor I showed no more than 50% removal, while for Reactor II the values were much higher, and it reached around 80% removal.

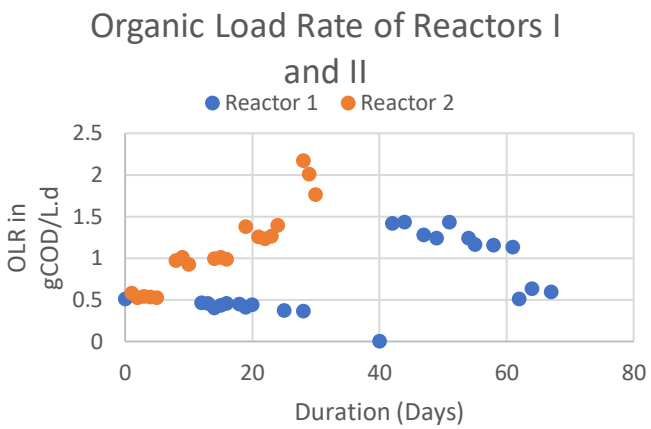


Figure 4.1 OLR of Reactors I and II

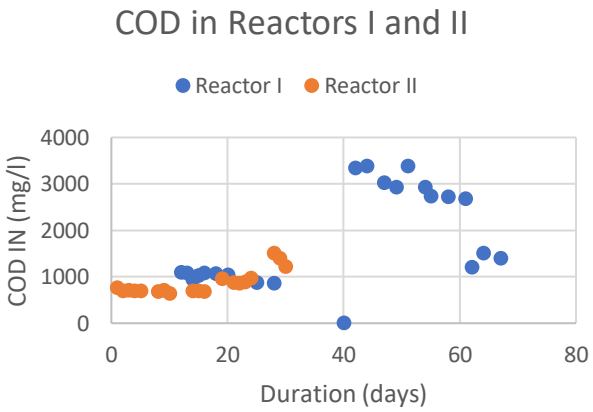


Figure 4.2 COD inlets for reactor I and II

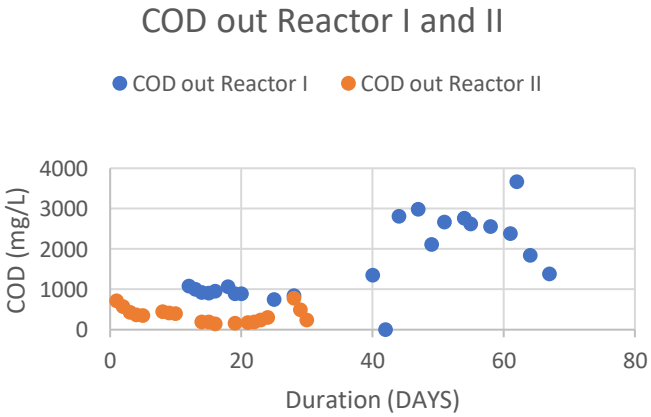


Figure 4.3 COD outlets for reactor I and II

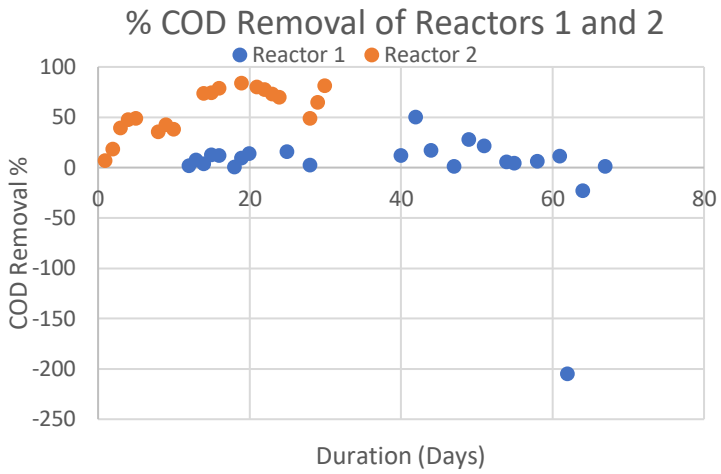


Figure 4.4 % of COD removed for reactor I and II

In Reactor I, the removal efficiency was higher when the OLR was at its maximum values (40-50 days). While in Reactor II, the removal efficiency was increasing gradually and when the OLR was increased, the maximum value of removal efficiency was recorded on the last day of the experiment (30 days).

4.1.2 Methane Production

Due to a certain failure in the gas separator, methane production was barely noticed. In Reactor I there were few spikes of total gas produced (260 ml only) during the whole sixty-seven days. The data is presented in a table.1 in the appendix due to the poor values. This issue will be interpreted in the Discussion section. Regarding Reactor II, the first significant biogas production was recorded after 27 days of setting up the reactor. It is also presented in table.2 in the appendix for the same reason. Unfortunately, around 1% only of the produced gas was methane. Many issues could influence the production and will be discussed in the next section. Note that bubbles were spotted in Reactor II continuously.

4.1.3 COD Balance and Fraction

For experiment I, it was hard to expect the amount of methane that should be recovered due to the long storage time. While running the experiment, the liquid (0.5M HCl) presented in the first gas counter that measures the total gas (methane and CO2) should have a liquid (0.5M HCL) inside it, which disappeared suddenly. It is usual for the HCl volume to decrease over time, but not a complete runoff. Even the gas production was not obvious, but the liquid runoff made the reading of the gas traces even impossible (bubbles were noticed during the experiment, which is an indication for gas production).

For experiment II, the liquid inside the gas counters was within the average quantity throughout the whole month of running the reactor. The bubbles inside the reactor tend to be more evident on the 8th day of running the reactor. That raised the suspicion toward any leakage, so we brought a 4-Gas Meter WatchGas (QCM), but we did not find any error within the connecting tubes. The biogas was appropriately noticed after 27 days of initiating the experiment. The methane gas detector was not showing any methane production, though all the setup and the required chemicals were checked several times. The biogas generation reached a maximum value of 50 ml/hour and varied from 20 to 40 ml/hour otherwise. The expected methane generation was 0.375 g CH4. COD/ g VS. d (provided from the company).

4.1.4 pH, Alkalinity, and VFA Variability

For Reactor I, 20 grams of buffer was added twice. The first one was on the 5^{th} day while the second one was on the day 47. The aim of this addition is to maintain an appropriate amount of buffer to counteract the low pH recorded during the experiment. Figure 4.5 shows the pH values of Reactors I and II where the average pH wasn't very far away from the optimal one (pH=7) in reactor II, while in Reactor I the pH was fluctuating, and it exceeded the boundaries of the optimal one. Figures 4.6 and 4.7 shows the VFA and alkalinity profiles for Reactors I and II respectively.

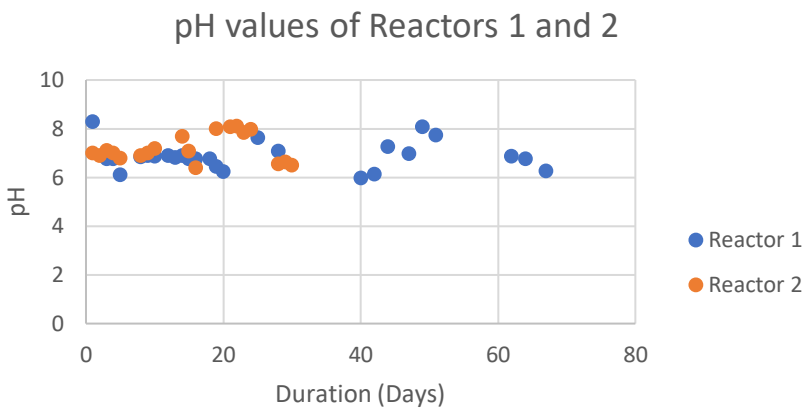


Figure 4.5 pH values of both Reactors I and II

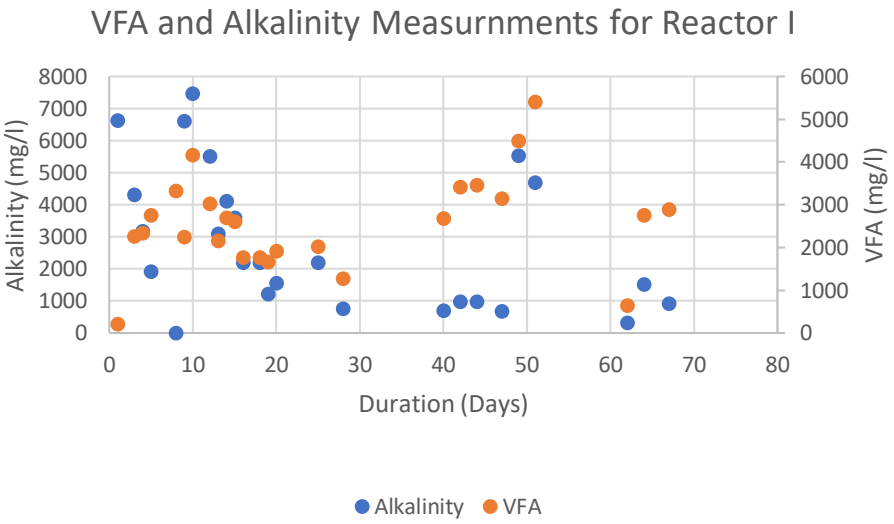


Figure 4.6 VFA and Alkalinity profiles for Reactor I

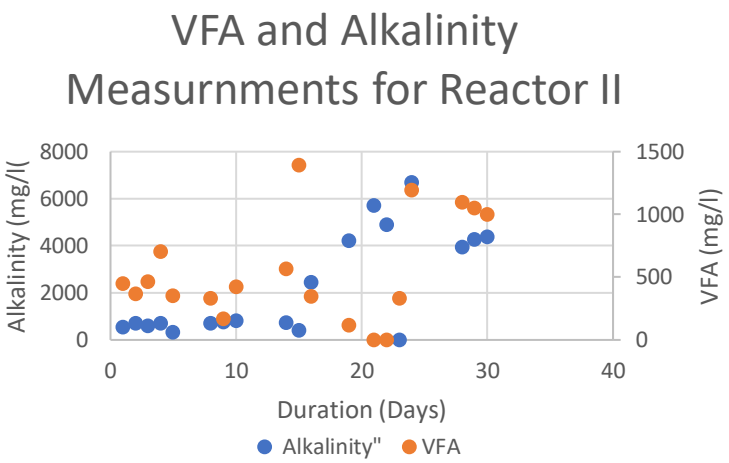


Figure 4.7 VFA and Alkalinity profiles for Reactor II

Figure 4.6 shows that the VFA and alkalinity values were extremely high in Reactor I. A 5000mg/l is the maximum amount of VFA recorded in Reactor I. Also, the addition of buffer lead to a noticeable increase in the alkalinity after days 5 and 40. Regarding reactor II, the VFA and alkalinity concentrations were rounding on the average, with an average of 500mg VFA/L and around 2000 mg/L for alkalinity.

4.1.5 Nutrients Availability

Nutrient measurements were done on a regular basis, like the COD and the rest of units measured. The collected data is summarized and presented in table 4.1. The error margin could be higher in this section since the filtration of the wastewater before measurements should not be done when sampling. In general, both Reactors I and II did not perform well in the nutrient removal efficiency which is going to be discussed in the next section. An average of 35% removal efficiency was not achieved here, where many times the values in the outlet were higher than that in the inlet.

Date	Total Phosphorous (mg/l)		Total Nitrogen (mg/l)	
	Inlet	Outlet	Inlet	Outlet
Reactor I				
Day 13	16.5	20	14	28
Day 25	13.7	13.4	12	22
Day 40	14.9	14.2	28	24
Day 51	13.9	12.9	4	34
Day 67	8.4	9.5	12	19
Reactor II				
Day 1	12.9	14.2	22	26
Day 10	13.2	12.9	18.3	31.2
Day 19	7.2	8.7	5	4
Day 30	6	6.1	16	17

Table 4.1 The concentrations of TP and TN in the inlet and outlet within time.

4.1.6 Granular Density

Granules density for experiment I decreased from 0.96 g/ml before initiating the experiment to reach 0.75 g/ml. note that a noticeable quantity of granules was dissolved in water and were not in a semi-solid state.

Regarding Experiment II, the measurement afterwards did not occur since the experiment is still going for further studies.

5. Discussion

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

5.1 Reactor Acclimatization

The up-flow velocity in the Reactor is driven by the feed injection to the Reactor, along with the reflux through the recirculation pump. Both are connected to form a single inlet which is entering the Reactor from the bottom. The average velocity within the UASB reactor ranges between 0.5 to 1 m/h [34].

Due to the high HRT (3 and a half days) used in Reactor I (to avoid a high OLR), up-flow velocities were around 0.01 m/h, far away from the optimal values. This low value was set to avoid a sudden shock for the inactivated granules. At that time, the Reactor was in a critical situation with old granules and low performances, which requires gentle mixing in the system. The choice of the inlet flow rate was taken based on trial and error for different flow rates. The recirculation factor should be high enough for proper mixing. However, it should still be a gentle mixing mode to avoid too many granules being washed out or deformed, and high sludge retention cannot be accomplished. A good UASB should have high sludge retention. The COD removal was not responding within time (once 50% removal), so the Vup kept constant. The acclimatization of Reactor I was not achieved since the activity was almost null.

Regarding Reactor II, the acclimatization took eight days to happen. First, the Vup was 0.03 m/h in the first seven days, which was increased to 0.05 m/h on the 8th day. The COD removal was doubled after this period (from 40 to 80%).

The Reactors recirculation ratio was similar to the 100:1 ratio, the recirculation flow concerning the inlet flow rate. While for Reactor II, it was a more efficient value with a 13:1 ratio. An effective ratio is set to be around 10:1 [32]. This will create more agitation and contact between the inlet and the granules found in the Reactor.

The food to the mass of the substrate ration (F:M) is the balance between the food injected into the system to the mass of available biomass (granules mass). An average ratio of F:M (average) can enhance the removal efficiency and granulation process. At the same time, a high ratio can lead to disturbing methane production and COD removal [21]. In Reactor I, F:M was higher than what was recorded in Reactor II due to the higher COD numbers in the inlet. That explains the lower percentage of removal and the traces of biogas production.

5.2 Reactor Performance

In this sub-chapter, reactor performance is analysed and interpreted checking the following parameters i) COD recovery, ii) methane production, and iii) COD balance. These are the main parameters to determine how effective was the anaerobic treatment. A comparison between Reactors I and II is discussed as well.

5.2.1 COD Removal Efficiency

Figures 4.2 and 4.3 demonstrate the COD in the inlet and the outlet of both reactors. The removal efficiency is calculated and appropriately expressed in figure 4.6. at the same time, the OLR is presented separately in figure 4.3 for both reactors. Generally speaking, when the OLR is increased, the COD removal efficiency is noticed a sudden decrease. The high OLR provokes the performance of the bacteria inside the Reactor, which stops the high conversion of the COD into biogas. After the medium is stabilized with the new concentrations, we have noticed a massive increase in the efficiency values (50% on day 42 for Reactor I and an increase of 37 % on day 13 in Reactor II) by decrease the value of COD in the effluent after achieving a steady-state condition. It can also refer to the adaptation of the bacteria to the new conditions.

In other words, the increase in COD concentration within the system will proportionally influence the specific growth rate, which will increase to reach the maximum specific growth rate (μmax). The food to mass (F:M) ratio is supposed to decrease over time due to the presence of biomass in the Reactor. A low F:M ratio means that the available food is limited. Thereby the bacteria form a dense floc which settles in the bottom. This will lead to an increase in the removal efficiency and enhance the granulation process. Usually, it is stable when the conditions are optimal, which leads to an increase in the activity and reproductivity of the bacteria (maximal population found at the ideal F:M ratio).

In Reactor I, during the whole process, the Reactor showed instability except day 42. The removal percentage was ranging between 5-30 %. While doing the sampling from the Reactor, a daily washout of granules was occurring, which explains the high COD values in the effluent. Furthermore, a decrease of 4cm in the granule's height was noticed from the start until the end of the Reactor, which can be explained in the hydrodynamics of the granules (next section). Another explanation for that can be the fluctuating pH recorded many times during the 67 days (8-5.9). These values were monitored precisely, and pH values could be lower than those without adding the buffer solution (NaHCO3) to the wastewater in the influent. Also, the 0.5M HCl liquid in the gas counter washed out of its place, and it might have reached the Reactor due to a non-stabilized pressure gradient (the gas counter was in a higher position over the Reactor).

Regarding Reactor II, an efficient COD removal was detected after 14 days of initiating the Reactor (74%). While the maximum removal occurred on day 19 with efficiency equals 84%. In general, the average COD removal efficiency decreases with increasing OLR. The decreased efficiency also was observed with the increasing OLR. The increasing OLR increases the biomass growth rate until it reaches a maximum specific growth rate (μmax). The COD in the inlet was gradually increased, from 700 to 1500. The Reactor first could not keep its removal rate high until it is adapted to the new modifications. Even though the COD inlet was considered high on day 30 (1250 mg COD/L), the Reactor was still able to remove more than 80% of the COD presented. Therefore, μmax was not achieved at low inlet substrate.

5.2.2 Methane Production

In general, the performance of a UASB reactor is evaluated by inspecting the methane production during the anaerobic digestion besides the COD removal efficiency. Granules in Reactor I had no specific methane production percentage. Unfortunately, there was no continuous biogas production during the whole interval (67 days) due to the previously mentioned reasons. The liquid runoff back to the system from the gas meter can also prevent the gas from flowing up from the UASB reactor, which might either be stuck in the upper zone of the Reactor or run off to the effluent. Another cause can be related to the gas separator that might not be perfectly working. Also, the granules washed out in the effluent relates directly to the decrease in biogas production [Anissa's thesis]. In addition, there might be gas leakage that was not detected, and the methane might have a high solubility in the wastewater, which is the main reason for methane loss.

At STP, the theoretical value of methane production corresponds to 0.375 l CH_4/g COD when the COD efficiency removal is 100% for granules used in Reactor II. While for the same reasons mentioned above, around 50ml/hr of biogas was generated during the last days of operating the Reactor (27-30 days). Nevertheless, the amount of methane generation is not clear enough.

We were not lucky enough to interpret the influence of the OLR and HRT on the production rate of biogas and methane in both reactors. The biogas quality is determined by the percentage of methane in the biogas, where other gases such as sulfides are excluded from the assumption to make it easier [23].

5.2.3 COD Balance

Another monitor for the performance of a UASB reactor is the COD balance. It allows the researcher to interfere whenever needed to counteract an uncontrolled situation. The overall average of COD recoveries was around 30% and above 80% in Reactors I and II, respectively. The calculation excludes COD in biomass/ sludge since COD biomass was not determined. Total COD is a fractionation of the inlet and outlet of the reactor. A certain number of granules were washed out of the system in Reactor I, which might be the main reason behind a low COD recovery (only 30%). Regarding Reactor II, the proportion of COD recovered is almost 80% (on the last days of the experiment), while the COD converted to biomass stands for 20%.

The presence of LCFA found in the substrates leads to a high removal efficiency of COD while the CH4 production rates are low. This makes the COD balance unstable [23]. The experimental results did not match the theory, especially when it comes to removal rates, but it can explain the deficient production of gases in Reactor I.

Things are different in Reactor II, where LCFA are not found in the vegetables and potato residues. On the other hand, the granules are rich in SO_4^{2-} and NO_3^-, where their presence can make a loss in electrons since they are potent oxidizing agents. Biogas specifications and the ions

5.3 Environmental Factors

For The VFA were monitored and measured periodically by the previously mentioned titration method to maintain and counteract any unusual values, which might hit the continuity of the anaerobic process. However, specific monitoring for the compounds found in the effluent was not carried out, such as propanoic or butyric acids, due to the lack of machinery (GC).

In Reactor I, up until the formation of VFA in the fermentation process was working well. The last process, methanogenesis, was not performing correctly. Figure 4.6 shows the high values of VFA's accumulated in the effluent. The recorded data shows that the amount of VFA presented in the effluent is about 20 times higher than the average values. A record of 5400 mg HAc/ l was reported on day 51. To counteract the extremely high values, buffer solution was injected to influent many times throughout the whole period to stabilize the pH (on days 5 and 47), which explains the high alkalinity values (3000mg CaCO3/L). Also, excessive availability of a buffer solution within the system will raise the pH and make it far from the required one (pH 7). The pH on day 51 was 7.74, as shown in Figure 4.5. These conditions will lead to more accumulation of VFA within the anaerobic processes. Measuring the VFA throughout the titration shows the available acetic acid generated in the system. The high accumulation of VFA led to a decrease in pH in several days (especially after day 40), which affected the productivity of the Reactor by inhibiting the methanogenic bacteria (efficiency stayed low, and no further biogas production). Adding sodium bicarbonate, especially within the first days of initiating the Reactor, is required for maintaining a pH of 7, which keeps the system stable [50]. After more than 60 days to save the granules, it was impossible to have a better result due to their vanished activity.

Meanwhile, in Reactor II, the situation is different. The VFA was within the typical values throughout the period except for days 15 and 24, shown in figure 4.7. The alkalinity was stable despite some high values, which can be interpreted as right before adding new wastewater, which happened on days 17 and 27 (settlement of buffer in the bottom of the Reactor).

The presence of H_2 controls the generation of the acids in the system (acetic, propanoic, and butyric). [23]

Nutrient availability is an essential factor in analysing the performance of the Reactor. The bacteria consume phosphorous and nitrogen for the growth process in an anaerobic medium by providing raw materials for cytoplasmic materials synthesis, serve as an energy source and electron acceptors [23].

5.4 Economy and Energy Recovery

A successful UASB plant is measured by the production of the product, methane biogas. The construction cost of anaerobic treatment systems is more expensive than the aerobic ones, while the operational expenses are more diminutive in the aerobic process. The operational cost is solved by the value of the methane and especially when it is presented in good quality (no presence of sulfides) [23].

An average of 1.5kWh of electricity can be produced from 13.5 MJ CH_4 energy/ kgCOD assuming a 40% conversion, while the rest will be in the form of heat. [9].

Unfortunately, the methane yield in Reactor I was neglected since the conversion rate was also low.

On the other hand, despite the gas meter failure, the removal rate ranges between 1 to 2 gCOD/ l.d. The optimum biogas conversion efficiency of 70% could have been reached but was not recorded due to machinery failure.

6. Challenges and Limitations

This chapter discusses the challenges that faces students while running a UASB especially using long stored granules. The following Sub-chapters discusses the pressure instability, pH, alkalinity and VFA values, Gas Measurement Failure, and Granule's washout.

6.1 Pressure Instability

While running Reactor I, a pressure drop occurred after 40 days of running the experiment. The liquid present in the gas counter (0.5M of HCl) disappeared once in a sudden from its place. There were no traces of leakage beside the counter, so the only explanation is that the liquid flow back to the system due to a failure in the pressure. Traces for the liquid were found in the connecting tube between the Reactor and the first gas counter.

After this incident, a rotten layer started to form at the top of the Reactor, as shown in figure 6.1. This might have happened due to the formation of high quantities of H_2S, which eventually turned rotten. [23]

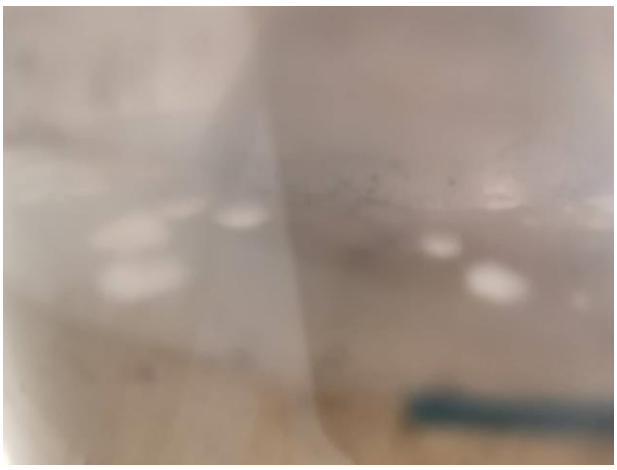


Figure 6.1 the rotten found in the top region of the reactor (in the liquid-gas separation section)

Also, it was hard to collect a sample from the effluent during the same period. The liquid flow out of the system was prolonged, although the volume of the liquid was above the effluent line. This can only be explained by a sudden pressure drop that might occur by the density and viscosity difference and the fluid speed in the gas and liquid form [53].

6.2 pH, Alkalinity, and VFA

In general, a neutral pH is a perfect medium for granules formation. Getting low to pH of 6.3 can still run the process until it reaches the methanogenic process (most sensitive to the pH) [8]. The acidity in the effluent has the ability to withstand pH changes caused by the bases, Carbon dioxide, VFAs, and alcohols. It is mainly controlled by free carbon dioxide, VFA, and hydrogen sulphide, synthesized during digestion. Balancing the VFA in an anaerobic reactor is essential since the biochemical process is ruined in high concentrations. This leads to disturbing the anaerobic digestion; Thus, the Reactor will collapse. A value over 150mg/l for VFA indicates that the Reactor is not performing ideally [40]. In Reactor I, various samples showed a pH below 6.3 (days 5, 40-42, and the last day) influenced by the high VFA values recorded. Also, HCl is an acidic compound, and this leakage to the system is another reason for affecting the pH within the system.

After day 5, exactly, 20grams of buffer ($NaHCO_3$) was added to the system to counteract the sharp pressure drop. The step was repeated few times throughout the 67 days. The buffer addition creates an increase in the alkalinity, which recorded 7500 mg/ L. Also, the degradation of proteins and lipids will result in ammonium bicarbonate generation (buffer) after the reaction between ammonium and carbonic acid [40]. The optimum alkalinity value should range between 0.16 and 0.18 of COD value in the inlet [45,53]. The excessive amount of buffer also inhibits the limiting nutrient reaction within the system, which affects the anaerobic respiration of the bacteria.

6.3 Gas Measurement Failure

While running experiment I, few bubbles were spotted flowing from the granules to the upper section of the reactor. Although the number of bubbles was not noticeable, this is an indicator of a gas flow. The gas counter kept on showing just spikes of measurements which are not continuous. After a while, the backflow of the liquid, which is described previously, happened. We filled it in several times and witnessed. The water disappeared suddenly, and traces were found in the connecting tubes. The gas meter was located in a position that is higher than the gas outlet of the reactor. To solve this issue, we managed to decrease the gas

indicator's height to below the reactor's level. The liquid inside the reactor stayed packed until the end of the interval.

In Reactor II, we kept the gas equipment in a fixed location. The bubbles inside the reactor were evident after ten days of initiating the reactor. Although everything looked normal inside the gas meter, gas production did not reach the desired values even. No leakage was detected after we run a gas leakage detector test. A probable crack happened to the gas meter after losing its liquid. This affected the whole gas counting process since the methane gas measurement was not recorded as well. It could also be that some of the liquid ran off to the gas separator beaker, besides turning back to the reactor.

We could have avoided this issue by installing a new connecting system and machines before starting the second experiment.

6.4 Granules Washout

Most of the samples taken out of Reactor I included a noticeable quantity of granules. They are carried up by the water coming in the inlet, along with the gas bubbles. Approximately a quarter of the granules was washed out during the 67 days. Usually, this can happen due to a) inappropriate up-flow velocity, b) daily flow concentration, c) concentration of the biomass and d) Sedimentation [20]. As the HRT is high, the up-flow velocity has no influence. The size of the granules was big (around 3mm); they were also semi-dry and hydrophobic due to the repulsion when we put them in the Reactor. It explains that the sedimentation process did not occur appropriately since the size is already occupied, and the granules are almost in their most considerable size.

The washout has caused a false reading for most nutrients, VFA, alkalinity, and even conductivity. This affects the role of the researcher in following and fixing the error. Also, it has caused clogging in most tubes due to the re-flux used in the design (we had to interfere many times to avoid shortcuts).

Economically speaking, this washout has a high cost since the desired product (biogas) will decrease.

7. Conclusion

UASB reactor is becoming more common worldwide to produce renewable biogas and treat municipal and industrial wastewater streams. In this review, a comparison was made between the performance of inactive granules (Reactor I) and the active ones (Reactor II) under the following conditions for both reactors: psychrophilic conditions (16°C for the Reactor, and 0 to 4°C for the inlet wastewater), similar reflux ratio, same apparatus used, the exact recipe for the synthetic wastewater, while the HRT was different, 84 hours and 31-17 hours for Reactors I and II respectively. The COD removal rate did not reach the expectation and barely counted for 20-30% removal at up-flow-velocity of 0.01m/h in Reactor I, while in Reactor II, it was above 80% just within ten days of initiating the Reactor under the up-flow velocity of 0.05 m/h.

The barriers that challenged us while running both reactors are adequately explained in this review. The instrumental failure prevented us from recording the actual quantities of biogas produced. Also, the nutrient removal in both reactors was not satisfying, although the nutrients in the inlet were constantly monitored to avoid subjecting the anaerobic digestion to a limiting nutrient condition. Modelling techniques could be implemented (such as ADM1) to study the reactors' kinetics and hydrodynamics [53]. It is essential to precisely observe the granulation process by knowing the quality and size of the granules throughout the timeline.

The high VFA accumulation in Reactor I raises concerns about the feasibility of fixing the situation inside the Reactor. An alternative could be applied to get the optimal benefits of the Reactor by changing the goal of the Reactor from producing biogas to increase the VFA

Despite the high COD removal efficiencies achieved in Reactor II, more than 20% of COD remains in the effluent, which requires post-treatment action. Besides, a limit of nutrient concentrations is obliged before disposal, which indicates that the process still requires nitrification, denitrification (which always does), or phosphate removal.

8. Recommendations

Biogas produced during AD could be a useful resource to substitute the traditional electricity generation techniques which can be managed locally. A broader research on the techno economic scale-up feasibility to integrate biogas as a renewable energy, despite focusing on different substrates. A detailed process modelling that is based on criteria indicators and Life cycle assessment (LCA) allows the optimization of energy recovery and other environmental factors within the biogas exploitation [7,34].

This chapter is divided into sub-chapters to discuss i) Pre-treatment, ii) Post Treatment,

8.1 Pre-treatment

Pre-treatment is a preparatory procedure to get rid of the impurities that can cause adverse effects. In our case, it is used to elevate the methane yield, which depends on the characteristics and physicochemical composition of the substrate. The possible techniques can be chemical (acidity, alkalinity), biological (enzymes), thermochemical (supercritical points, oxidation), thermophysical (microwave, ultrasound), and physical (reducing the size). For example, a pre-hydrolysis reduces the fraction of particulate COD that degrades slow, ensuring a higher performance of the UASB reactor even with high OLR. To improve the biogas yield and reduce any accumulation of VFA, a packed bed reactor could be installed straight before the UASB reactor as a chemical pre-treatment method in onion waste treatment. Press liquid, or leachate, is a mechanical method to separate the solids from the liquid and produce a highly degradable fluid that efficiently produces a higher methane yield. E. coli and ultrasound reduce the concentrations of LCFA, which protect the methanogenic bacteria from being inhibited. The recent studies focus on increasing the biodegradability of the substrate in the terms of lignocellulosic steams, but it is limited by favouring the VFA accumulation over methanogenesis. It is essential to investigate the effectiveness of scaling up a pre-treatment process through techno-economic and environmental aspects. Will the increased production of methane cover the cost of installation and running those technologies? Also, it is vital to know the impact of the used chemicals on the environment Reactor II can introduce these techniques due to its stability and performance, while it is hard to apply on Reactor I due to its condition [7,34].

8.2 post-Treatment

It is necessary to run such a process to comply with the legal standards of disposal of nutrients, solids, and pathogens. An innovative post-treatment technique based on high-rate algal ponds is being developed, which operates under standard temperatures. This step ensures 60% more removal of nutrients and COD, besides 25% more methane produced. A successive microalgae post-treatment apparatus (using Chlorella sorokiniana) proven to have higher removal efficiencies despite fluctuation in the OLR [7,34].

Also, electrochemical treatment is another well-established technique for removing polyaromatics, lignin, phenolic compounds, and humic acids. The experiment can be performed using a filter-press cell with a two-plate electrode, separated by a Teflon layer of width that can be determined on purpose (usually 5mm). This system has a very high operating cost due to the consumption of power and oxygen besides other chemicals. Also, sludge production could be problematic due to its ethical and legal disposal [11].

Electrocoagulation, another used technology for post-effluent treatment, is a complex process of the following steps: electrolytic reactions, coagulants formation, adsorption of pollutants on the coagulant, and then removed by flotation or sedimentation. The solubility of metal hydroxides, besides the pH, directly influences the efficiency of the process.

9 Proposal

There is a recent interest in biofuel production from renewable biomass resources to create an alternative for traditional crude oil to decrease greenhouse gases (GHG) emissions after being burnt. VFAs consist of propanoic, butyric, and acetic acid. Those organic chemicals can be used in various applications such as food processing, pharmaceutical, industrial, and biofuels production. VFAs can produce alcohols after undergoing a simple hydrogenation reaction and ketones by the ketonization process [19,29]. The produced ketones can fit replacing the gasoline since the molecular size will be similar after an aldol condensation of the ketones. However, the effectiveness of biofuel production depends on the conversion cost [50].
Based on the high VFAs produced in Reactor I (up to 40 times than the average value), the optimal benefits from the reactor could be by changing the aim from biogas production into VFAs utilization. In other words, inactive granular digestion in a UASB by synthetic wastewater aiming to produce VFA.
The product (VFAs) will be beneficial and have a wide broad range of usage. Here are the industrial applications of these chemicals: a) **Bioplastic production**, the chemical structure of the VFA controls the composition and the properties of the produced plastic, even though the end product will be efficient due to the economic and environmental feasibility. Polyhydroxyalkanoates are synthesized from the VFAs while being biodegradable and depending on renewable sources. b) **Hydrogen Source,** under anaerobic conditions, hydrogen is produced from VFAs by the fermentation of *Rhodobacter Sphaeroides* bacterium by hydrogenase enzyme. Also, hydrogen production is strictly affected by the type of chemicals that formulate the VFAs. C)**Biological nutrient removal** can replace methane or methanol usage during anoxic denitrification processes. Also, it can be used as an electricity source (without any treatment) and microbial lipids for biodiesel. Besides, VFAs are key building blocks in most chemical industries such as textile, plastic, and synthetic fibers and are considered attractive for the food and pharmaceutical industries [6].
A techno-economic analysis is conducted to check on the cost of production of carboxylic acid from VFAs. The total operational cost (including the 10% return rate) was just below 400$, while the standard value of carboxylic acid ranges between 1200 to 1500$ [16].
As the rise of energy production from renewable resources has a considerable rise, especially in Norway, it is also essential to decrease the dependence on fossil fuels in petrochemical products which stands for 28% of the annual consumption of oil [47].

Starting the reactor: the design can be exactly the same as we did for Reactor I, psychrophilic condition to inhibit the methanogens bacteria, a low pH (less than 6.4), and using inactive granules after being stored for a determined period. The goal is to employ the required conditions to produce the desired product. It is important to know that this process will not require a pre-treatment installation, and if low-lignin biomass is used will not require further post treatment. The biomass cost counts for 40-80% of the total operational expenses which classify it economically feasible. The reactor can treat a high strength wastewater which could be beneficial for both, industrial and urban wastewaters. A reactor can convert around 0.37g VFA/g COD with an inlet COD of 12g/L.D from Chlorella vulgaris [29,55].
The reactor can be kept running in the same way as a normal UASB is monitored, in order to interfere in case it is needed to do so.
This process is convenient for Norway because of the following: First, almost all the electricity production in Norway comes from renewable energy (hydropower, thermal, and wind turbines) [37]. Also, the continuous calls from the Norwegian society to stop the exploration for oil in the North Sea which support the entrepreneurs for launching their innovative ideas with full support provided by the investors and the public sector [52].
The application can improve Norway's innovative chemical industries, while keeping the environmental concerns as a priority.
Around 2700 kgCOD (give or take) needed to produce a ton of the VFAs (the desired product) [55]. An average of 190 L/ d of wastewater are generated per capita. Four and a half million in Norway had access to wastewater treatment facilities in 2017 [56]. An average of 800mg COD/ L is taken into consideration in this study. So, around 150 gCOD are produced per capita daily. A production of 675000 Kg of COD is produced daily in Norway. This can produce around 250 tons of VFAs per day with a minimal value that ranges between 4000 to 5000 NOK for ton. An estimation of 400 million NOK could be collected from the production of VFAs. This counts for around 5% of the total cost of operation in Norway (7.9 Billion NOK in 2017) [56].

KgCOD generation Per Day	Tons of VFA generated Per Day	Tons of VFA generation per Year	Average Price of a ton of VFA (NOK)	Annual Revenues (Million NOK)	Annual Operational Cost (Billion NOK)	% of Cost covered
675000	250	91250	4500	405	7.9	5

Table 9.1 A detailed presentation of the percentage of total operational cost of wastewater treatment in Norway, covered by the generated VFA.

In conclusion, the less non-biodegradable substances trapped in the lands and oceans is on the same level of importance of the economic revenue. Our duty toward the current and future generations forces us to keep the eyes open on every single innovative idea that could protect their life and continuity.

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Appendixes:

Appendix 1. Biogas production data of Reactor I

DAYS	GAS PRODUCTION						METHANE PRODUCTION					
	V1	Time	V2	Time	V3	Time	V1	Time	V2	Time	V3	Time
	ml	-	ml	-	ml	-	ml	-	ml	-	ml	-
0												
1												
3												
4												
5												
8												
9												
10												
12	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
13	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
14	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
15	6.52	8am	6.52	10am	0	12pm	0	8am	0	10am	0	12pm
16	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
18	166.3	8am	166.3	10am	169.5	12pm	97.44	8am	97.44	10am	97.44	12pm
19	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
20	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
21	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
22	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
23	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
24	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
25	3.62	8am	3.62	10am	3.62	12pm	0	8am	0	10am	0	12pm
26	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
27	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
28	6.52	8am	6.52	10am	6.52	12pm	0	8am	0	10am	0	12pm
29	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
30	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
31	35.86	8am	35.86	10am	35.86	12pm	6.96	8am	6.96	10am	6.96	12pm
32	26.08	8am	26.08	10am	26.08	12pm	0	8am	0	10am	0	12pm
33	0	8am	0	10am	0	12pm	0	8am	0	10am	0	12pm
40	3.48	8am	3.48	10am	3.48	12pm						
42	0	8am	0	10am	0	12pm						
44	0	8am	0	10am	0	12pm						
47	0	8am	0	10am	0	12pm						
49	0	8am	0	10am	0	12pm						
51	0	8am	0	10am	0	12pm						
54	0	8am	0	10am	0	12pm						
55	0	8am	0	10am	0	12pm						
58	0	8am	0	10am	0	12pm						
61	0	8am	0	10am	0	12pm						
62	0	8am	0	10am	0	12pm						
64	0	8am	0	10am	0	12pm						
67	0	8am	0	10am	0	12pm						

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

Appendix 2. Biogas production data of Reactor II.

DAYS	GAS PRODUCTION						METHANE PRODUCTION					
	V1	Time	V2	Time	V3	Time	V1	Time	V2	Time	V3	Time
	ml	-	ml	-	ml	-	ml	-	ml	-	ml	-
1	0	8am	0	10am	0	12pm						
2	0	8am	0	10am	0	12pm						
3	0	8am	0	10am	0	12pm						
4	0	8am	0	10am	0	12pm						
5	0	8am	0	10am	0	12pm						
8	0	8am	0	10am	0	12pm						
9	0	8am	0	10am	0	12pm						
10	0	8am	0	10am	0	12pm						
14	0	8am	0	10am	0	12pm						
15	0	8am	0	10am	0	12pm						
16	0	8am	0	10am	0	12pm						
19	0	8am	0	10am	0	12pm						
21	588.1	8am	0	10am	0	12pm						
22	0	8am	0	10am	0	12pm						
23	0	8am	0	10am	0	12pm						
24	0	8am	0	10am	0	12pm						
27	2200	8am	2300	10am	2400	12p,	3.26	8am	3.26	10am	3.26	12pm
28	1124	8am	1172	10am	1220	12p,						
29	220	8am	245	10am	271	12p,						
30	365	8am	400	10am	438	12p,						

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

Appendix 3. Total COD Analysis Data

Duration (day)	COD in Reactor I(mg/l)	COD in Reactor II(mg/l)
0		
1		756
2		689
3		702
4		699
5		689
8		677
9		703
10		643
12	1100	
13	1074	
14	944	690
15	1026	700
16	1074	684
18	1060	
19	974	958
20	1038	
21		872
22		856
23		880
24		966
25	876	
26		
27		
28	864	1510
29		1398
30		1224
31		
32		
33		
40	>1500	
42	3334	
44	3380	
47	3020	
49	2920	
51	3380	
54	2930	
55	2740	
58	2720	
61	2680	
62	1200	
64	1500	
67	1400	

Duration (day)	COD out Reactor I (mg/l)	COD out Reactor II (mg/l)
1		705
2		563
3		426
4		364
5		351
8		436
9		402
10		399
12	1077	
13	992	
14	910	180
15	894	180
16	942	146
18	1054	
19	880	154
20	892	
21		174
22		192
23		238
24		294
25	740	
26		
27		
28	844	768
29		496
30		232
31		
32		
33		
40	1348	
42	>1500	
44	2800	
47	2980	
49	2100	
51	2660	
54	2760	
55	2620	
58	2550	
61	2380	
62	3660	
64	1840	
67	1380	

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

Appendix 4. OLR in Reactors I and II

Duration (day)	COD in Reactor I (mg/l)	OLR Reactor I (ml/min)	Duration (day)	COD in Reactor II (mg/l)	OLR Reactor II (ml/min)
0	1200	0.508984	1	756	0.580608
1			2	689	0.529152
3			3	702	0.539136
4			4	699	0.536832
5			5	689	0.529152
8			8	677	0.97488
9			9	703	1.01232
10			10	643	0.92592
12	1100	0.466568	14	690	0.9936
13	1074	0.455541	15	700	1.008
14	944	0.400401	16	684	0.98496
15	1026	0.435181			0
16	1074	0.455541	19	958	1.37952
18	1060	0.449602	21	872	1.25568
19	974	0.413125	22	856	1.23264
20	1038	0.440271	23	880	1.2672
21			24	966	1.39104
22					0
23					0
24			28	1510	2.1744
25	876	0.371558	29	1398	2.01312
26			30	1224	1.76256
27					
28	864	0.366468			
29					
30					
31					
32					
33					
40	>1500				
42	3334	1.414127			
44	3380	1.433638			
47	3020	1.280943			
49	2920	1.238527			
51	3380	1.433638			
54	2930	1.242769			
55	2740	1.16218			
58	2720	1.153697			
61	2680	1.13673			
62	1200	0.508984			
64	1500	0.63623			
67	1400	0.593814			

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

Appendix 5. COD removal percentage for Reactors I and II

Days	COD removal % Reactor 1	Days	COD removal % in Reactor 2
12	2.09	1	6.75
13	7.64	2	18.3
14	3.6	3	39.3
15	12.87	4	47.9
16	12.3	5	49.1
18	0.57	8	35.6
19	9.65	9	42.8
20	14.1	10	37.95
25	15.53	14	73.91
28	2.31	15	74.3
40	12.3	16	78.7
42	50	17	
44	17.2	19	83.9
47	1.32	21	80.05
49	28.1	22	77.6
51	21.3	23	73
54	5.8	24	69.6
55	4.38	25	
58	6.25	26	
61	11.2	28	49.1
62	-205	29	64.5
64	-22.667	30	81.05
67	1.43		

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

Appendix 6. Alkalinity and VFA values for reactors I and II

	Reactor1			Reactor 2	
Duration (day)	Alk(mg/l)	VFA(mg/l)	**Duration (day)**	Alk(mg/l)	VFA(mg/l)
1	6626.1	205.2	1	532	450
3	4318	2266.1	2	693.5	369
4	3182.9	2336.7	3	602.2	463
5	1915.1	2748.8	4	706.5	703
8	10, 631.6	3318.3	5	320.5	352
9	6600.5	2252.7	8	695.5	333
10	7464.5	4159.4	9	762.2	169
12	5513	3029.4	10	804	423
13	3087	2159.1	14	738.9	565.8
14	4117	2693.6	15	412.1	1390.6
15	3596.1	2605	16	2436.4	347.7
16	2197.1	1760.2			
18	2197.1	1760.2	19	4213.5	114.9
19	1222.7	1657.9	21	5708.6	0
20	1559.9	1917.5	22	4891.2	0
21			23	4723,9	332
22			24	6692.4	1194.3
23					
24					
25	2199.8	2017.8	28	3957.7	1096.6
26			29	4276.7	1051.7
27			30	4367.6	998.5
28	760.6	1266.5			
29					
30					
31					
32					
33					
40	695.8	2685			
42	970.3	3411.2			
44	975.1	3453.8			
47	687.3	3141.3			
49	5529.6	4492.7			
51	4682.7	5401.1			
54					
55					
58					
61					
62	327.1	647.7			
64	1512.9	2747.5			
67	923.6	2888.4			

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

Appendix 7. Recorded pH values for reactors I and II.

Duration (day)	pH in reactor 1	pH in reactor II
1	8.29	7
2		6.9
3	6.76	7.1
4	6.76	7
5	6.12	6.8
8	6.84	6.9
9	6.91	7
10	6.87	7.2
12	6.91	
13	6.82	
14	6.9	7.7
15	6.76	7.08
16	6.78	6.41
18	6.78	
19	6.46	8.01
20	6.24	8.07
21		8.12
22		7.84
23		7.97
24		
25	7.64	
26		
27		
28	7.08	6.55
29		6.63
30		6.5
31		
32		
33		
40	5.97	
42	6.14	
44	7.27	
47	6.97	
49	8.08	
51	7.74	
54		
55		
58		
61		
62	6.88	
64	6.76	
67	6.27	

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