# FACULTY OF SCIENCE AND TECHNOLOGY

# BACHELOR'S THESIS

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# ANAEROBIC TREATMENT OF LOW TO MEDIUM STRENGTH MUNICIPAL WASTEWATER IN LABORATORY SCALE UP-FLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTORS

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# Abstract

The focus of this study is assessing and exploring the potential of anaerobic treatment for municipal wastewater of low- to medium strength (i.e., 212 mg/l COD and 931 mg/l COD) for converting organic matter into methane in up-flow anaerobic sludge blanket (UASB) reactors. Two laboratory-scale UASB reactors were set up in the study and were investigated for 76 days; one reactor (Reactor Vik) treated medium strength wastewater; and the other reactor (Reactor SNJ) treated low strength wastewater. The performance of the reactors was assessed based on the efficiency of chemical oxygen demand (COD) removal and their ability to generate methane. Reactor Vik obtained an average COD removal efficiency of 60% towards the end of the operating period, while Reactor SNJ attained an average COD removal efficiency of a negative 3%. The methane production increased with an average of 40 ml/d when the OLR increased in Reactor Vik. However, there was an overall lack of methane production despite increase in OLR in Reactor SNJ. The results obtained indicate a significant COD removal efficiency and methane production when treating medium strength wastewater in Reactor Vik, in contrast to the treatment of low strength wastewater in Reactor SNJ. The observation of high sulfate concentration in Reactor SNJ led to a hypothesis suggesting that the reactor is inhibited by hydrogen sulfide. The average methane yields obtained in the last 10 days of observation in Reactor Vik were 0.43 $gCODCH_4/gtCOD$ removed and 0.49 $gCODCH_4/gsCOD$ removed. Under these conditions, approximately 41.6 GJ/d of energy which is equivalent to 4624 kWh/d of electricity could be recovered at IVAR Vik wastewater treatment plant which has approximately 8000 $m^3/d$ of average hydraulic loading based on an estimated daily removal of 3083 kg soluble COD. Anaerobic treatment using UASB reactor for treating the medium-strength wastewater at IVAR Vik wastewater treatment plant represents a feasible and promising alternative as pre-treatment for the aerobic biological treatment. Through this process, a reduction to 250 – 300 mg/l soluble COD is achieved, and organic matter is converted into economically valuable product as methane with a production rate of 1080 $m^3/d$.

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

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Stavanger, May 2023

Belinda Quale, Ingvild Moe and Sara Lovise Haga

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# Abbreviations

| | |
|---|---|
| AMB | Acetoclastic Methanogenic Bacteria |
| ATP | Adenosine Triphosphate |
| BOD | Biochemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| EPS | Extracellular Polymeric Substances |
| ESGB | Expanded Sludge Granular Blanket |
| GC | Gas chromatography |
| HMB | Hydrogenotrophic Methanogenetic Bacteria |
| HRT | Hydraulic Retention Time |
| IC | Ionic Chromatography |
| IVAR | Interkommunalt Vann Avløp og Renovasjon |
| LCFA | Long Chain Fatty Acid |
| OHPB | Obligate Hydrogen Producing Bacteria |
| OLR | Organic Loading Rate |
| pCOD | Particular Chemical Oxygen Demand |
| SCFA | Short Chain Fatty Acid |
| sCOD | Soluble Chemical Oxygen Demand |
| SNJ | Sentralrenseanlegget Nord-Jæren |
| SRT | Sludge Retention Time |
| tCOD | Total Chemical Oxygen Demand |
| TDC | Thermal Conductivity Detector |
| TSS | Total Suspended Solids |
| UASB | Up-flow Anaerobic Sludge Blanket |
| VFA | Volatile Fatty Acid |
| VSS | Volatile Suspended Solid |
| WWTP | Wastewater treatment plant |

# 1 Introduction

## 1.1 Background and motivation

The demand for water in the world is continuously increasing, and it is expected to do so in the coming decades. The United Nations has predicted the world would face a global clean water deficit of 40% by 2030 (The United Nations, 2021). Two-thirds of the global population live in areas that face water scarcity for at least one month a year, and about 500 million people reside in areas where water consumption exceeds locally renewable water resources by a factor of two (Mekonnen & Hoekstra, 2016). The capability to access adequate and good water supplies is getting more challenging due to the increasing world population, climate change and the COVID-19 pandemic (Boretti, 2022; The United Nations, 2021). Since there is a water shortage in the world, wastewater treatment and water reuse have become significant (Karri et al., 2021).

Globally, an estimated 48% of wastewater is released into the environment without undergoing any form of treatment (World Health Organization, 2022), resulting in harmful impacts on both human health and ecosystems. Untreated wastewater that is microbiologically contaminated can contribute to spreading diseases like diarrhea, cholera, dysentery, typhoid and polio (World Health Organization, 2022). It is estimated that 842 000 people, mostly children in undeveloped nations, die of diarrhea caused by poor sanitation and hygiene annually (Prüss-Üstün et al., 2016). Pollution of oceans and rivers is primarily caused by the discharge of inadequately treated industrial and municipal wastewater (Latif et al., 2011). High concentrations of organic matter in wastewater effluent can lead to environmental problems such as eutrophication in the receiving water body. Therefore, it is important to monitor and control the concentration of organic matter in wastewater through effective wastewater treatment processes (Shanableh & Ginige, 1999).

Wastewater is an often overlooked and undervalued resource. It is essential to develop a more circular and sustainable wastewater treatment, where wastewater is fully taken advantage of as a potential resource with a low carbon footprint. Raw wastewater mainly consists of three resource components: organic material, nutrients and water (Safitri, 2022). Biotechnology provides an efficient and flexible solution for extracting and transforming resources from wastewater into valuable products, laying the foundation for a circular bio-based economy. Biogas can be produced through anaerobic treatment of organic matter in wastewater, which can then be used as an energy source (Puyol et al., 2017). Wastewater contains dissolved and particulate bound macronutrients which can be recovered and used as nutrients in agriculture as fertilizer. Treated wastewater can also be recovered with the intention that it can be reused (Safitri, 2022).

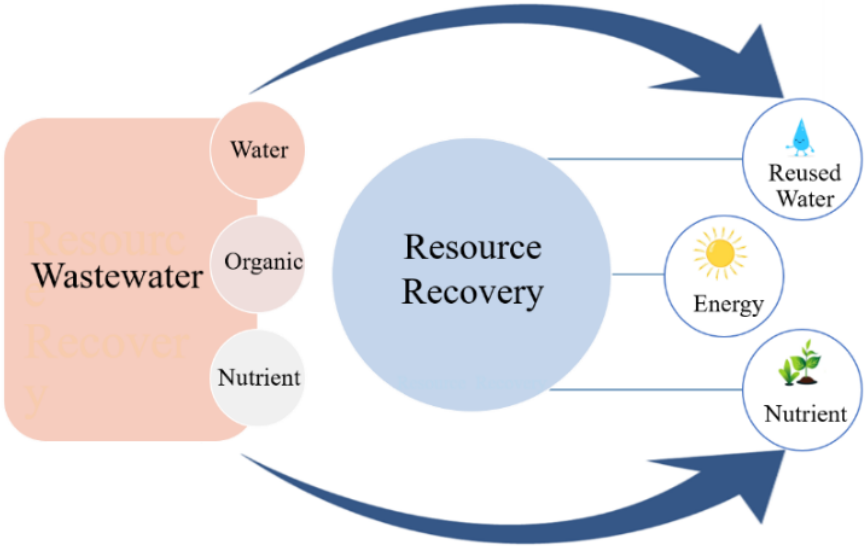

***Figure 1.1*** *Wastewater is a resource for reused water, renewable energy, and nutrients (Safitri, 2022).*

Most wastewater treatment technologies are relatively energy-intensive and become increasingly expensive over time (Rojas & Zhelev, 2012). Wastewater can be treated aerobically or anaerobically. Aerobic processes involve the use of free or dissolved oxygen by microorganisms in the conversion of organ wastes to biomass and carbon dioxide. Anaerobic processes degrade complex organic wastes into methane, carbon dioxide and water through four basic steps in the absence of oxygen: the hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic wastewater treatment is generally preferred over aerobic wastewater treatment in certain situations because it can be more energy efficient, produce less sludge, and be better suited for treating high-strength wastewater (Gomec, 2010). Aerobic biological processes are more commonly used as treatment of organic wastewaters for achieving high degree of treatment efficiency, as the effluent quality is generally higher than effluent from anaerobic systems.  On the other hand, anaerobic treatment has had considerable progress based on the concept of resource recovery and utilization while still achieving the object of pollution control (Chan et al., 2009).

Both aerobic and anaerobic processes can achieve high organic removal efficiency. Aerobic systems are suitable for treatment of low strength wastewaters, where the Chemical Oxygen

Demand (COD) concentrations are less than 1000 mg/l, and anaerobic systems are suitable for high strength wastewaters, where the COD concentrations are over 4000 mg/l (Chan et al., 2009). Due to the aerobic system having greater treatment efficiency and the anaerobic systems having better resource recovery, the anaerobic system is considered to be an intermediate step in the biological treatment prior to the aerobic process. This solution is called an anaerobic-aerobic system (Gray, 2017). The anaerobic-aerobic systems provide the advantages of both systems. The advantages include great potential of resource recovery, high overall treatment efficiency, less disposal of sludge, low energy consumption and removing the possibility of volatilization (Gray, 2017). By digesting excess aerobic sludge in the anaerobic tank, a minimum stabilized total sludge is produces which leads to less sludge disposal cost. This also results in an additional benefit which is a higher gas yield. Anaerobic pretreatment, which has an influent equalization effect, reduces the diurnal variations of the oxygen demand. This leads to further reduction of the required maximum aeration capacity, resulting in lower energy consumption. Lastly, the potential for volatilization during aerobic treatment is eliminated because of the breakdown of the volatile compound in the anaerobic treatment (Cervantes et al., 2006). A great alternative for the anaerobic process in an anaerobic-aerobic system is the up-flow anaerobic sludge blanket (UASB).

New technologies have been implemented over the years to improve the anaerobic-aerobic system, such as the UASB, to provide a treatment process which is both technologically and economically viable with the dual goals of resource recovery and compliance with current legislation for effluent discharge (Chan et al., 2009). The UASB reactor system, which was introduced by Lettinga and his colleagues in the 1970s, has received widespread acceptance and has been used successfully in the treatment of several types of wastewaters (Hickey et al., 1991). Chernicharo et al. (2019) summarized six full-scale UASB-based domestic wastewater treatment reactors which all were currently operated in Brazil. The overall organic removal efficiencies of COD in their full-scale UASB systems were within the range of 56 – 91% with approximately 75% methane fraction of the biogas produced. The produced methane was recovered and used as an energy source for the sludge drying system (Chernicharo et al., 2019).

Despite the excellent performance of removing organic carbon by the UASB system (in wastewater treatment up to 90%), it is frequently asserted that the process is limited due to methanogens having relatively slow growth rate and being sensitive to environmental conditions, particularly under psychrophilic conditions (Lettinga et al., 2001). Anaerobic treatment is also vulnerable to organic overloading. This is due to it disturbing the bioconversion and affecting the microbial community (Cardinali-Rezende et al., 2013; da Silva Martins et al., 2017). Low-temperature anaerobic processes has financial benefits, particularly for some high latitude countries, because it requires less heating and has potential to produce more bioenergy. The development of well-balanced community substrate-product interaction within the granules is a critical factor to assure stable granulated anaerobic treatment performance at low-temperatures and high loadings (McKeown et al., 2009).

There are several parameters that affect the operation of wastewater treatment using the UASB reactor. The smaller the concentration of total suspended solids (TSS) is in the wastewater, the less problematical the treatment will be. The maximum acceptable TSS in the wastewater is dependent on the concentration of organic matter. A typical domestic sewage has a low concentration of waste, where the COD concentration is about 0.5 g/l. The UASB reactor is proven to successfully treat typical domestic wastewaters (Souza, 1986). Another issue of UASB reactor treatment is the presence of compounds that is potentially toxic to the bacteria. In particular are sulfates, which is a toxic compound, an area of concern due to their presence in many wastewaters. In the methanogenesis step of the anaerobic degradation of organic matter, the sulfate-reducing bacteria will compete with the methanogens for hydrogen in the presence of sulfates (Souza, 1986). The product of the reduction of sulfate, $H_2S$ is highly toxic to the bacteria and can cause odor and corrosion problems. Temperature is another important factor for the economic feasibility for the reactor. The process of anaerobic digestion is optimal at a temperature of 37°C, but occurs at an acceptable rate between 15 – 25°C and a high rate between 30 – 40°C. For diluted wastewater the temperature is much more important because the quantity of methane produced is low in relation to the volume of wastewater to be heated, and as a consequence, when heating is necessary and external energy source would have to be used (Souza, 1986). Safitri et al., 2022 found that the UASB system was maintained stable at low temperatures (2.5 - 25°C) and variable organic loading rates (OLR) (1.0 -15.0 gCOD/l.d). The reactor has significant COD removal efficiency and methane production, which demonstrated the feasibility of UASB system application treating municipal wastewater at low-temperatures and variable loadings (Safitri, 2022).

In anaerobic wastewater treatment the loss of dissolved methane is an often-overlooked downside. Souza et al. (2011) and Wu et al. (2017) discovered that dissolved methane is in the liquid phase of an anaerobic bioreactor effluent (saturation factor of 1.03 -1.67). This dissolved methane in the effluent increases with the solubility of the methane at decreasing temperatures. As a result, considerable amounts of dissolved methane are lost in the effluent, and this is increased in low-temperature wastewater and high-flow through situations (Brandt et al., 2019). Once the effluent from the treatment is discharged and exposed to ambient methane partial pressures the methane degasses into the atmosphere. The energy recovery from biogas in anaerobic wastewater treatment has a positive climate effect, but this methane loss significantly diminishes and even offsets the effect (Safitri, 2022).

The wastewater is a mixture of sewage, agricultural drainage, industrial waste effluents and hospitals discharge. Untreated wastewater may contain different range of pathogens including bacteria, parasites, viruses and toxic chemicals such as heavy metals and organic pollutants from agriculture, industrial and domestic sources (Andrew et al., 1997; Drechsel & Evans, 2010). These pollutants must be brought down to permissible limits in order to safely dispose of the wastewater. Subsequently, the environmental contaminants and health hazards is minimized (Manju et al., 1998; Poots et al., 1978). In addition, UASB systems for treatments of municipal wastewaters of variable loading conditions is attractive for resource recovery. Anaerobic treatment processes, like the UASB, would appear to meet criteria like utilizing resources as efficiently as possible and minimize environmental harm while increasing industrial productivity, and improving quality of life (McCarty, 2001). The UASB system is suitable as a pre-treatment to aerobic processes and would influence the applicability of anaerobic treatment by UASB systems of municipal wastewater treatment. The wastewater discharge would meet the standards of safely disposal, as well as high-methane biogas and nutrients will be produced in the process. Limited knowledge and experience may cause limited use of anaerobic treatment, including granulated sludge wastewater treatment, which may result in reluctance of anaerobic technology providers to market their process solutions for low mesophilic and psychrotolerant use (Lettinga., 2018). To conclude, the motivation relies on sustainable wastewater treatment and resource recovery, as embedded in several United Nations sustainability goals.

## 1.2 Scope of work

This study was conducted by the Water and Environmental Engineering Study Program at the University of Stavanger and supported by IVAR. IVAR (*Interkommunalt Vann Avløp og Renovasjon*) is a norwegian public company managing water, wastewater, and waste in the region of Rogaland. The company operates several water and wastewater treatment plants (WWTP), waste management facilities, and provides services such as collection, treatment, and distribution of drinking water, collection and treatment of wastewater, and collection and recycling of waste. Two in-house designed laboratory-scale UASB reactors were operated continuously treating wastewater from two WWTP located in south-west Norway. One reactor receiving primary treated municipal wastewater from Vik WWTP, and the other reactor receiving primary treated municipal wastewater from Mekjarvik SNJ (Sentralanlegget Nord-Jæren) WWTP. Both treatment plants are operated by IVAR, Norway. Figure 1.2 and Figure 1.3 shows the processes configuration at IVAR Vik WWTP and IVAR SNJ WWTP, respectively.

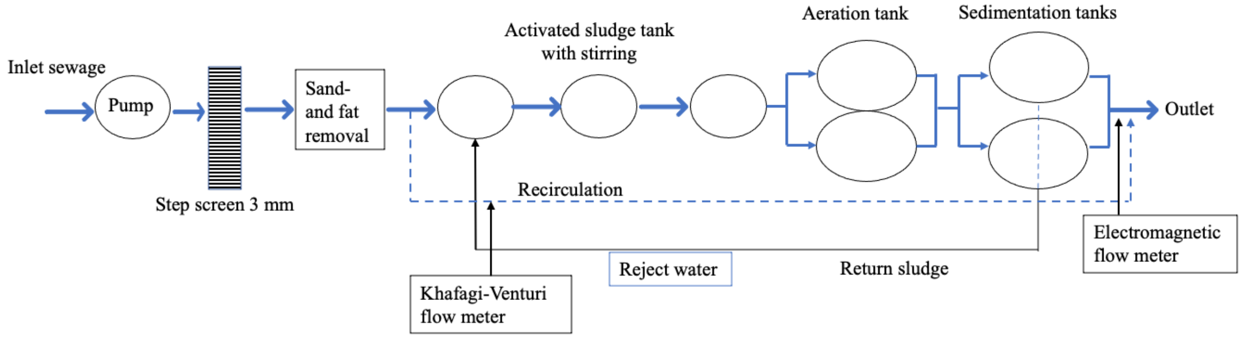

***Figure 1.2*** *The process configuration of IVAR Vik (De Lara, 2023).*

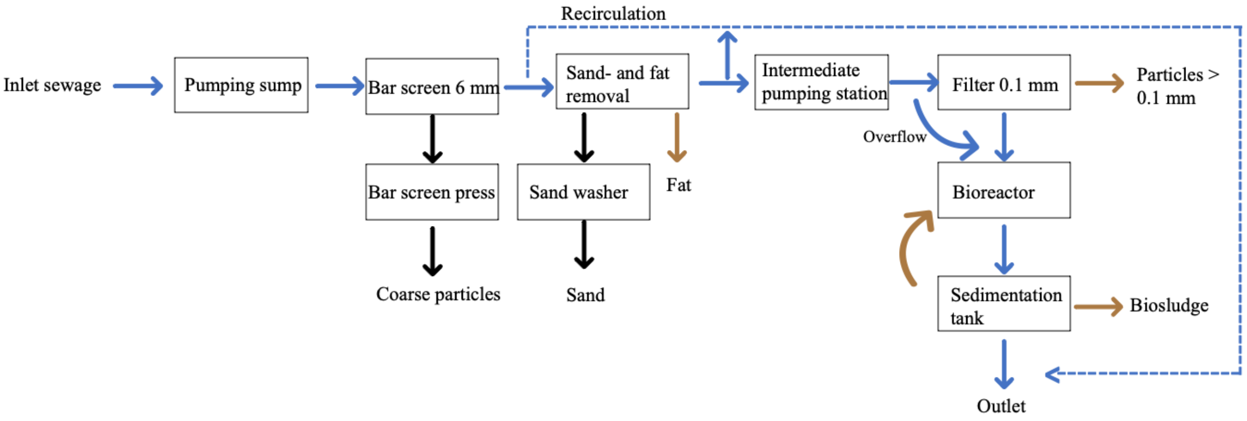

***Figure 1.3*** *The process configuration of IVAR SNJ (Wold & Furre, 2023).*

At IVAR Vik the wastewater is initially pumped through a fine step screen (3 mm) and is subsequently led to an aerated sand and fat removal tank before being pumped into the secondary biological treatment process. The screens and sand/fat tank remove sewage debris, sand and fat from the wastewater (IVAR, 2021a).

The primary treatments at IVAR SNJ consists of a bar screen (6 mm opening), sand and grit removal, fat removal, and filters (0.1 mm opening). The bar screen removes coarse particles and debris. The waste is sent for incineration, while the water is directed to a basin where fat and sand is removed. A small amount of air is added, causing the sand to settle and the fat to rise to the surface. The sand is suctioned from the bottom and washed before being deposited, while the fat is scraped off and further processed. Subsequently, the water is directed to a filtering system equipped with 20 drum filters, which remove all particles larger than 0.1 mm. The sludge accumulated on the filter screens is flushed away and pumped to biogas and fertilizer production (IVAR, 2021b).

The wastewater treated at Vik may be characterized as a municipal wastewater with significant contributions from Time and Klepp municipal (approx. 50 000 p.e.). The WWTP also has significant contributions from agriculture and food processing industries, like the dairy production at Q-Meieriene AS and potato industry HOFF SA Jæren (De Lara, 2023; Ravndal et al., 2018). The inlet wastewater at IVAR Vik WWTP has a flow rate of 8 000 $m^3/d$. The wastewater treated at SNJ WWTP is also characterized as municipal wastewater and is mainly contributed from 300 000 inhabitants from Randaberg, Stavanger, Sola, Sandnes and Gjesdal municipal (IVAR, 2021b). The wastewater IVAR SNJ WWTP receives consists of 70% municipal wastewater from households, while the remaining wastewater emerge from industry, like small-scale service industries and the slaughterhouses Nortura SA (Forus) and Fatland Jæren AS (Hommersåk) (Ravndal et al., 2018; Wold & Furre, 2023). The inlet wastewater at IVAR SNJ WWTP has a flow rate of 100 000 $m^3/d$. The two types of wastewaters investigated in this study are; (1) a medium-strength wastewater with dominant industrial loading (Vik) and (2) a typical low-strength municipal wastewater (SNJ) (Ravndal et al., 2018).

## 1.3 Thesis objectives

The main objective of this bachelor thesis was to investigate the up-flow anaerobic sludge blanket (UASB) reactor performances treating low to medium organic concentrations.

## 1.4 Thesis structure

The title of this thesis is “Anaerobic Treatment of Low to Medium Strength Municipal Wastewater in Laboratory Scale Up-flow Anaerobic Sludge Blanket (UASB) Reactors”. The thesis comprises of seven chapters. Chapter 1 provides an overview of the research background and motivation, scope of work and general objectives. Chapter 2 reviews the literature relevant to the topic, while Chapter 3 outlines the methodology adopted for the experimental part of the study. In Chapter 4, the research findings are summarized and in Chapter 5 the results are discussed. Finally, Chapter 6 presents the conclusions and Chapter 7 is recommendations for future research.

1. Introduction
2. Literature review
3. Methodology
4. Results
5. Discussion
6. Conclusions
7. Recommendations

# 2 Literature Review

This chapter describes theoretical understanding of the anaerobic process as well as defines anaerobic wastewater treatment, along with a definition of anaerobic stoichiometry. Additionally, the chapter provides a concise review of the factors that impact the anaerobic treatment processes of UASB reactors, a detailed description of UASB reactors themselves, and their applications within the municipal wastewater treatment.

## 2.1 Anaerobic wastewater treatment

Anaerobic digestion is the fermentation process where organic materials are degraded to produce biogas, which mostly consists of methane and carbon dioxide. Anaerobic digestion processes occur in absence of oxygen, where redox potential is low, and organic material is available (Henze, 2008). In anaerobic treatment, several species from the two completely different biological kingdoms, the Bacteria and Archaea, collaborate to transform organic waste into methane gas through a variety of intermediates (McCarty, 2001). Anaerobic and aerobic process follow the same metabolic pathway in the breakdown of carbon and energy. There are two basic differences between anaerobic and aerobic process; (a) the terminal fate of electrons produced in the oxidation reactions; and (b) the amount of Adenosine triphosphate (ATP) which is formed by oxidative phosphorylation. The amount of ATP that is formed when a pair of electrons is passed through the electron transport system, depends on the differences in redox potential between the electron donor and acceptor. Hence, more ATP will usually be released in aerobic respiration (Benefield & Randall, 1980).

Figure 2.1 presents the outcomes of carbon and energy in aerobic and anaerobic wastewater treatment. It is assumed that the oxidation of 1 kg COD requires 1 kWh of aeration energy. Aerobic treatment is associated with high operational costs due to the energy needed to maintain optimal oxygen levels, and around 50% or more of the influent COD is transformed into new sludge. The principles of carbon and energy flow in aerobic and anaerobic conversion significantly influence the design and operation of the corresponding wastewater treatment system (Henze, 2008).

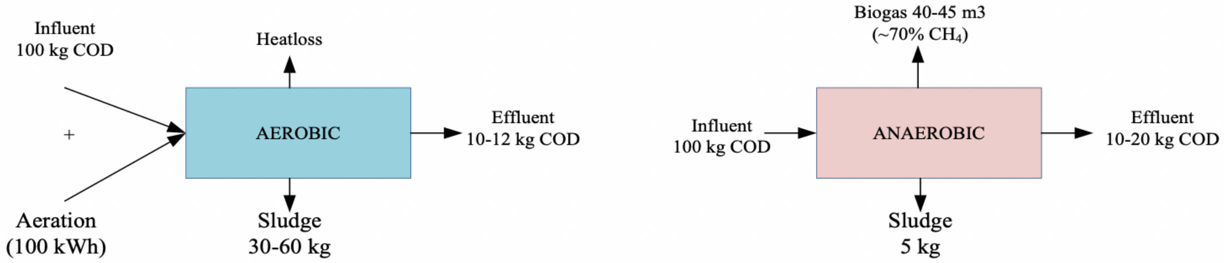

***Figure 2.1*** *The fate of carbon and energy in aerobic and anaerobic wastewater treatment (Henze, 2008).*

According to Figure 2.1, the anaerobic process offers several advantages over the aerobic process. Firstly, it produces less sludge per unit of substrate, and the amount of excess sludge is well stabilized. Additionally, the granular anaerobic sludge produced in the bioreactor has a market value. Secondly, the methane generated during the treatment process has an economic value. Lastly, the anaerobic process has a higher organic loading potential as it is not limited by the oxygen transfer capability at high oxygen utilization rates (Henze, 2008).

## 2.2 Microbiology of anaerobic conversion

Four essential steps make up the complex biological process of anaerobic carbon conversion, which depends on the natural activity of mixed anaerobic consortia that produce methane (Angelidaki et al., 2011). This process identifies acidogenic- and acetogenic bacteria and methanogenic archaea. These communities work together to maintain stability and continuity in the process. Simply said, the anaerobic degradation pathway of organic matter is a four-stage process with multiple series and parallel reactions. The four stages are 1) disintegration and hydrolysis, 2) acidogenesis, 3) acetogenesis and 4) methanogenesis (Henze, 2008). These stages are as shown in Figure 2.2 and discussed below.

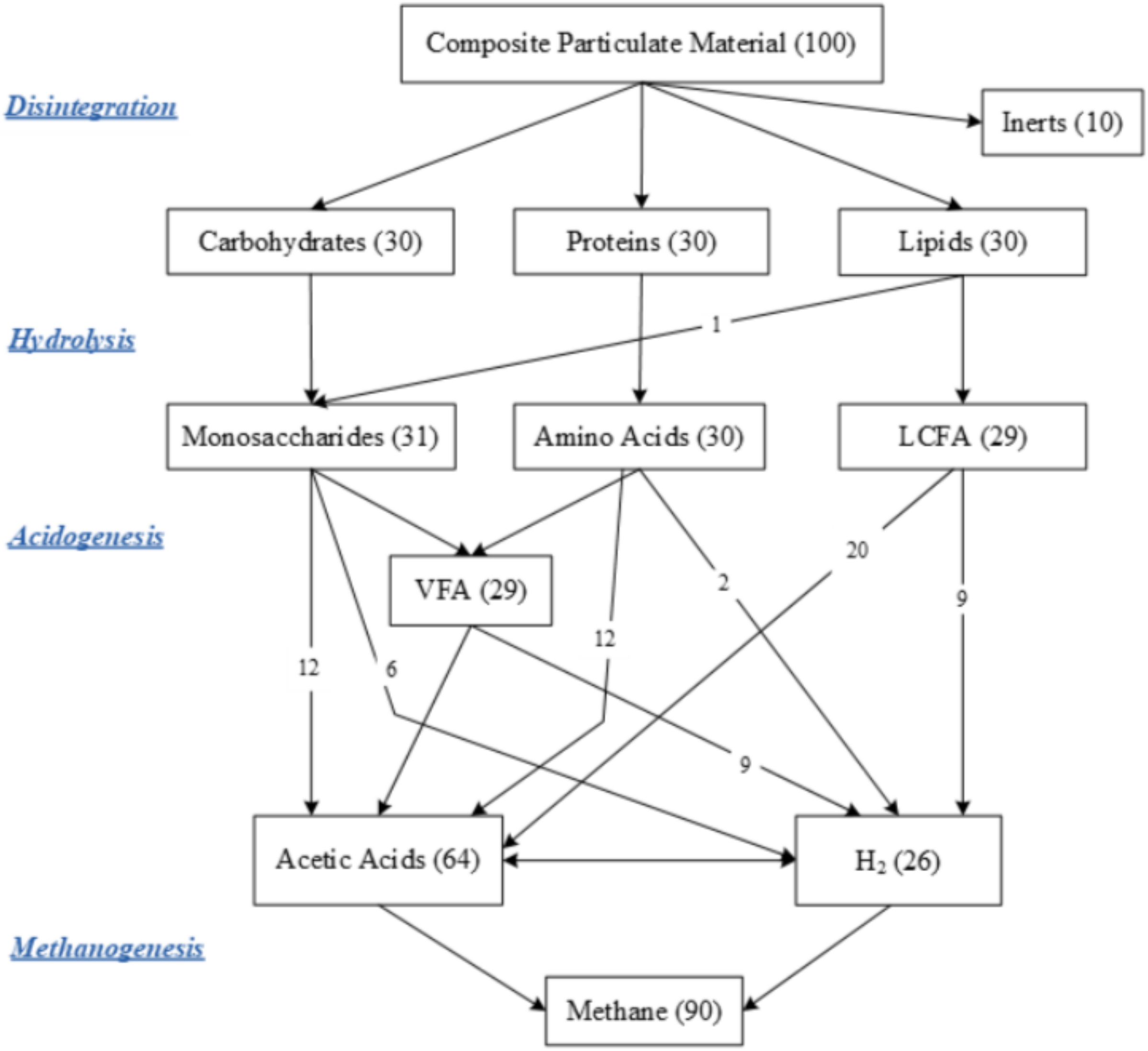

***Figure 2.2*** *Multistep of anaerobic processes and chemical oxygen demand (COD) flow in percent unit (Batstone et al., 2002).*

### 2.2.1 Disintegration and Hydrolysis

Disintegration and hydrolysis are extracellular biological and non-biological processes mediating the breakdown and solubilization of complex organic material to soluble substrates. The substrates are complex composite particulates and particulate carbohydrates, proteins, and lipids. The last three substrates are also products resulting from the disintegration of composite particulates (Batstone et al., 2002). Hydrolysis is a process mediating the breakdown of complex, undissolved material into less complex, dissolved compounds which can pass through the cell walls and membranes of the fermentative bacteria. Polysaccharides, proteins, and lipids are unable to be degraded directly by microorganisms because they are large polymeric compounds, and therefore they have to be depolymerized to allow for passage through the cell walls and membranes. In the hydrolysis step, proteins are hydrolyzed to amino acids, polysaccharides to simple monosaccharides and lipids to long chain fatty acids (LCFA) through the action of extracellular enzymes. The major groups of enzymes consist of proteases (which act on proteins), cellulases, amylases and glucanases (which act on polysaccharides), and lipases (which act on fats, oils, and lipids). Hydrolysis is a chemical reaction where compounds interact with water, leading to a decomposition of both the compound and water, and by simpler means is the cleavage of chemical bonds by the addition of water (Henze, 2008).

In dilute wastewaters with high TSS/COD ratio, such as distillery slops and low temperature sewage, the hydrolysis step may determine the overall process and therefore be a rate-limiting step during the anaerobic digestions of complex substrates. Hydrolysis is very sensitive to temperature and temperature fluctuations, and therefore determining the reactor design is essential for reactors that handle dilute wastewater like low temperature domestic sewage. The products of hydrolysis are the substrates for the acidogenic bacteria in the acidogenesis (Henze, 2008). Hydrolysis reactions require an input of energy, which is derived from the catabolism of smaller molecules produced by hydrolysis and synthesis. Stoichiometrically, polymers are broken down into readily biodegradable substrates of their monomeric compositions, although certain lipopolysaccharides are transformed into monosaccharides and LCFAs (Safitri, 2016).

### 2.2.2 Acidogenesis

The second step in the process of degradation of organic matter is acidogenesis. Acidogenesis is the fermentation stage where acidogenic bacteria break down the hydrolyzed products. Through the cell membrane, the hydrolyzed products diffuse inside the bacterial cells and are subsequently fermented or anaerobically oxidized. Soluble organic monomers of sugars and amino acids are degraded to produce alcohols, aldehydes, volatile fatty acids (VFAs), and acetate, together with $H_2$ and $CO_2$. Acidogenesis is a microbiological process that produces anaerobic acid without the use of an additional electron acceptor or donor (Henze, 2008). Acidogens are responsible for carrying out fermentation, which occurs at a relatively fast pace. The growth rate of acidogenic bacteria is similar to aerobic rates, typically ranging from 2-7 $\mu_m$ per day (Batstone et al., 2002). The processes can take place at high hydrogen or formate concentrations and provide significant biomass yields, as acidogenesis can occur without an additional electron acceptor and free energy yields are normally higher (Henze, 2008).

As mentioned, short chain fatty acids (SCFA), also known as VFAs, such as acetic, propionic, and butyric acids, are the principal end products of acidogenesis. Along with lactic and formic acid, alcohols can also be generated, including methanol and ethanol. A variety of growth parameters, including substrate composition, environmental factors, and operational factors, influence the composition of fermentation products. Environmental factors include, among others, pH and temperature, while operational factors include loading rate and retention time. The substantial portion of energy associated with the excreted fermentation products limits the amount of energy available for growth, which results in a low growth yield (Henze, 2008).

Table 2.1 lists several acidogenic reactions with sucrose from thermodynamically aspect. Evidently, the conditions in the reactor medium determine the type of end products. According to the table, the free energy change ($\Delta G$) of the reactions is dependent on the concentration of dissolved $H_2$. If $H_2$ accumulates, more reduced products such as propionate, butyrate, alcohols, and lactate are likely to appear. However, if $H_2$ is efficiently removed, by $H_2$ scavenger organisms like methanogens, acetate will be the main product. Therefore, these more reduced intermediate products are frequently present in the effluents of anaerobic reactors that are overworked or otherwise disturbed (Henze, 2008). Acetic acid and $H_2$ serve as direct substrates and energy sources for methanogenesis. The other fermentation products that appear when $H_2$ accumulates, need to undergo an acetogenesis process to be converted into acetic acid and $H_2$, before they can be substrates in the methanogenesis (Henze, 2008; McHugh et al., 2003).

***Table 2.1*** *Acidogenic reactions with sucrose as the substrate and the corresponding free energy change ΔG°´ at 25°C (Henze, 2008).*

| **Reactions** | **ΔG°´(kJ/mol)** |
|---|---|
| $C_{12}H_{22}O_{11}+9H_2O \rightarrow 4CH_3COO^{-}+4HCO_3^{-}+8H^{+}+8H_2$ | $-457.5$ |
| $C_{12}H_{22}O11+5H_2O \rightarrow 2CH_3CH_2CH_2COO^{-}+4HCO_3^{-}+6H^{+}+4H_2$ | $-554.1$ |
| $C_{12}H_{22}O_{11}+3H_2O \rightarrow 2CH_3COO^{-}+2CH_3CH_2COO^{-}+2HCO_3^{-}+6H^{+}+2H_2$ | $-610.5$ |

The acidogenesis step is where conversion happens the fastest of all the anaerobic operations. Among all the anaerobic processes, the acidogenesis reaction have the highest free energy. However, due to the large amount of energy obtained from the oxidation of $H_2$, the biomass growth yield is limited, which results in a low fermentation step growth yield of approximately Y ~ 0.1 – 0.2 gVSS/g COD. Table 2.2 compares the kinetic properties of acidogenesis and methanogenesis, and subsequently the growth and conversion rates of acidogenesis are higher compared to methanogens (Henze et al., 2008; McHugh et al., 2003; Tchobanoglous et al., 2003). The yield coefficient (Y) is the amount of biogas produced per unit of substrate consumed measured in gVSS/gCOD. The half saturation constant ($K_s$) is a measure of the affinity of the acidogenic bacteria and methanogenic archaea for organic matter in wastewater. $K_s$ represents the concentration of organic matter at which the microbial growth rate is half of the maximum growth rate, and it is expressed in mgCOD/l. The maximum specific growth rate ($\mu_m$) is the maximum rate at which the acidogens and methanogens can grow under favorable conditions, measured in units of per day (Henze, 2008).

***Table 2.2*** *Averaged kinetic properties of acidifiers and methanogens (Henze, 2008).*

| **Process** | **Conversion rate gCOD/gVSS.d** | **Y gVSS/gCOD** | **$K_s$ mgCOD/l** | **$\mu_m$ 1/d** |
|---|---|---|---|---|
| Acidogenesis | 13 | 0.15 | 200 | 2.00 |
| Methanogenesis | 3 | 0.03 | 30 | 0.12 |
| Overall | 2 | 0.03-0.18 | - | 0.12 |

Due to the rapidness of the acidogenesis, anaerobic reactors are subjected to souring, which is characterized by a sudden drop in pH, when reactors are overloaded or disturbed by toxic compounds. After the generated acids start to consume alkalinity, the pH begins to drop, resulting in a larger concentration of non-dissociated VFAs and a more severe inhibition of methanogens. The latter, naturally, causes an even faster accumulation of VFAs and subsequent pH reduction (Henze, 2008). Figure 2.3 shows the viscous cycle of methanogenic overloading and accumulating VFAs.

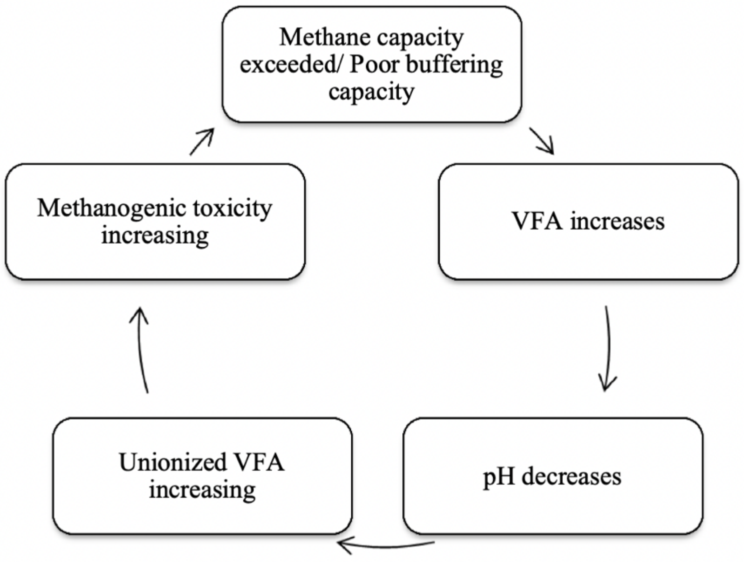

***Figure 2.3** Reactor pH drop as a result of methanogenic overloading and accumulating VFAs (Henze, 2008).*

The fact that the acidifiers function even at low pH levels, the reactor can and will reduce to a pH of 4 to 5, when the methanogenic capacity of the system is exceeded. The Stickland reaction is typically followed in the acidogenic conversion of amino acids. In this reaction, an amino acid is de-ammonified by anaerobic oxidation, producing additional VFAs and $H_2$, along with the reductive deammonification of other amino acids, which consumes the produced $H_2$. Both processes result in the release of $NH_3$, which later acts as a proton acceptor, leading to a pH increase. In this reaction there is no net proton production and no potential for a pH drop in the reactor (Henze, 2008).

### 2.2.3 Acetogenesis

The third step of the anaerobic conversion of organic matter to methane and carbon dioxide is the acetogenesis. Acetogenesis is a process where the SCFAs, other than acetate, which are produced in the acidogenesis step are further converted to acetate, hydrogen, and carbon dioxide by the acetogenic bacteria. Propionate and butyrate, which are SCFAs produced in the acidogenesis when $H_2$ accumulates, are the most important substrates in acetogenesis and are key intermediates in the anaerobic digestion process. The other products of the acidogenesis like lactate, ethanol and methanol is also converted to acetate in the acetogenesis. $H_2$ and $CO_2$ are even converted to acetate in the homoacetogenesis. LCFAs are converted to acetate by specific acetogenic bacteria following the b-oxidation, and LCFAs with uneven carbon atoms yield propionate in addition to acetate. LCFAs that are non-saturated, like oleate and linoleate, are first saturated by $H_2$ and then the b-oxidation happens (Henze, 2008). The level of $H_2$ in the environment is regulated by the $H_2$-producing acetogenic bacteria in the acetogenesis, and the $H_2$-consuming methanogenic bacteria in the following step, methanogenesis. Under standard conditions these reactions are unfavorable since ΔG° is positive. Consequently, butyrate, ethanol, propionate and the LCFA palmitate will not be produced under standard conditions since the ΔG° is positive, which leads to a negative bacterial energy yield (Henze, 2008), as shown in Table 2.3. Stochiometric conversion reactions follow propionate conversion as an example in Equation 2-1.

$$\Delta \mathrm{G} = \Delta \mathrm{G} + \mathrm{RT}\, ln \cdot [\mathrm{CO_2}] \cdot \frac{[\mathrm{Acerate}] \cdot [\mathrm{CO_2}] \cdot [\mathrm{H_2}]^3}{[\mathrm{Propionate}]}$$

Equation 2 -1

***Table 2.3*** *Stoichiometry and free energy change ΔG for some acetogenic reactions (Henze, 2008).*

| **Compound** | **Reaction** | **ΔG° (kJ/mol)** |
|---|---|---|
| Lactate | $CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$ | -4.2 |
| Ethanol | $CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$ | +9.6 |
| Butyrate | $CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$ | +48.1 |
| Propionate | $CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$ | +76.1 |
| Methanol | $4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O$ | -2.9 |
| Hydrogen-$CO_2$ | $2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$ | -70.3 |
| Palmitate | $CH_3\text{-}(CH_2)_{14}\text{-}COO^- + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2$ | +345.6 |

The hydrogen partial pressure is maintained at an extremely low level by effective uptake of $H_2$, by the $H_2$-consuming methanogenic bacteria or sulphate reducing bacteria. The methanogenic bacteria convert molecular hydrogen in the anaerobic digester, and since the reactions occurs so rapidly, the hydrogen partial pressure drops below $10^{-4}$ atm. The occurrence of the acetogenesis reactions is now possible as a result of a now negative free energy ΔG°′ due to the pressure drop, as shown in Figure 2.4.

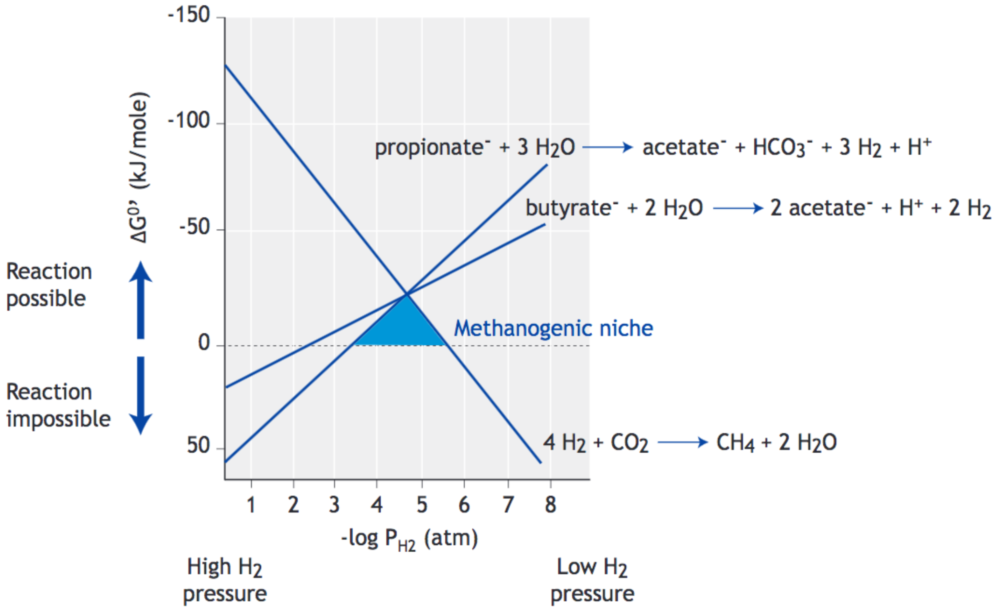

***Figure 2.4*** *Free energy change as a function of the $H_2$ partial pressure, a negative ΔG°' indicates possible occurrence of the mentioned reaction (Henze, 2008).*

This indicates that the breakdown of LCFAs and alcohols is highly dependent on the activity of electron collecting organisms such as the methanogenic bacteria. The $H_2$-producing organism can only grow in the presence of a $H_2$-consuming organism. This microbial association is called syntrophic association. The hydrogen pressure will not exceed $10^{-4}$ atm and is generally between $10^{-4}$ -$10^{-6}$ atm for a properly functioning methane-producing reactor. This pressure range is called the Methanogenic niche which set the upper limit for acetogens and a lower limit for methanogens for the $H_2$ concentration as shown in Figure 2.4. Consequently, the degradation of ethanol, butyrate, propionate, and palmitate becomes exergonic and will yield energy for the acetogens at such low hydrogen concentrations (Henze, 2008).

LCFA often limits the entire digestion process as a consequence of LCFA conversion being highly endergonic. LCFA tend to absorb to the sludge forming fatty clumps of biomass with little if any methanogenic activity in up-flow anaerobic sludge blanket (UASB) reactors. This results in only partly successful reactors (Henze, 2008).

### 2.2.4 Methanogenesis

The final step in the overall anaerobic conversion of organic matter to methane and carbon dioxide is methanogenesis, which is carried out by methanogens. Methanogens are a group of microorganisms that produce methane as a byproduct of their metabolism. During the methanogenesis, a group of anaerobic archaea both reduce the carbon dioxide using hydrogen as an electron donor and decarboxylate acetate to form methane. This is the only stage where the influent COD leaves the reactor in gaseous form (Henze, 2008).

The two most common pathways for methanogenesis to occur in freshwater systems are hydrogenotrophic methanogenesis and acetoclastic methanogenesis (Mobilian & Craft, 2022). Acetoclastic methanogens use acetate as an electron acceptor, to split the acetic acid into methane and $CO_2$. Hydrogenotrophic methanogens use $H_2$ as electron donor to reduce $CO_2$ to methane. Acetate serves as the primary precursor for over 70 % of the methane produced, while $H_2$ and $CO_2$ are mostly responsible for the remainder (Henze, 2008). The acetoclastic methanogens have very slow growth rates (0.12 per day), which leads to doubling times of several days or even longer. Due to the extremely low growth rates, high sludge concentrations are desired and anaerobic reactors need a very extended start-up time with unadapted seed material. Hydrogenotrophic bacteria can develop at a significantly faster rate (2.85 per day) than acetoclastic bacteria. As a result of this feature, and despite the acetogenic reaction step, anaerobic high-rate reactor systems exhibit remarkable stability under varying conditions (Henze, 2008). Table 2.4 lists the kinetic properties of the two most common types of methanogic bacteria.

***Table 2.4*** *Most important methanogenic reactions with some kinetic properties (Henze, 2008).*

| **Functional Step** | **Reaction** | **ΔG** (kJ/mol) | **$m_{max}$** (1/d) | **$T_d$** (d) | **$K_s$** (mg COD/l) |
|---|---|---|---|---|---|
| **Acetotrophic methanogenesis** | $CH_3^- + COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ | -31 | 0.12 | 5.8 | 30 |
| **Hydrogenotrophic methanogenesis** | $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ | -131 | 2.85 | 0.2 | 0.06 |

Generally, the biogas produced during the methanogenesis step consists of 65% methane and 35% carbon dioxide. The optimal pH range for the methanogenesis step is approximately 6.8 – 7.6 to achieve the highest efficiency (Henze, 2008).

## 2.3 Anaerobic Stoichiometry

The measurement and characterization of organic material in wastewater are usually based on the amount of oxygen that is consumed during the process of organic material oxidation. In the upcoming sub-sections, the stoichiometry of anaerobic processes will be discussed in detail.

### 2.3.1 Chemical Oxygen Demand (COD)

COD is a common parameter for evaluating the strength of contaminants in wastewaters. It measures the ability of water to consume oxygen during the breakdown of organic matter and the oxidation of inorganic substances like ammonia (Abdul Syukor et al., 2021). To determine the COD value, the oxidation of organic compounds must be carried out at a specific temperature and for a specified period. The amount of oxygen consumed by a strong oxidizing agent is expressed in terms of oxygen equivalent, typically measured in $gO_2/m^3$ (or $mgO_2/l$). This value can be calculated by considering that 1 mole of $O_2$ weighs 32 grams and has 4 electron equivalents. Thus, 1 electron equivalent (eeq) corresponds to 8 grams of COD (Henze et al., 2008). The relationship between COD and electron equivalents is shown in Equation 2-2.

$$\frac{1}{2}H_2O \rightarrow H^+ + \frac{1}{4}O_2 + e^- \Longrightarrow \frac{1}{4}mol\ O_2 \cdot 32\frac{g}{mol} = 8\ gram\ O_2$$
$$1eeq = 8\ g\ COD$$

Equation 2-2

The theoretical COD can be derived from the chemical oxidation reaction, assuming a complete oxidation, as shown in Equation 2-3. This Equation expresses 1 mole of organic material demands ¼(4n+a-2b) mole of $O_2$ or 8(4n+1-2b) $gO_2$ (Henze, 2008).

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

Equation 2-3

Equation 2-4 needs to be adjusted when dealing with organic material containing nitrogen compounds. The number of electrons that will stay with N and the total weight of N in the compound needs to be corrected (Henze, 2008).

$$C_nH_aO_bN_d + \frac{1}{2}(2n + 0.5a - 1.5d - b)O_2 \rightarrow nCO_2 + d\ NH_3 + \frac{a-3}{2}d\ H_2O$$

Equation 2-4

The theoretical COD per unit mass of organic compounds can vary depending on the specific compounds. For instance, to fully oxidize 1 mole of methane to carbon dioxide and water, 2 moles of oxygen are required (Daigger, 2011). Equation 2-5 illustrates how to calculate the COD equivalent of methane, where the oxidation number of carbon varies from -4 (as in $CH_4$) to a +4 (as in $CO_2$) (Henze, 2008).

$$CH_4 + O_2 \rightarrow CO_2 + H_2O$$

$$\frac{COD}{CH_4} = 2 \cdot \frac{32}{16} = 4 \frac{gCOD}{gCH_4}$$

Equation 2-5

The lower the average oxidation state is in the given compound the greater the potential for the compound to bind with oxygen, and consequently the greater COD value. In case of the compound ($C_nH_aO_bN_d$) the Buswell Equation (Equation 2-6) can be used to determine the theoretical amount of methane gas, assuming that the compound is completely biodegradable and would be entirely converted by the anaerobic organisms, which means no sludge yield, into $CH_4$, $CO_2$ and $NH_3$ (Henze, 2008).

$$C_nH_aO_bN_d + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3d}{4}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3d}{8}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} \cdot \frac{3d}{4}\right) CO_2 + NH_3$$

Equation 2-6

### 2.3.2 COD Fraction

The total amount of COD in wastewater can be divided into two fractions based on biodegradability: Biodegradable COD and Non-Biodegradable COD as shown in Figure 2.5. Biodegradable COD is the fraction, which is treatable sewage, which presents the part of the wastewater that can be biologically degraded in anaerobic conditions. Both biodegradable and non-biodegradable COD can further be divided into two categories, particulate and soluble. Microorganisms can easily degrade soluble biodegradable COD, whereas particulate biodegradable COD needs to undergo hydrolysis into smaller molecules before microorganisms can use it for growth. Hence, soluble COD are readily biodegradable and particulate COD are slowly biodegradable. Slowly biodegradable components are composed of high molecular weight that requires significant metabolism to convert them into simple monomers, in the hydrolysis, prior to being substrates for acidogenic bacteria. Consequently, a longer sludge retention time (SRT) may be necessary to break down these materials (Lettinga & Hulshoff Pol, 1991). In contrast, microorganisms cannot utilize particulate non-biodegradable COD, since it will adsorb to biomass and accumulate in the sludge. On the other hand, soluble non-biodegradable COD will not be degraded by microorganisms and will not accumulate in the sludge. Instead, it will pass through with the effluent and be unaffected by any biological or physical process.

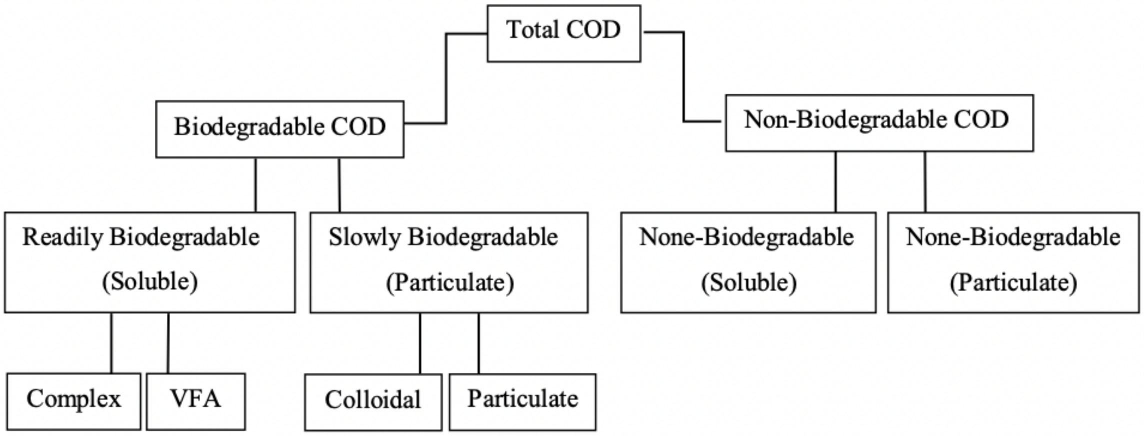

***Figure 2.5*** *Fraction of COD and wastewater (Tchobanoglous et al., 2003).*

Equation 2-7 shows the calculation of total COD (TCOD).

$$TCOD = COD_{soluble\ bio} + COD_{particulate\ bio} + COD_{non\ bio\ soluble} + COD_{non\ bio\ particulate}$$

Equation 2-7

### 2.3.3 Growth and Substrate Consumption Rate

Bacterial growth can be categorized into four distinct phases, as follows; (1) Lag phase, which represents the duration required for bacteria to adapt to their environment prior to initiating growth; (2) Growth phase, where cell division takes place, and the biomass exhibits exponential growth. In this phase, bacterial sensitivity is considerably high; (3) Stationary phase, which denotes a period when the biomass concentration remains constant in function of time; (4) Death phase, characterized by the cessation of growth and the death rate exceeds the growth rate (Tchobanoglus et al., 2003). The bacterial growth shows an exponential increase and is the first-order reaction based on the biomass concentration, as demonstrated by Equation 2-8. The $\frac{dX_B}{dt}$ is the biomass growth rate; $t_g$ is generation time; $\mu$ is specific growth rate (gVSS/gVSS.d); and $X_B$ is biomass (gVSS/l).

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

Equation 2-8

The specific growth rate depends on the concentration of limiting factors, such as carbon source, electron donor, nitrogen, phosphorus, or other essential growth factors. It can be explained mathematically using different formulas, where the most common is the Monod Equation (Equation 2-9), which employs Monod kinetics (Figure 2.6). The half saturation constant ($K_s$) is defined as the substrate concentration at which $\mu$ equals half of the maximum specific growth rate ($\mu_{max}$) measured in gVV/gVSS.d, while $C_s$ represents the growth-limiting substrate concentration (g/l) (Tchobanoglous et al., 2003). Based on the Monod Equation, when $C_s$ is much larger than $K_s$, $\mu$ is approximately equal to $\mu_{max}$, and the biomass grows at its maximum rate independent of substrate concentration.

$$\mu = \frac{\mu_{max} \cdot C_s}{K_s + C_s} \qquad \frac{dX_B}{dt} = \mu \,.\, X_B = \frac{\mu_{max} \cdot C_s}{K_s + C_s} \,.\, X_B$$

Equation 2-9

The relationship between substrate consumption and biomass growth can be expressed in terms of the growth yield factor (Y), which represents a proportional coefficient derived from Equation 2-8. The substrate consumption rate can be calculated using Equation 2-10 and biomass growth can be estimated using Equation 2-11. In Equation 2-10, $\frac{dC}{dt}$ is the substrate consumption rate (gCOD/l.d) and $\frac{\mu}{Y}$ ($k_m$) is specific substrate consumption rate (gCOD/gVSS.d).

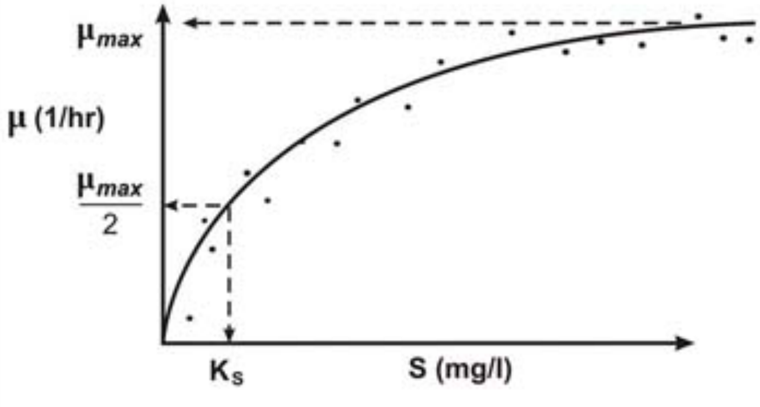

***Figure 2.6*** *Monod kinetic (Cunningham et al., 2010).*

$$\frac{dX}{dt} = Y\frac{dC}{dt} \qquad \frac{dC}{dt} = \frac{\mu \,. X_B}{Y} \qquad \frac{dC}{dt} = k_m . X_B$$

Equation 2-10

The specific substrate consumption rate (k) is a key parameter that reflects substrate removal in the reactor and is subjected to the same substrate concentration effects as μ. The maximum specific substrate consumption rate ($k_{max}$) corresponds to the maximum bacterial growth rate $\left(\frac{\mu_{max}}{Y}\right)$. The amount of methane produced can be estimated by calculating the percentage of substrate that remains unutilized for growth and is subsequently converted to methane (1-Y), as demonstrated in Equation 2-11.

$$\frac{dM}{dt} = (1-Y)\frac{dC}{dt} = (1-Y)\frac{\mu \cdot X_B}{Y} = (1-Y) \cdot k_m \cdot X_B$$

Equation 2-11

### 2.3.4 Methane Production

The amount of $CH_4$ produced during the anaerobic process is dependent on the removal of organic matter within the system, since $CH_4$ is equivalent to a certain amount of COD. In general, not all organic matter is capable of biodegradation, and a portion of the organic substrate will be utilized for cell synthesis. Equation 2-12 can be used to estimate the anticipated $CH_4$ production rate based on the fundamental influent variables, such as flow rate, COD concentrations, and information on the biodegradability of the COD. The stoichiometry of $CH_4$ produced is followed Equation 2-12 (Henze et al., 2008).

$$CH_4 + O_2 \rightarrow \; CO_2 + H_2O$$

Equation 2-12

One mole of methane needs two moles of oxygen to be converted into carbon dioxide and water. The COD equivalent of methane is therefore 4 kg COD/kg $CH_4$. This means that 0.35 $m^3$ of methane for every kg of COD (22.41 $m^3$/64 kg COD) is converted to methane at STP (standard temperature and pressure) of 0°C and 1 atm. At temperature 35°C, 0.40 $m^3$ of methane produced per kg of COD (25.29 $m^3$/64 kg COD) is converted to methane.

### 2.3.5 COD Balance

To ensure proper operation and control of an anaerobic process, it is essential to monitor relevant parameters and evaluate measurements regularly. As shown in Figure 2.7, all COD that enters the system ultimately ends up in the end product of $CH_4$, except for what is included into the biomass. Therefore, COD is a valuable control tool for anaerobic systems because it allows for the establishment of mass balance using COD as parameter. This is showed in Equation 2-14.

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

Equation 2-13

A thorough analysis of the gaseous, liquid and solid outlets is necessary to determine the fate of COD in an anerobic reactor (Henze, 2008). The theoretical COD equivalent for 1 kg bacterial VSS, with an estimate composition of $C_5H_7O_2N$, can be calculated as 1.42 kg COD/ kg VSS based on the basic influent characteristics. If the influent and effluent are measured properly, a balance can be established by expressing both $CH_4$ and newly grown bacteria as COD (Grady Jr et al., 2011; Henze, 2008).

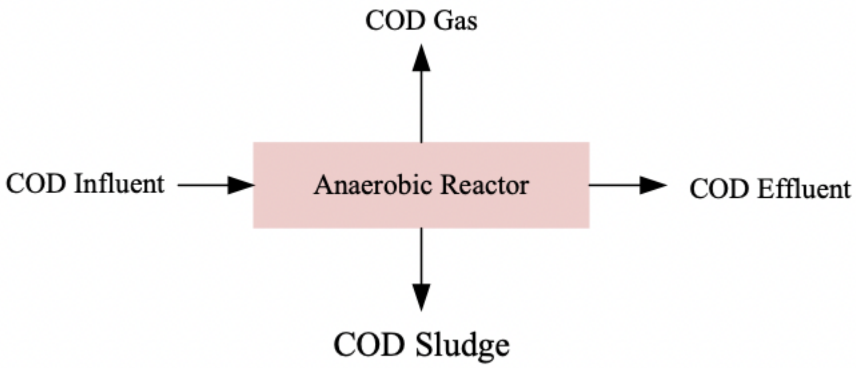

***Figure 2.7*** *COD balance (Henze, 2008).*

## 2.4 Parameters affecting anaerobic digestion

There are many parameters that affect the efficiency performance of anaerobic digestion. These parameters include SRT, hydraulic retention time (HRT), organic concentration and loading rate, temperature, pH, alkalinity, and nutrients. A wide variety of inhibitory substances are the primary cause of anaerobic digester upset or failure since they are present in substantial concentrations in the wastewater. The most common inhibitors present in the wastewater is VFAs, ammonia, sulfate, salinity, light metal ions, heavy metals, and organics (Chen et al., 2008). In the following sub-sections, factors affecting the anaerobic process will be described.

### 2.4.1 Sludge retention time (SRT)

The performance of all anaerobic processes is primarily determined by the SRT, which is a crucial control parameter. It impacts the kind of microorganisms that can grow in the bioreactor and their activity, affecting the effluent quality. The SRT can be calculated by dividing the mass of sludge present in the system by the amount of sludge that is wasted or produced for the system, shown in Equation 2-15 (Henze et al., 2008).

$$SRT = \frac{Mass\ of\ sludge\ in\ system}{Mass\ of\ sludge\ wasted\ from\ system}$$

Equation 2-14

In biological terms SRT is linked to the growth rate of the biomass. Equation 2-16 relates the specific growth rate (μ) and the Endogenous decay coefficient ($k_d$) to the SRT. Microorganisms with low growth rates, such as methanogens, require longer SRT in order to grow in the system. In practice, the SRT is regulated by the sludge wasting rate, as demonstrated in Equation 2-16.

$$\frac{1}{SRT} = \mu - k_d \rightarrow SRT = \frac{1}{\mu - k_d}$$

Equation 2-15

In general, a higher SRT promotes greater biodiversity and supports a wider range of functions. On the other hand, an SRT that is too low runs the risk of compromising certain functions, which is particularly problematic for nitrification - a critical process for ammonia removal, as denitrification is contingent on nitrification. Table 2.5 provides a summary of the impact of SRT on various activated sludge functions (Smith et al., 2015).

***Table 2.5*** *Impact of solids retention time on wastewater treatment processes (Smith et al., 2015).*

| **Activated sludge function** | **Impact SRT** | **Optimum SRT** |
|---|---|---|
| **COD removal** | Too low: Elevated Biochemical Oxygen Demand (BOD) in treated effluent.<br>Too high: increased oxygen requirements; increased energy use. | As low as possible to permit development of a flocculent sludge and nitrification, if required. |
| **Nitrification** | Too low: incomplete nitrification; elevated ammonia in treated effluent; denitrification not possible<br>Too high: increased oxygen requirements; increased energy consumption; increased nitrate (degrades denitrification performance). | 4 to 8 days |
| **Denitrification** | Too low: incomplete denitrification; elevated nitrogen in treated effluent; higher chemical usage in denitrification filters<br>Too high: increased oxygen requirements; increased energy usage | 10 to 15 days |
| **Phosphorus Removal** | Too low: Enhanced biological phosphorus removal (EBPR) will not occur. Increased chemical usage.<br>Too high: Phosphorus uptake rate is reduced. Supplemental chemical addition could be required. | 5 to 12 days |

| | | |
|---|---|---|
| **Final Settling** | Too high: Increased solids loading; filamentous bulking; increased TSS in treated effluent.<br>Too low: poor settling; increased TSS in effluent. | Variable – depends on treatment goals |
| **Sludge Treatment** | Too low: higher sludge production.<br>Too high: reduced sludge dewaterability; lower sludge digestability; reduced biogas production. | Variable – depends on treatment goals |

To ensure successful biochemical transformations, the chosen SRT must always exceed the minimum SRT necessary for the microorganisms responsible for that specific function. The minimum SRT is the threshold at which a particular group of microorganisms is incapable of growing in a suspended growth reactor. Figure 2.8 illustrates the typical SRT values for different anaerobic conversion processes at temperature of 35°C. Lower temperatures will generally require longer SRT values (Grady Jr et al., 2011).

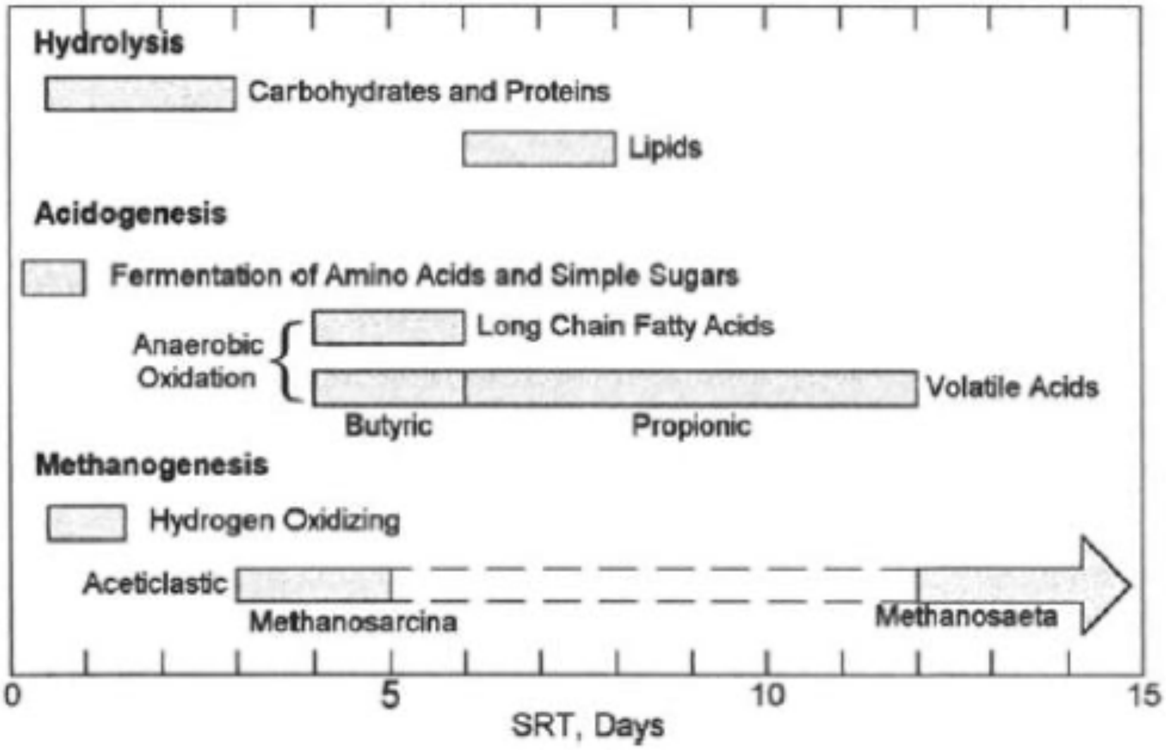

***Figure 2.8*** *Typical SRT ranges for various biochemical conversions in anaerobic bioreactors at 35°C (Grady Jr et al., 2011).*

The hydrolysis of particulate carbohydrates and proteins to produce monosaccharides and amino acids is a relatively rapid reaction that occurs within approximately three days. In contrast, the hydrolysis of lipids to form LCFAs and other soluble reaction products is a much slower reaction that generally does not occur for SRT values less than about six days. Figure 2.8 demonstrates the relative effects of SRT on the growth of the various types of microorganisms found in anaerobic bioreactors and the resulting impact on the types of biochemical conversion that will occur (G. H. Chen et al., 2020; Grady Jr et al., 2011).

The anaerobic treatment of wastewater containing carbohydrates and proteins with the production of methane can be achieved at SRT values of approximately eight days. However, significant methane formation will occur at SRT values as low as five to six days. Nevertheless, in such cases, significant quantities of propionic acid may accumulate, as the SRT is too short to allow the growth of bacteria that anaerobically oxidize propionic acid to acetic acid and hydrogen. SRT values in excess of eight days will be required to stabilize wastewaters containing significant quantities of lipids, such as primary sludge from domestic treatment systems. Generally, a minimum SRT of approximately ten days is specified to ensure complete and reliable degradation of lipids in anaerobic bioreactors (G. H. Chen et al., 2020; Grady Jr et al., 2011; Jördening & Winter, 2005).

### 2.4.2 Hydraulic retention time (HRT)

When a UASB reactor is used to treat municipal wastewater, HRT is one of the most crucial factors impacting the performance. The volume of process per unit of flow rate of influent is known as the HRT as shown in Equation 2-17 (Henze, 2008).

$$HRT = \frac{Volume\ of\ aeration\ tank}{Influent\ flow\ rate} = \frac{V}{Q_{in}}$$

Equation 2-16

The up-flow velocity is directly related to the HRT and is vital for capturing suspended solids. Reduced up-flow velocity causes a rise in HRT, which improves the ability of the system to remove suspended solids. At high up-flow velocity, the COD removal efficiency of a UASB reactor declines. This is due to the fact that higher up-flow velocity causes the sludge granules to be smashed and reduces the amount of time it spends in contact with the wastewater, leading to a larger washout of solids (Rizvi et al., 2015). A relatively small HRT is frequently suggested to scale down wastewater treatment projects, which corresponds with a high up-flow velocity for the UASB reactor. The ability of the reactor to remove COD degrades when the HRT is being shortened (H. Chen et al., 2020).

SRT and HRT are both time-related concepts in the anaerobic treatment system. SRT represents the sludge age, or the duration of material retention in the reactor, while HRT refers to the nominal value of the liquid and dissolved retention time of the material. If there is no sludge recycle or retention, SRT and HRT is equal. However, in reactor with sludge recycle, SRT differs from HRT. The link between SRT and HRT is neither proportional nor linear. The relationship between SRT and HRT depends more on factors such as COD, BOD, and TSS (Henze, 2008)

### 2.4.3 Organic Concentration and Organic Loading Rate (OLR)

The economics of choosing anaerobic treatment depend significantly on the strength of the wastewater. To produce enough methane to heat the wastewater, assuming it is at ambient temperature, generally biodegradable COD concentrations larger than 1500 to 2000 mg/l are required (Tchobanoglous et al., 2014). Low influent COD concentrations will result in low biogas production rate and low substrate levels (50 – 100 mgCOD/l) (Lettinga et al., 2001; Rebac et al., 1995a). Consequently, a decrease in mixing intensity and poor substrate-biomass interaction are anticipated. Treating low strength wastewater with the highest OLR attainable in relation to the maximum sludge COD conversion capacity, may result in significant hydraulic sludge washout (Rebac et al., 1995a).

The OLR is the amount of organic matter per unit reactor volume, which is subjected to the anaerobic digestion process in the reactor in a given unit time (Grangeiro et al., 2019). The OLR is the key design factor to determine the bioreactor volume, and also one of the main factors that influence the production of biogas in the process. Equation 2-18 displays the volumetric OLR expressed as kgCOD/m$^3$.d or gCOD/l.d. Where Q is the flow rate (l/d); $C_{in}$ is the influent COD concentration (gCOD/l); and V is the volume of the reactor (l) (Tchobanoglous et al., 2003).

$$OLR = \frac{Q \cdot Cin}{V}$$

Equation 2-17

The OLR for anaerobic treatment processes ranges from 1 to 50 kgCOD/m$^3$.d, which is greater than the OLR level of 0.5 to 3.2 kgCOD/m$^3$.d for aerobic treatment processes. The OLRs are impacted by the chosen anaerobic process, the wastewater type and the temperature (Tchobanoglous et al., 2003). As we can see from Equation 2-17 and 2-19 the relationship between OLR and HRT is displayed. The OLR is inversely proportional to the HRT (Grady Jr et al., 2011).

$$OLR = \frac{Cin}{HRT}$$

Equation 2-18

As mentioned, in a reactor without sludge recycle, the loading is related to SRT only because the SRT and HRT is equal. However, in a reactor with sludge recycle, the SRT is independent of HRT. The relationship between SRT and OLR is inversely proportional to each other (Grady Jr et al., 2011; Tchobanoglous et al., 2014). Equation 2-20 displays the inverse proportion between SRT and volume (V), and that the volume is related to SRT through biomass concentration (X) in the bioreactor. Greater biomass concentration leads to increased OLR, which enables the bioreactor to have a smaller volume (Grady Jr et al., 2011).

$$SRT = \frac{X \cdot V}{Y \cdot Q \cdot Cin} = \frac{X}{Y \cdot OLR}$$

Equation 2-19

In anaerobic systems, considerable variations in influent flowrate and organic loads can disturb the balance between acid fermentation and methanogenesis. The acidogenic reactions may occur more quickly, potentially lowering pH and raising hydrogen and VFA concentrations to levels that prevent methanogenesis (Tchobanoglous et al., 2014).

### 2.4.4 Temperature

Temperature is a crucial factor that has an influence on the rate of all chemical reactions that involve growth processes. The rate of microbial growth and the total amount of growth can be affected by temperature. Once the temperature reaches a certain threshold, the rate of growth reaches its maximum. As the temperature continues to increase, heat-sensitive cell-components such as enzymes become denatured leading to a drop in the growth rate. The temperature impact is expressed in Equation 2-21.

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

Equation 2-20

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

Microorganisms are classified into temperature classes based on the optimum temperature and the temperature range in which the species are able to grow and metabolize, as shown in Figure 2.9. The groups are psychrophilic (0 – 20°C), mesophilic (20-42°C) and thermophilic (42 - 75°C) (Grady Jr et al., 2011; Lettinga, 1995). The overlapping between the growth temperature ranges in Figure 2.9 indicated that there is no clear boundary between the groups of microorganisms.

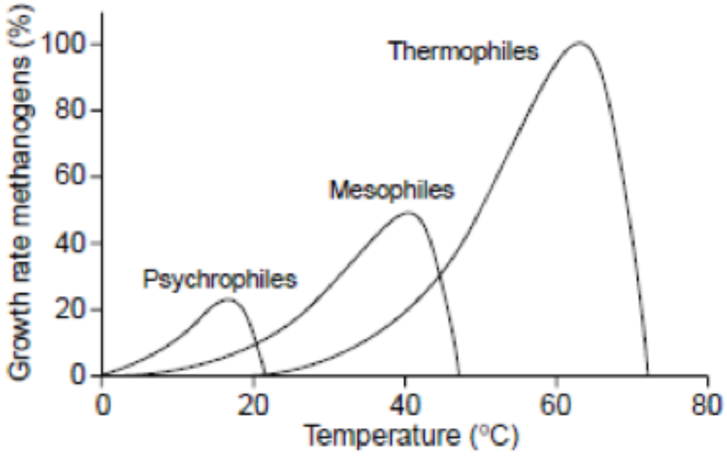

***Figure 2.9*** *Relative growth rates of methanogens with different temperature (Lettinga et al., 2001).*

Most reactions in the biodegradation of organic matter require more energy to proceed at low temperature than at a temperature optimum (Lettinga et al., 2001). Through studies it has been discovered that in general, low temperature leads to a decrease in the maximum specific growth and substrate utilization rates. On the other hand, it might also lead to an increased net biomass yield (g biomass/g substrate converted) of methanogenic population or acidogenic sludge (Lettinga et al., 1997; Lettinga et al., 1999).

Lower temperatures result in slower reaction rates, which necessitates the use of longer solid retention time (SRT), larger reactor volumes, and lower organic COD loadings. The solids loss in an anaerobic reactor becomes a crucial limiting factor when larger SRTs are required. With effluent TSS concentrations for suspended growth processes in the 100 mg/l range, anaerobic reactors typically create more evenly distributed and less flocculent particles than aerobic systems. The potential SRT of the procedure and treatment will be constrained for diluted wastewaters by the effluent TSS concentrations. Either poor treatment performance happens, or the reactor needs to be run at a higher temperature (Tchobanoglous et al., 2014). Greater SRT values are required for operation at lower temperatures, which calls for either very low effluent volatile suspended solids (VSS) concentrations for weaker wastewaters or more biodegradable COD in the influent.
The temperature has a significant impact on the development and survival of microorganisms. All three temperature ranges (psychrophilic, mesophilic and thermophilic) allow for anaerobic treatment, but low temperatures usually cause a drop in the maximal specific growth rate and methanogenic activity. At low temperature range, it takes 10 - 20 times more biomass in the reactor, or a higher SRT and HRT, to achieve the same COD removal efficiency as at 35°C. This is because the methanogenic activity at these temperatures is 10 - 20 times lower than the activity at 35°C (Rizvi et al., 2015).

In order to enable more appropriate biological reaction rates and provide more stable treatment, reactor temperatures between 25 and 35 °C are typically preferred (Tchobanoglous et al., 2014). The process of anaerobic digestion is optimal at a temperature of 37°C, but occurs at an acceptable rate between 15 – 25°C and a high rate between 30 – 40°C. For diluted wastewater the temperature is much more important because the quantity of methane produced is low in relation to the volume of wastewater to be heated, and as a consequence, when heating is necessary and external energy source would have to be used (Souza, 1986). Safitri et al., 2022 found that the UASB system was maintained stable at low temperatures (2.5 - 25°C) and variable OLRs (1.0 -15.0 gCOD/l.d). The reactor has significant COD removal efficiency and methane production, which demonstrated the feasibility of UASB system application treating municipal wastewater at low-temperatures and variable loadings (Safitri, 2022). Research examining the influence on anaerobic processes indicates a significant decrease in metabolic activity among mesophilic anaerobic methanogenic bacteria as temperatures decreases, as presented in Figure 2.10. This finding suggests that during start-up under low-temperatures, the capacity of an anaerobic reactor containing mesophilic biomass will decrease rapidly (Lettinga et al., 2001).

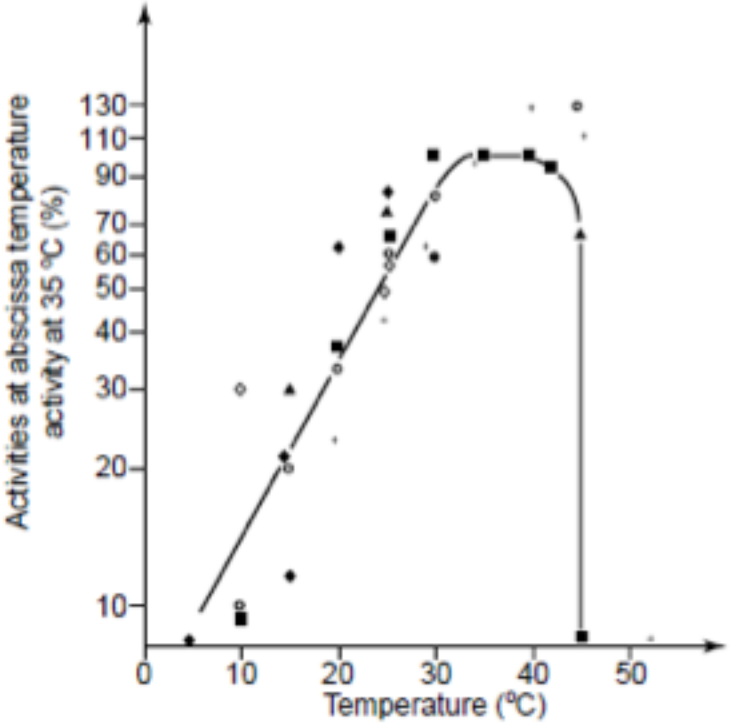

***Figure 2.10*** *Temperature dependency of the methane production rate of mesophilic anaerobic processes from different researchers, white diamond, black circle, blank square, and cross are research conducted by different researchers (Lettinga et al., 2001).*

### 2.4.5 pH

The pH level of wastewater can have a significant impact on the bacteria activities in anaerobic treatment process. The microorganisms that break down the organic matter are sensitive to changes in pH. Therefore, maintaining the proper pH range is critical for the success of the anaerobic treatment process. The optimal pH range for adequate activity of microorganisms are in the range 6.0 – 8.0, whereas a pH range of 6.8 – 7.4 provides optimum conditions for methane microorganisms. If the pH falls below 6.8 or rises above 7.4 within the 6.0 – 8.0 range, the methanogenic activity will have a significant decline (Grady Jr et al., 2011; Henze, 2008). Both acetoclastic methanogenic bacteria (AMB) and hydrogenotrophic methanogenic bacteria (HMB) have very low free energies, causing them to depend on reversed electron flow though the cell membrane powered by proton or cation motive force (Henze, 2008). The hydrogen-ion concentration is considered to be one of the most important factors that influence enzyme activity (Benefield & Randall, 1980).

If the pH level falls outside this range, the microorganisms' activity may be inhibited, leading to reduced treatment efficiency or complete process failure (Jain et al., 2015). If the pH level is too low, it can cause the accumulation of VFAs, which can inhibit the activity of methane-producing microorganisms, leading to reduced biogas production and treatment efficiency. Additionally, low pH levels can lead to the accumulation of toxic substances such as hydrogen sulfide, which can be harmful to both the microorganisms and the treatment system (Khanal, 2011). On the other hand, if the pH level is too high, it can lead to the precipitation of certain compounds, such as calcium and magnesium, which can clog pipes and other equipment, reducing treatment efficiency. High pH levels can also cause the loss of ammonia, which is a critical nitrogen source for the microorganisms involved in the treatment process (Khanal, 2011). As a result, maintaining the proper pH range is essential for the successful anaerobic treatment of wastewater. pH monitoring and control are critical for ensuring that the treatment process operates within the optimal range, leading to efficient treatment and biogas production.

### 2.4.6 Alkalinity

To maintain a stable pH the anaerobic system must have a high alkalinity. During anaerobic treatment, fermentation reactions result in the production of gas bubbles with a high $CO_2$ content (25 - 35%) in the liquid, which raises the dissolved $CO_2$ concentration in the liquid. To counteract the dissolved carbonic acid and keep the pH at or near neutral, a high alkalinity concentration, in the range of 2000 to 4000 mg/L as $CaCO_3$, is often required. Amino acids or proteins can be metabolized and deaminated during anaerobic treatment to produce alkalinity as $NH_4(HCO_3)$ in wastewaters that are low in alkalinity. The alkalinity requirements will often be substantially higher for wastewaters with higher total dissolved solids concentrations and ionic strengths (Tchobanoglous et al., 2014).

Chemicals commonly utilized as buffers comprise lime, sodium carbonate, sodium bicarbonate and sodium hydroxide. Among these options, sodium bicarbonate ($NaHCO_3$) is the preferred choice because it can effectively adjust the equilibrium to the desired value without disrupting the physical and chemical balance of the delicate microbial population. Adding sodium bicarbonate during the start-up phase, is crucial for maintaining a pH level around 7 and ensuring the stability of the system (Choi et al., 2007; López-López et al., 2015).

### 2.4.7 Nutrients

For microorganisms, nutrients; (1) serve as acceptor for the electrons released in the energy-yielding reaction; (2) serve as an energy source for cell growth and biosynthetic reactions; and (3) supply the material needed for the synthesis of cytoplasmic material (Benefield & Randall, 1980). The mass of cells formed per unit mass of COD removed anaerobically is much lower than it is aerobically. This is due to the loss of energy in the original substrate as the liquid is converted to methane. As a result, less sludge is produced by anaerobic processes than aerobic process, which means less nitrogen and phosphorus are needed for biomass growth. Many industrial wastewaters may not contain enough nutrients, thus adding nitrogen and/or phosphorus may be necessary (Tchobanoglous et al., 2014). The needed COD:N:P ratio for a typical activated sludge process is 100: 5: 1, whereas the ideal C:N:P ratio for maximizing methane yield has been found to be 100: 2.5: 0.5 (Droste & Gehr, 2018).

The growth of methanogenic bacteria in anaerobic processes requires the presence of the trace amounts of iron, nickel, cobalt, and molybdenum. In several instances, it has been demonstrated that adding trace metals improves the effectiveness of COD removal in anaerobic processes. In order to determine the precise amount of trace metals that are beneficial for high-rate anaerobic processes, which can differ for different wastewaters, successive trails are utilized (Tchobanoglous et al., 2014). The biodegradable COD concentration of the wastewater, cell yield, and the nutrient concentration in bacterial cells can be used to determine the minimum concentration of macro- and micronutrients. Generally, the influent nutrient concentration should be adjusted to twice the minimum nutrient concentration required to ensure that a small excess is present and that the process is not limited by the availability of nutrients (Tchobanoglous et al., 2014; Ydstebø, 2005).

### 2.4.8 Inhibitory Substances

There is considerable effect of the concentration of any material on the specific growth rate of bacteria when all the materials are present in excess. As the concentration of the material increases, the specific growth rate will also increase until it reaches the maximum specific growth rate $\mu_{max}$ (Grady Jr et al., 2011). Further increase in concentration lead to a point where no effect is observed, but beyond that point, a threshold value will be reached, and the specific growth rate will start to decline. This threshold value marks the onset of toxicity, and any concentration exceeding this threshold is considered toxic. Toxicity severity will continue to increase with concentration above the threshold value.

Toxic compounds can have a significant impact on the performance of anaerobic treatment processes. The presence of toxic compounds can inhibit the growth and activity of microorganisms responsible for the breakdown of organic matter. This can lead to a decrease in treatment efficiency and slower degradation rates. Although, the presence of a toxic substance does not imply that an anaerobic process cannot function. Anaerobic methanogenic reaction rates can be inhibited by some toxic compounds, but the process can be maintained if there is enough biomass, and the loading is kept at a manageable level. The ability to adapt to toxic concentrations has also been demonstrated (Speece, 1996), although in order to avoid toxicity issues during the anaerobic degradation process, pretreatment steps may be required. In the following sub-chapters, a few specific materials will be described.

#### 2.4.8.1 Volatile Fatty Acids (VFAs)

In anaerobic reactors, accumulation of acids affects the pH of the medium. When the pH is held constant near neutral pH, neither acetic nor butyric acids have any significant toxic effects upon hydrogen-utilizing methanogenic bacteria at concentrations up to 10 000 mg/l (Wang et al., 2009). However, propionic acid exhibits partly toxicity to methanogenic bacteria at a concentration of 1000 mg/l at neutral pH (Grady Jr et al., 2011; Wang et al., 2009). Therefore, it seems that at neutral pH only propionic acid is likely to cause toxic effects in anaerobic operations, and only at relatively high concentrations. Further research is needed to determine the generality of this pH-volatile acid interaction for acetic and butyric acids (Henze, 2008). Overall, it can be concluded that at neutral pH there is little inhibition by VFAs.

As mentioned in previous sub-chapters, a decrease in pH will affect the methanogenic bacteria activity. Resulting in low HMB and AMB activity, which will reduce the acetic acid production and the acidic fermentation products, or VFA will be accumulated. If the production of VFAs exceed the maximum capacity of methanogenic consuming acetic acid and hydrogen, it will lead to further accumulation and decrease pH, as previously displayed in Figure 2.3.

### 2.4.8.2 Ammonia

Ammonia inhibition is a common problem in anaerobic treatment systems where there are high levels of ammonia in the influent. Ammonia is toxic to many microorganisms and in high concentrations it can disrupt cellular functions, reduce growth rates, and cause cell death. Wastewater high in protein content will produce significant amount of ammonia. As the protein is degraded, the nitrogen is released as ammonia (Droste & Gehr, 2018; Henze, 2008). The form of ammonia depends on the pH, either ammonium ion ($NH_4^+$) or dissolved free ammonia ($NH_3$). Ammonia is a weak base and dissociates in water, as shown Equation 2-22.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

Equation 2-21

$NH_3$ has been suggested to be the main cause of inhibition since it is freely membrane permeable (De Baere et al., 1984; Kroeker et al., 1979). Both species are inhibitory but at different concentrations. Severe toxicity occurs when the concentration of free ammonia surpasses 150 mg/l, while an ammonium ion concentration greater than 3000 mg/l is required to produce the same effect (Grady Jr et al., 2011). An increase in pH would result in increased toxicity due to the shift to a higher ionized ammonia ($NH_4^+$) ratio. Inhibition and instability of the process due to ammonia often results in VFA accumulation, which again leads to decrease in pH and resultingly a declining concentration of $NH_3$. The interaction of $NH_3$, VFAs and pH may lead to an “inhibited steady state", which is a condition where the process is running stably but with a lower methane production (Angelidaki & Ahring, 1993; Angelidaki et al., 1993). Therefore, controlling the pH within the growth optimum of microorganisms by addition of acid is important to reduce ammonia toxicity (Bhattacharya & Parkin, 1989).

Temperature change has an effect on the $NH_3$ concentration, where an increased temperature results in a higher concentration of $NH_3$. Several studies have found that anaerobic treatment of wastewater with a high concentration of ammonia in the influent was more easily inhibited and less stable at thermophilic temperature than at mesophilic temperatures (Braun et al., 1981; Parkin & Miller, 1983). Certain ions such as $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ have been found to be antagonistic to ammonia inhibition. This is a phenomenon in which the toxicity of one ion is decreased by the presence of other ions (Braun et al., 1981; Hendriksen & Ahring, 1991; McCarty & McKinney, 1961). Lastly, acclimation is a factor that can influence the degree of ammonia inhibition. If the methanogens adapt to the inhibitory substances, the microorganisms can retain viability at concentrations far exceeding the initial inhibitory concentrations (Kroeker et al., 1979).

#### 2.4.8.3 Sulfate

Sulfate is a common constituent of many wastewaters due to its presence in many industrial chemicals which are used in a variety of industrial processes (O'Flaherty et al., 1998). In anaerobic wastewater treatment, sulfate is converted to hydrogen sulfide ($H_2S$) by the sulfate-reducing bacteria (SRB) (Hilton & Oleszkiewicz, 1988; Koster et al., 1986). The production of $H_2S$ can result in problems in anaerobic treatment. Two stages of inhibition exist as a result of sulfate reduction. Primary inhibition is due to competition for common organic and inorganic substrates from SRB, which restrain methane production (Harada et al., 1994). SRB, methanogens and/or obligate hydrogen producing bacteria (OHPB) compete for the same substrates, hydrogen and acetate, as they operate in the same environmental conditions. The competition between methanogens and SRB is very complex and is determined by the growth rates of the bacteria. The bacteria with the fastest growth rate will prevail in this competition (Henze, 2008). Secondary inhibition results from the toxicity of sulfide to various bacteria groups (Anderson et al., 1982; Colleran et al., 1995; Colleran et al., 1998; Stefanie et al., 1994).

If the concentration of soluble sulfates exceeds 200 mg/l, the metabolic activity of the methanogenic population will experience significant inhibition. However, after acclimatization, concentrations ranging between 100 – 200 mg/l may be tolerated (Grady Jr et al., 2011). At a neutral pH, hydrogen sulfide acts as a weak acid, and thus, is present in equilibrium with the hydrogen sulfide ion, as shown in Equation 2-23.

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

Equation 2-22

Hydrogen sulfide have limited solubility in water and tends to distribute itself between the liquid and gas phases. This leads to a reduction in methane yield per unit of degraded organic waste, which has a negative effect on the overall energy balance of the process. In addition, the quality of the biogas is reduced since a part of the sulfide produced ends up as $H_2S$ in the biogas (Henze, 2008). The $H_2S$ can lead to odor issues, corrosion of equipment and formation of sulfur oxides occur when the gas is burned (Grady Jr et al., 2011).

#### 2.4.8.4 Salinity

Chloride compounds, like NaCl, can inhibit the activity of anaerobic microorganisms responsible for breaking down organic matter in the wastewater, thereby leading to decreased removal efficiency of COD and TSS. For saline wastewater, salt is considered to be a significant inhibitor of anaerobic treatment. However, the amount of salt inhibition varies based on the quality of the wastewater, specifically the types of substrates that support bacterial growth (Xiao & Roberts, 2010).

Aslan and Şekerdağ investigated the impact of salt concentration on the anaerobic treatment of synthetic high salinity wastewater using the UASB reactor, focusing on the salt inhibition on COD removal rate and efficiency, as well as methane production. The results indicated a significant decline in COD removal rate and efficiency as the salt concentration increased from 0 to 50 g/l (Aslan & Şekerdağ, 2016).

#### 2.4.8.5 Heavy metals

Heavy metals such as ferrous, zinc, cadmium, cuprous and cupric are essential micronutriens required for growth and survival of bacteria, however excessive amounts of these metals can be toxic and inhibit cellular reactions. Heavy metals may not pose a significant problem in anaerobic reactors, despite their potential for extreme toxicity, as only soluble forms of these metals can have an impact on the process. Moreover, the soluble concentrations of these metals can be reduced to non-toxic levels by precipitating them with sulfides produced during the process. In situations where inadequate sulfide is produced, sulfur can be added. This must be done with caution as sulfides are inhibitors by preventing methanogens from producing methane, as mentioned above.

To precipitate 1 mg of heavy metal, approximately 0.5 mg of sulfides is required. Ferrous sulfide is an ideal source of supplemental sulfide, as it is less inhibitory than other heavy metals, as shown in Table 2.6. Additionally, the sulfide precipitates of more inhibitory heavy metals are more insoluble than ferrous sulfide, allowing the added sulfide to maintain low concentrations of those heavy metals. As long as the pH remains at or above 6.4, any excess iron will form iron carbonate and prevent inhibition caused by soluble iron (Grady Jr et al., 2011)..

***Table 2.6*** *Concentration of soluble heavy metals exhibiting 50% inhibition of anaerobic processes (Grady Jr et al., 2011).*

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

#### 2.4.8.6 Light metal cations

Maintaining a neutral pH in the reactor usually involves the addition of a base. However, this must be taken with caution as light metal cations present in most bases can have toxic effects on the microbial community. Of particular concern are sodium, potassium, calcium, and magnesium due to their widespread use and their toxicity exhibits a complex interaction. These cations are necessary for microbial growth and can affect the specific growth rate, similar to other nutrients. If the concentration of one cation is less than the required maximum growth concentration, the toxicity exhibited by another cation will be more severe (Grady Jr et al., 2011). Further, the simultaneous presence of two cations at toxic concentrations will have a more significant effect than either cation individually. Despite these complexities, some generalizations about the effects of various cation concentrations can be made and are summarized in Table 2.7.

***Table 2.7*** *Stimulatory and inhibitory concentration of light metal cations in anaerobic processes (Grady Jr et al., 2011).*

| **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 concentration which is listed as stimulatory are those that allow for the maximum reaction rates, ensuring the optimal metabolic activity for the bacteria under normal conditions. The concentrations listed as moderate inhibitory are adaptable after a period of acclimatization, as long as they are applied steadily. However, a sudden increase in these concentrations can significantly disrupt the performance of the reactor for several days. On the other hand, strongly inhibitory concentrations can severely hinder bacterial growth, requiring excessively long SRTs to prevent process failure. If the toxic effect of a light metal cation cannot be countered by adding stimulatory concentrations of other cations, diluting the wastes becomes necessary. Table 2.8 summarizes antagonistic responses for the light metal cations and ammonia.

***Table 2.8*** *Antagonistic responses for light metal cations and ammonia.*

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

### 2.4.9 Other organic compounds

Organic compounds that are typically associated with aerobic processes have a wide range of potential to cause inhibition in anaerobic processes. These organic compounds can also be biodegraded to a significant extent with sufficient acclimatization. Certain organic compounds, which exhibit low solubility in water or adhere to biomass, have the potential to accumulate and disrupt anaerobic processes. Examples of such compounds include Ethyl benzene, Formaldehyde, Ethyl dibromide, chloroform, and Alkyl benzene sulphonate detergent (Grady Jr et al., 2011). During acclimatization, the activity of methanogenic bacteria community may decrease considerably.

## 2.5 Up-flow Anaerobic Sludge Blanket (UASB) Reactor

The following sub-sections are divided into two parts. The first section presents a comprehensive overview of the general concept of UASB reactor, including typical operating conditions required for a successful application. The second section delves into the specific application of UASB reactor for the treatment of municipal wastewater.

### 2.5.1 General concept

One of the most effective high-rate anaerobic technologies utilized for the treatment of various types of wastewaters is the UASB reactor, which has been widely adopted and proven successful. The ability of the UASB reactor to maintain a high sludge concentration and achieve efficient separation of solids, liquids, and water phases is the key factor behind its success.

The UASB reactor consists of a rectangular or circular tank in which water or sludge flows in an upward direction through an activated anaerobic sludge bed. The influent wastewater enters the bottom of the bioreactor via a distribution system that is engineered to deliver a uniform flow across its cross-section. The sludge bed, which is made up of highly settleable granules or flocs, containing mixed cultures of bacteria, fills about half of the volume of the reactor. As the wastewater or sludge passes through these granules, VFAs and biogas are formed in the porous particles (Grady Jr et al., 2011; Henze, 2008). Biogas generated during the process rises automatically to the top of the reactor, carrying water and solid particles, including biological sludge and residual solids. Via baffles, the biogas bubbles are directed towards a gas-liquid surface located at the upper part of the reactor, which leads to an efficient gas-liquid-solid separation. The upward flow of gas bubbles and liquid result in the ascent of certain granular and flocculent solids, which then enter the gas-liquid-solid separator. These solid particles fall back to the top of the sludge blanket, while the released gases are captured in an inverted cone or similar structure located at the top of the reactor. As water flows through the apertures between the baffles, it carries some solid particles that settle in the settling area. This is due to a reduction in upward velocity caused by an increase in the cross-sectional area. After settling, the solids slide back into the sludge blanket, while the water exits the settlers via overflow weirs. The basic UASB reactor is illustrated in Figure 2.11.

In order to improve the treatment efficiency of municipal wastewater at low temperatures the Expanded Sludge Granular Blanket (ESGB) reactor was introduced. Effluent recirculation combined with a taller (or a high height/diameter ratio) reactor led to a better influent distribution than the basic UASB reactor. The ESGB utilizes an up-flow liquid velocity greater than 4 m/h to expand the granular sludge blanket and eliminate dead zones, resulting in improved sludge-wastewater contact and prevention of excess sludge accumulation (Lettinga et al., 1999; Rebac et al., 1995a). A taller reactor provides a large surface area for the attachment and growth of microorganisms. This leads to a more stable system with better performance (Seghezzo et al., 1998). It has been extensively demonstrated that reactors with recirculation can effectively treat low strength wastewater, even though the recirculation of the effluent serves to dilute the influent concentration (Kato, 1994; Kato et al., 1994). Additionally, the ESGB can accommodate higher OLRs compared to the UASB, and consequently the gas production is also higher. It is important to consider the impact of gas loading and hydrostatic pressure at the bottom of tall reactors since it can be higher than in short reactors, and the effect of these parameters on the performance of the process also has to be considered (Seghezzo et al., 1998). According to research conducted by Rebac et al., the ESGB reactor exhibits promising potential as a high-rate treatment system for low-strength wastewater under psychrophilic conditions (below 12°C) (Rebac et al., 1995b). The study achieved COD removal efficiencies exceeding 90% at OLRs of up to 12 gCOD/l per day and HRT as low as 1.6 hours using a VFA-mixture as feed. Measuring VFAs can aid in identifying the factors responsible for changes in removal efficiency (Seghezzo et al., 1998; van Lier et al., 1997). The ESGB reactor compared to the UASB reactor is shown in a schematic diagram in Figure 2.11.

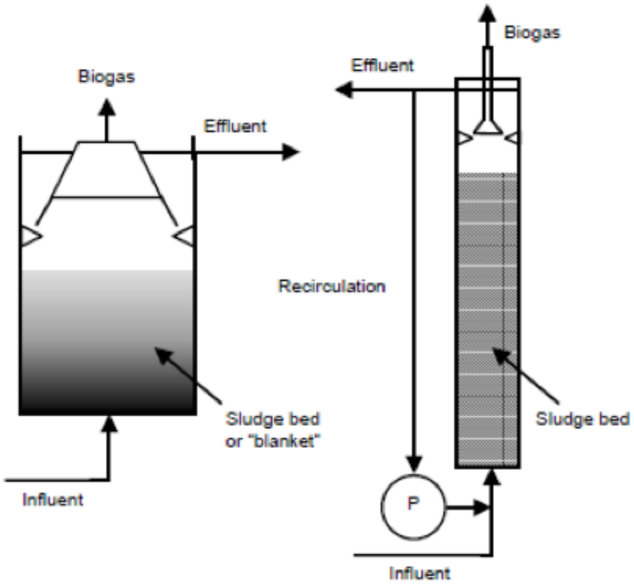

***Figure 2.11*** *Schematic diagram of the UASB (left) and ESGB (right) bioreactor (Seghezzo et al., 1998).*

### 2.5.2 Granulated anaerobic wastewater treatment

The UASB reactor uses granulated biomass and has been successfully applied for treatment for both municipal wastewater and domestic sewage (Lettinga, 2010; Singh & Viraraghavan, 1998). The biomass in UASB reactors is in the form of compact granules that contain a complex community of microorganisms embedded in the extracellular polymeric substances (EPS) matrix, that is biofilm (Safitri, 2022). From studies on the microbial composition of the granules, it appears that the surface is made up of coccid bacteria, while rod-shape *Methanosaeta* are dominant in the interior and provide a filamentous structural backbone for the granulation. The physical characteristics of the granules provide a complex microbial ecology producer. A specific methane production activity of 0.1 g COD/VSS per day in the granular particles has been reported (Henze et al., 2008; Seghezzo et al., 1998).

Anaerobic microorganisms can form granules through self-immobilization of bacterial cells, and the performance of the UASB system is strongly dependent upon granulation process with a particular wastewater. Anaerobic granular sludge consists of millions of organisms per gram biomass. None of the individual species can fully break down the incoming organic matter, but they work together through complex interactions to completely degrade it. Granular sludge reactors are useful in wastewater treatment because they can maintain a high number of organisms, and rapidly transform and treat large volumes of organic matter in a small space (Liu & Sung, 2002). The granules in these reactors are large and dense, allowing them to settle quickly, making it easy to separate purified effluent from the biomass (Batstone & Keller, 2001; Noyola & Moreno, 1994).

The size distribution and the settleability are important characteristics that affect the retention of granules. The ability of granules to settle indicates that the active biomass which is driving the treatment does not get washed out of the system. This allows for high volumetric rates and high OLRs, thereby facilitating compact and reduced design costs of WWTPs (Hulshoff Pol et al., 2004). The size distribution and density of the granules determines the settleability and consequently the retention. Hydrodynamics and the type of feed influence the psycho-chemical characteristics of anaerobic granules (Batstone & Keller, 2001). The diameter of anaerobic granules ranges from 0,1 mm to 8 mm, depending on the waste treated and hydraulic and gas shear forces (Henze, 2008; Trego et al., 2020; Wu et al., 2016), and they are usually dark in color and spherical. Generally, particle densities are in the range of 1.0 to 1.05 g/l (Henze, 2008), and they have settling velocities around 60 m/h (Hulshoff Pol et al., 2004). This is 20 – 30 times the critical velocity of activated sludge flocs (Satoh et al., 2007). Anaerobic granules are naturally porous and layered, and as their size increases their porosity does as well. This is important for mass transfer and activity (Wu et al., 2016).

The development of a granular sludge bed can take many months, but this is normally avoided today by seeding with granular sludge waste from other UASB reactors. Schmidt and Ahring (1996) describe a four-step process for the development of granular sludge: (1) attachment of cells to an uncolonized inert material or other cells, (2) initial adsorption of other colloidal or bacteria particles by reversible physiochemical forces, (3) irreversible attachment of microbial organisms due to microbial extracellular polymers, and (4) multiplication of cells from substrate diffusion into the granular structure (Henze, 2008).

### 2.5.3 Municipal Wastewater Treatment Application

The UASB reactor was originally designed for treating high strength wastewaters, and was first conducted in Cali, Colombia in the early 1980s (De Man et al., 1986; Lettinga, 1995; Lettinga & Hulshoff Pol, 1991; Seghezzo et al., 1998). It was a 64 m$^3$ UASB pilot scale reactor, operated to demonstrate the effectiveness in treating municipal wastewater under prevailing environmental conditions. The pilot scale reactor showed promising results and was shortly followed by constructions of full-scale reactors in Colombia, Brazil and India. Subsequently, research on the UASB reactor was initiated to investigate the potential to treat different types of wastewaters, under various temperature conditions, and in both full scale and pilot scale systems (Henze, 2008). The technology of anaerobic treatment has evolved from focused laboratory-scale experiments to successful full-scale implementations (Van Lier et al., 2015). Most full-scale UASB reactors are treating industrial wastewater, but application of full-scale UASB reactors treating municipal wastewater has largely increased over the last decade. The majority of the full-scale UASB reactors treating municipal wastewater lay in warm climate regions such as Brazil and Columbia (Khan, 2011).

Researchers have studied the use of UASB reactors for treating high strength municipal wastewater under psychrophilic and low mesophilic conditions since 1976. Seghezzo et al. (1998) operated a 6 m$^3$ UASB reactor seeded with digested sewage sludge at HRT of 14 – 17 h and observed COD reduction of 85 - 65% and 70 - 55% at 20°C and 13 - 17°C, respectively. He concluded that the UASB technology was a cost-effective, compact, and simple approach for treating sewage even at lower temperatures. De Man et al. (1986) conducted research on various UASB reactors (0.120, 0.240, 6, and 20 m$^3$) and determined that anaerobic treatment of low to high strength domestic sewage (500-1500 mgCOD/l) could be achieved at 12-18°C with an HRT of 7-12 h, resulting in total COD removal efficiencies of 40 - 60%.

A study conducted in India investigated the application of UASB reactors treating low to medium strength municipal wastewater under field conditions. The UASB reactors represent a robust and efficient technology for sewage pre-treatment with COD removal of 51 ± 13%. It was capable to generate renewable energy with a biogas yield of 0.20–0.40 $m^3$/kgCOD removed under the conditions prevailing in India. The UASB was utilized to treat low strength wastewater with COD concentration of 300 mg/l, resulting in effluent COD of around 120 mg/l.

## 2.6 Post-treatment technology development for anaerobic treatment effluents

Traditional effluent limits are still exceeded for various organic compounds, nutrients, and pathogens in the anaerobic effluent. The discharge of wastewater into norwegian waters is regulated by the Norwegian Pollution Control Act (Forurensningsloven) and the Water Resources Act (Vannressursloven). Under these acts, the WWTPs in Norway are subjected to a discharge limit of removing 75% of the COD supplied to the treatment plant (Lovdata, 2007). As a result, post-treatment unit processes are usually required for UASB reactors to meet water quality standards, safeguard receiving water bodies, and/or make the effluent suitable for water reuse (such as agricultural irrigation) (Safitri, 2022). The main role of the post-treatment is to complete the removal of organic matter, as well as to remove constituents little affected by the anaerobic treatment, such as nutrients (nitrogen and phosphorus) and pathogenic organisms (viruses, bacteria, pro-tozoans and helminths) (Chernicharo, 2006). To polish the anaerobic effluent aerobic post-treatment is used to achieve high overall treatment efficiency. The aerobic treatment also smoothes out fluctuations in the quality of the anaerobic effluent (Chernicharo, 2006). A common approach to remove nitrogen is to send the effluent from the anaerobic treatment to an aerobic reactor, where nitrification and denitrification occur. Phosphorus removal can also be achieved in the aerobic reactor using chemical precipitation or biological processes (Wang & Wang, 2013). Discharge of nutrients into recipient water bodies contributes to the eutrophication process. The eutrophication process is abnormal algae growth due to the nutrients discharged, which can cause hypoxia (or oxygen depletion) and harmful algal blooms (Chernicharo, 2006). Post-treatment processes that are commonly used for pathogen removal include disinfection methods such as ultraviolet radiation, ozonisation and chlorination (Chernicharo, 2006). Pathogenic organism removal is essential, especially in developing countries with poor sanitation and inadequate investment in health, to reduce the risk of human contamination (Chernicharo, 2006).

Several unit operations have been implemented for anaerobic effluent post-treatment to meet the water quality standards. These include activated sludge systems (Cao & Ang, 2009; Mungray & Patel, 2011), microalgal-based treatments (Ángeles et al., 2021; Torres-Franco et al., 2021), membrane filtrations (Bailey et al., 1994; Ozgun et al., 2015; Rivera et al., 2021) and biofilm-based treatments (Tawfik et al., 2003; Vieira et al., 2013).

## 2.7 Dissolved methane removal from anaerobic effluent

The atmospheric accumulation of greenhouse gases is considered to be the main cause of global climate change. Human activities and industrialization are increasing this accumulation, and it is recognized as one of the major problems facing humanity. Carbon dioxide and methane is the main contributors of the greenhouse gases in the atmosphere, where carbon dioxide being 77% of total emissions (considering both the volume and its global warming potential), and methane is emitted at a lesser extent (14%) but has a global warming potential 34 times greater than carbon dioxide (Ruiz-Ruiz et al., 2020). This means that biogas recovery from municipal wastewater through anaerobic processes might not be economically practical and environmentally friendly due to the fact that substantial amount methane is dissolved into treated effluent (Liu et al., 2014).

Soluble COD in municipal wastewater often ranges from 100-300 mg/l with an average of 200 mg/l. Theoretically, 350 ml of methane can be produced from 1 g of bCOD removed (Lawrence & McCarty, 1969; Liu et al., 2014). If the COD removal efficiency of the anaerobic process is as high as 90%, the produced methane is about 63 l/m$^3$ wastewater. This is equivalent to about 41 g/m$^3$ at 30°C. The solubility of methane at 30°C is 18.5 g/m$^3$, which means that for an anaerobic reactor being operated at 30°C the methane produced is 45% in its dissolved form. The lower the temperature the anaerobic system is operated at the higher fraction of the methane produced is in its dissolved form (Liu et al., 2014). Dissolved methane would lead to reduced energy efficiency of anaerobic process for municipal wastewater treatment, and it would increase the risk of being released into the environment. Methods and technologies for recovering dissolved methane for the anaerobic effluent is required to reduce the environmental risk of anaerobic treatment of municipal wastewater. Post-treatments that have been developed include degassing membrane-based recovery (Bandara et al., 2011; Cookney et al., 2016) and air stripping oxidation (Hatamoto et al., 2010; Matsuura et al., 2015).

## 2.8 Knowledge Gaps

There is a lack of research on the efficiency of UASB reactors treating low strength municipal wastewater, which is concerning since adaption to treat low strength wastewater is one of the most important challenges of the UASB process (Singh et al., 1996). COD concentrations can vary widely depending on the source of the wastewater, and industrial wastewater tends to have higher COD concentrations than domestic wastewater. In Norway, the average COD concentration in domestic wastewater according to the Norwegian Water Resources and Energy Directorate (NVE) is around 600 – 800 mg/l, which is relatively low compared to other countries (Norwegian Water Resources and Energy Directorate). In order to maximize the potential and efficiency of the UASB reactor in the conversion of organic matter into methane, it is imperative to conduct additional research on the UASB application for treating low strength wastewater.

## 2.9 Specific Objectives

This thesis investigates the treatment of low- and medium strength wastewater at room temperature using UASB technology. The main objective was to determine the effectiveness of the UASB reactor for converting organic matter to methane on wastewater with medium influent COD concentrations of IVAR WWTP Vik, and wastewater with low influent COD concentrations of IVAR WWTP SNJ. To achieve this objective, in-house designed laboratory scale, UASB reactor systems were set up. The wastewater from IVAR Vik and IVAR SNJ has average influent COD concentrations of 931 mg/l and 212 mg/l, respectively (De Lara, 2023; Wold & Furre, 2023). Based on the literature review and theory background, there is a need to conduct observations to evaluate the process performance and reliability of anerobic treatment processes in UASB reactors, specifically designed for treating low- to medium strength wastewater. To evaluate the productivity of the anaerobic granular sludge system, several parameters were studied. These included determining its efficiency in removing COD, measuring the rate of methane production, and assessing methane yield. Additionally, a COD balance analysis was conducted to assess reactor performance and investigate COD recovery and loss during operation. TSS was measured to have an indicator of the water quality and the effectiveness of the treatment process. The focus was also on identifying critical factors that affect the performance of UASB reactor. Critical factors include VFA composition- and specification, and sulfate- and chloride concentration. pH was measured frequently to ensure that the reactor was within the optimum range.

# 3 General Methodology

This chapter describes the laboratory-scale equipment applied in the anaerobic treatment experiment of wastewater from IVAR Vik (Vik, Norway) and IVAR SNJ (Mekjarvik, Norway) for energy production in the form of methane in an UASB reactor. Two UASB reactors were used in the study for investigating UASB reactor performance and analyzing biogas potential; one reactor (Reactor Vik) treated wastewater with primary treatment from IVAR WWTP Vik; and one reactor (Reactor SNJ) treated wastewater with primary treatment from IVAR WWTP SNJ. All laboratory analysis for this bachelor's thesis project were conducted at the University of Stavanger.

In this study, the performance of two laboratory-scale UASB reactors treating municipal wastewaters with low- to medium COD levels (100 - 1500 mgCOD/l) was investigated over 76 days (11th of February to 28th of April 2023). To assess the performance of the anaerobic granular sludge system, the capacity for converting and removing COD, the TSS removal efficiency, the specific methane production rate, and the methane yield of the reactors were measured. Reactor performance was determined by interpreting the observations using COD mass balance analysis. The pH variability, VFA composition and sulfate- and chloride concentrations was measured to detect problems in the reactor.

## 3.1 UASB systems and operation

The installation of critical instruments was necessary for the establishment of the laboratory-scale UASB reactors. The combined efforts of these instruments assured the anaerobic treatment process. This section provides a comprehensive account of the configuration of the two reactors utilized in this study.

### 3.1.1 The configuration of the UASB reactors

Figure 3.1 displays the identical UASB Reactor Vik and Reactor SNJ that were used for investigating the UASB performance at treating low to medium strength wastewater. The granules were obtained from Acevom NV in Gent, Belgium, and they originate from vegetable and potato processing companies. The methanogenic activity of the granules is in general 0.5 – 0.6 g $CH_4$·COD/g VS·d, and their size is about 1 – 3 mm in diameter. The configuration of the reactors is illustrated in the flow diagram of the laboratory-scale UASB reactors in Figure 3.2. As shown in Figure 3.2, the inlet wastewater is stored in a 30 l plastic container in a fridge to affirm stable feed composition of the wastewater. The feed wastewater was pumped into the reactor from the container using a peristaltic pump with an adjustable flow rate. The reactor is made from glassblower Mæhlum (Lillehammer), and the dimensions of the reactors include a height of 540 mm and a diameter of 68 mm, with a total volume of 1 liter. To measure the volume of produced biogas a gas counter was used. Table 3.1 describes the important equipment used in the set-up of the UASB reactors.

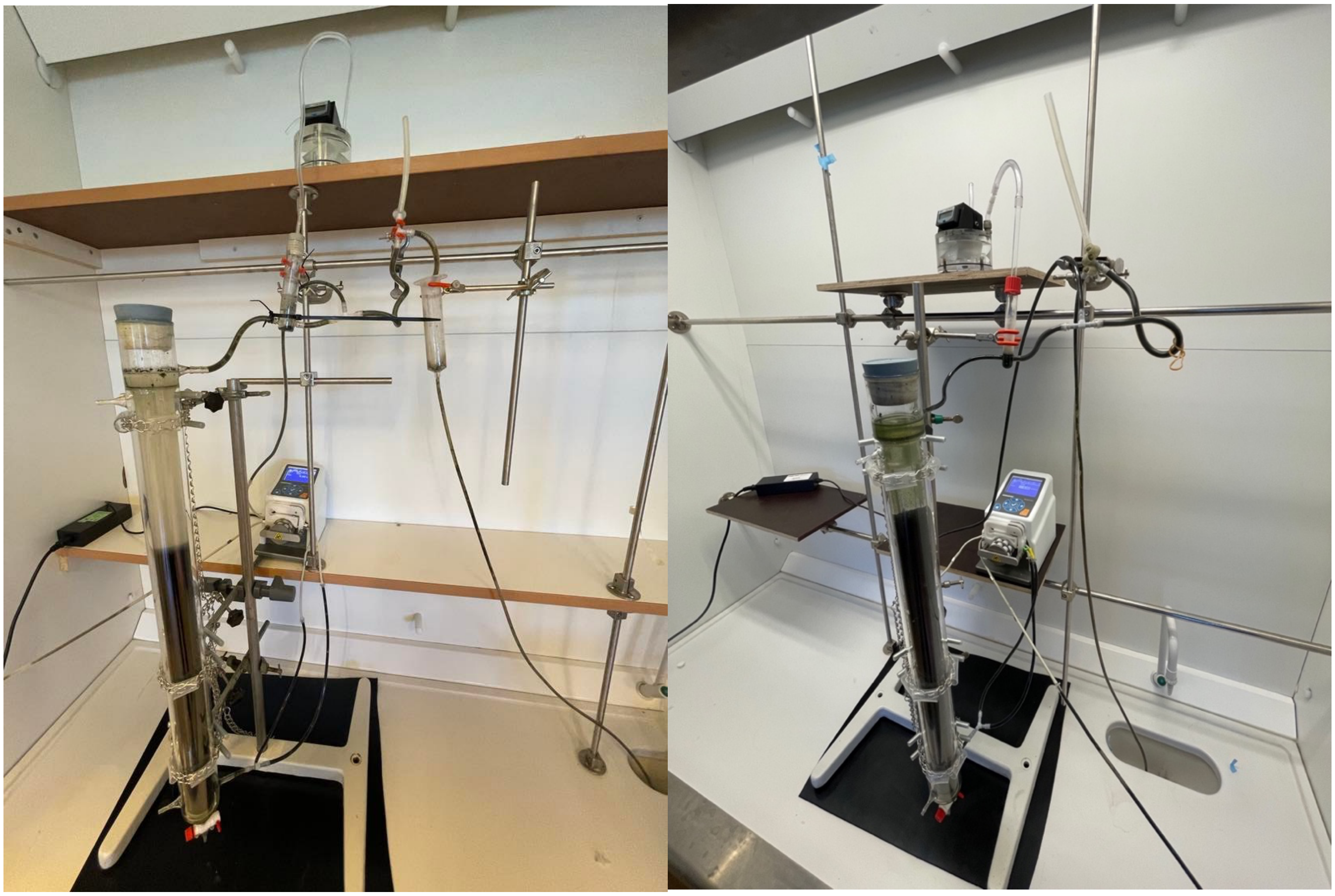

***Figure 3.1*** *Photo of the laboratory scale UASB Reactor Vik (left) and Reactor SNJ (right).*

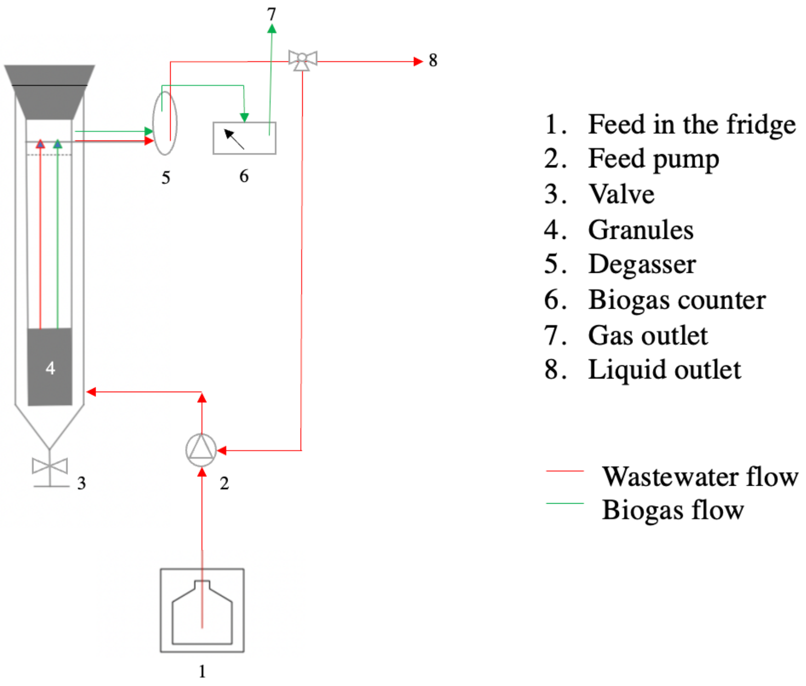

***Figure 3.2*** *The flow diagram of the laboratory scale UASB reactor Vik and SNJ.*

***Table 3.1*** *The properties of equipment used for the UASB reactors.*

| **Equipment** | **Manufacturer** | **Specification** | |
|---|---|---|---|
| Feed and recirculation pump | Ismatec | Type<br>Channel<br>Model<br>Flowrate | : peristaltic pump<br>: 4 independent controllable channels<br>: Reglo ICC<br>: 0 – 43 ml/min |
| Gas counter | Ritter | Model<br>Gas flowrate<br>Max.pressure<br>Min. pressure<br>Vol.measurement<br><br>Measuring accuracy | : MGC-1 V3.3 PMMA<br>: 1 ml/h- 1 l/h<br>: 100 mbar<br>: 5 mbar<br>: Reactor Vik → 3.29 ml<br>: Reactor SNJ → 3.25 ml<br>: less than approx. ±1% |

### 3.1.2 Starting-up UASB Reactors and Operational conditions

The experiment in Reactor Vik and Reactor SNJ was carried out to investigate the UASB performance and biogas potential for 76 days. The aim was to monitor the reactors towards achieving a steady state. The reactors were operated parallelly throughout the investigation. During the first five days of the experiment, the reactors were operated with diluted wastewater from IVAR Grødaland WWTP (Norway), with dissolved COD concentration of approximately 500 mgCOD/l. This particular wastewater was chosen as a start-up substrate owing to its high COD concentration. It was also to make sure that all essential equipment, instruments, and materials were appropriately arranged and installed. In this start-up period, the biogas production was the only parameter measured. By measuring the biogas production rate during the start-up process, adjustments can be made to the operational conditions of the reactor to promote the growth and activity of the anaerobic microorganisms. The reactors were filled 75% of their volume, consisting of approximately 350 ml of granules, where it is assumed that 50% of this volume constitutes water filling the interstitial spaces between the granules, and 650 ml of water. This result in a sludge volume of 50 – 60% v/v with respect to the UASB reactors used. These volumes were randomly selected, but with the consideration of having a sufficient amount of granules and adequate space for water outflow. The UASB reactors was started-up at low OLR of 1.0 – 1.5 gCOD/l·d. After five days, the inlet wastewater was replaced by the wastewater from IVAR Vik WWTP and IVAR SNJ WWTP. During operation of the bioreactor, flow rate was monitored daily by measuring the volume of the effluent liquid in a volumetric cylinder within a given time interval to ensure consistency with the flow rate displayed on the pump. This enabled adjustment of the flow rate to attain the desired rate.

## 3.2 Analytical Measurement

Prior to conducting any analytical experiment, it is essential to ensure that the sample to be analyzed was adequately homogenized by shaking, to achieve sufficient distribution of particles. Washing and/or dilution are necessary in analytical procedures. In this study, distilled water was used for dilution/washing purposes. The analytical methods used are described below.

### 3.2.1 pH Measurement

The pH measurement was preformed automatically by using Orion™ Versa Star Pro™ with Orion™ 8156BNUWP ROSS Ultra™ pH Electrode (Thermo Scientific™, US). Prior to each measurement, the electrode was rinsed with distilled water to remove any contaminants. Additionally, the pH meter was calibrated using a buffer solution with a pH value of 7.0 to ensure accurate readings. The pH value was read once the display of the pH meter had stabilized.

### 3.2.2 Total Suspended Solids (TSS) Measurement

TSS was determined according to Standard Methods 2540D and 2540E (Rice et al., 2013). Cytiva Whatman GF/C™ filters (47 mm diameter) with particle retention 1.2 µm (Cytiva, US) suitable for combustion were placed in a drying oven over night at 103 - 105°C, to ensure complete dryness of the filters. Aluminum dishes was used to support the filters. The filters were cooled in a desiccator, and the weight of the filters was measured using an analytical balance. The weight was noted as $m_{filter}$. One filter was transferred to the filtering device and fixed by adding vacuum, while the other filters were kept in the desiccator. Wastewater was gradually transferred from a cylinder to the filter while applying vacuum to the filtering device. The cylinder with wastewater was measured before and after filtration, and the difference is the sample volume, noted as $V_{sample}$. The wastewater was applied centrally to the filter disk to avoid particulate build up along the filtering device wall. The filter was vacuum-dried for 3 minutes, before carefully removed from the filtering device to the aluminum dish. The water in filtration device (filtrate) was retained for dissolved component analysis. The filter was dried in oven overnight before it was cooled in a desiccator and measured. The weight was noted as $m_{filter+solids.}$ The same procedure was repeated to both influent and effluent for the two wastewaters. TSS was calculated with Equation 3-1.

$$TSS\left[\frac{mg}{l}\right] = \frac{m_{filter+solids} - m_{filter}}{V_{sample}}$$

Equation 3-1

### 3.2.3 COD Measurement

COD was determined according to Standard Methods 5220D (Rice et al., 2013) with potassium dichromate in sulfuric acid (colorimetric) using Merck Spectroquant® COD test kits. The COD test kits used had Product Number 14541 COD cell test (25 – 1500 mg/l concentration range), Product Number 09773 Hg-free COD cell test (100 – 1500 mg/l concentration range) and Product Number 09772 Hg-free COD cell test (10 - 150 mg/l concentration range). Both total COD (tCOD) and dissolved COD (sCOD) were analyzed from both influent and effluent of UASB reactors. It was ensured that the wastewater analyzed were homogenized.

The procedure of the COD measurement was started by using a volumetric pipette to transfer 2-3 ml homogenized sample to a COD vial. The volume pipetted depends on the amount specified in the manual of the corresponding test kit. When the COD concentration was expected to exceed the concentration range of the cell test kit utilized, the sample was diluted with distilled water to fit in the range. The sample was digested in a thermo reactor (Spectroquant® Model TR 620, Germany) at 148°C for 2 hours. After 2 hours, the COD vials were removed from the reactor and placed in a metal test tube rack to cool until room temperature. Following a period of 10 minutes, the sample was swirled and returned to the rack for complete cooling, which took approximately 30 minutes. When the test tubes reached room temperature, the vials were placed in a spectrometer (Spectroquant® Prove 300, Germany). The mark on the test tube was aligned with the orientation mark as it was placed in a spectrometer cell compartment. The correct measuring method according to the corresponding test kit utilized was selected. The reading was equivalent to COD concentration. COD vial was eventually placed in prescribed container. The same procedure was repeated to determine dissolved COD (sCOD) of the filtrate collected in the filtration device, for both influent and effluent.

### 3.2.4 Total Volatile Fatty Acid (VFA) Measurement

A 5-point titration procedure was used for determination of total VFA. The measurements were performed using TitroLine® 500 Auto-titration (SI Analytics, Germany). Initially, 20 ml of each sample (SNJ influent, SNJ effluent, Vik influent and Vik effluent) was filtrated, and subsequently diluted to a final volume of 50 ml. The samples were then placed on a magnetic stirrer at low rotation (60-100 rpm) to minimize (avoid) $CO_2$ input or loss. Conductivity and temperature were measured on the sample. The initial pH was measured, and if the pH was less than, 6.7, NaOH was added until the pH was about 6.7 (± 0.1). The sample was then titrated with 0.082M HCl to pH 5.9 (± 0.1), and the volume of the acid added was noted. The titration was repeated to pH 5.2 (± 0.1) and 4.3 (± 0.1), and the volumes of the acid added were noted. The calculation of the results was conducted with the computer program TITRA 5 (Moosbrugger et al., 1993). Furthermore, VFA was also specified and confirmed by ion chromatography which will be described in the following section.

### 3.2.5 Ion chromatography (IC)

In addition to VFA specification, the concentration of sulfate ($SO_4^{2-}$) and Chloride ($Cl^-$) in four samples (SNJ influent, SNJ effluent, Vik influent and Vik effluent) were determined using a Dionex ICS-5000+EG IC system (Thermo Fisher Scientific Inc., US). The sulfate concentration was measured due to sulfate being able to be converted to hydrogen sulfide which is a toxic compound in the anaerobic digestion. The measurement of chloride was performed due to the use of mercury free COD test kits where chloride interferes with the COD results. An eluent generator was used to produce a potassium hydroxide (KOH) eluent. The reagents used were KOH from the eluent generator and deionized water with 18 MΩ-cm resistance. The standards used were $MgSO_4$ (VWR, Norway), $CaCl_2$ (VWR, Norway), formic acid (Merck, US), acetic acid (Merck, US), propionic (Riedel-de Haen, US) acid, and butyric acid (Riedel-de Haen, US).

Eight samples were prepared in advance before the IC analysis. At first, approximately 50-100 ml of each of the four samples was filtered in a vacuum filter with particle retention 1.2 µm. The filtrate was collected in eight sample bottles, where four of the bottles were diluted 5 times with destilled water. Once the samples were prepared, each sample was then filtrated again with 0.2 µm filters (VWR, Norway) and transferred to a 1.5 ml short thread vial (Fisher Scientific, Norway). Each sample was measured by IC in duplicate for determination of measurement. The chromatographic conditions applied are presented in Table 3.2.

***Table 3.2*** *The Chromatographic Condition.*

| Parameters | VFA specification | Sulphate and Chloride |
|---|---|---|
| **Column** | IonPac AS-20 (P/N 063148) | IonPac AS11-HC Analytical (P/N 52960) |
| **Flow rate** | 1.0 ml/min | 0.25 ml/min |
| **Column Temperature** | 30°C | 30°C |
| **Detection** | Suppressed conductivity, ASRS, AutoSupression recycle mode, 75 mA | Suppressed conductivity, ASRS, AutoSupression recycle mode, 75 mA |
| **Eluents** | Water<br>Potassium hydroxide (KOH) | Water<br>Potassium hydroxide (KOH) |
| **Method** | Gradient:<br>1 mM Potassium hydroxide (KOH), hold for initial concentration.<br>1 mM Potassium hydroxide (KOH), for 10 minutes.<br>15 mM Potassium hydroxide (KOH), for 10-22 minutes.<br>1 mM Potassium hydroxide (KOH), for 22-30 minutes. | Isocratic:<br>15 mM Potassium hydroxide (KOH), for 18 minutes; |
| **Typical System Operating Backpressure** | 13.8-15.2 MPa (2000-2200 psi) | 13.8-15.2 MPa (2000-2200 psi) |

### 3.2.6 Gas chromatography (GC)

The biogas composition (methane and $CO_2$ ratio) was confirmed by Agilent 7890B gas chromatography (Agilent, USA). Biogas sample was collected in a gas sampling bag which had a volume of 5 l (Tedlar®, Sigma Aldrich, Germany), and squeezed onto GC equipped with a thermal conductivity detector (TCD) (Agilent column, 0.32 mm diameter, 30 m length and 0.25 µm film).

# 4 Results

Results obtained from the experiments are presented in this chapter. This chapter is divided into two sub-chapters; (a) reactor performance including COD removal efficiency, methane production, COD balance analysis, and TSS; (b) pH variability, VFA generation availability- and composition and Sulfate ($SO_4^{2-}$) – and Chloride ($Cl^-$) concentrations. The data and figures that were presented have been summarized, while the raw data that was collected is included in the Appendixes.

## 4.1 Reactor Performances

In this sub-chapter, the performance of Reactor Vik and Reactor SNJ during the experiment is described. The reactor performances were characterized by their ability to remove COD and convert it into methane.

### 4.1.1 COD Removal Efficiency

The sCOD profile efficiency as a function of time for both influent and effluent for Vik wastewater are shown in Figure 4.1. The figure demonstrates that the influent sCOD is maintained at a higher value compared to the effluent sCOD, excluding from day 7 until day 12. This indicates a mostly positive COD removal efficiency. Average sCOD influent was 460 mg/l, and the sCOD effluent ranges from 70 – 650 mg/l. The lowest sCOD concentrations observed were from day 48 until day 61 while the wastewater samples were diluted with distilled water, due to usage of Hg free COD kit 09772 with concentration range 10 - 150 mg/l, to fit in the concentration range. On day 63 and day 65, the sCOD influent were measured with diluted samples as well. The average influent concentration when kit 09772 was used was 141 mg/l and the average effluent concentration was 123 mg/l. The average influent concentration when the other two kits was used, kit 09773 and 14541, was 460 mg/l and the average effluent concentration was 331 mg/l.

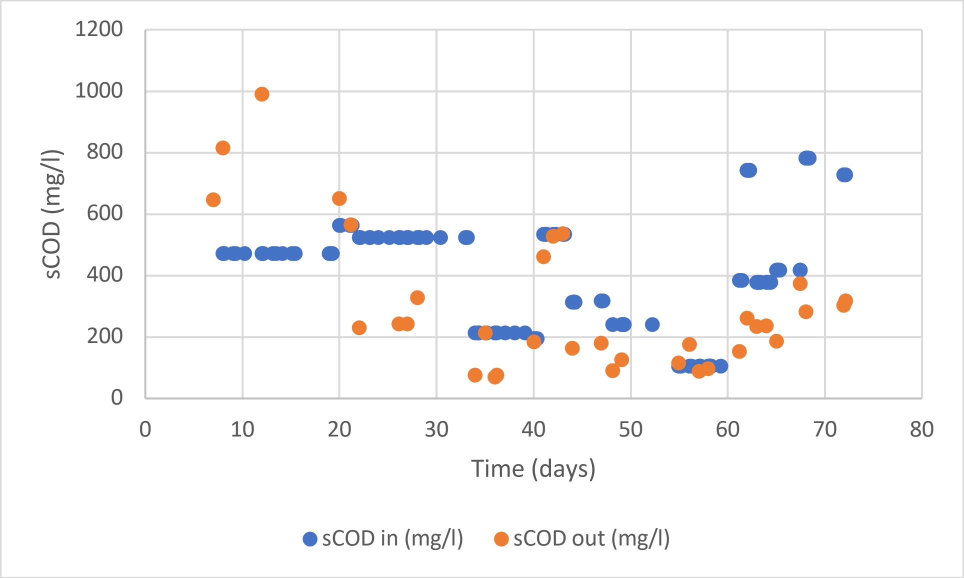

***Figure 4.1*** *Influent and effluent COD concentration of Reactor Vik as a function of time.*

Figure 4.2 illustrates the relationship between sCOD removal efficiency (%) and organic loading rate (OLR) as function of time. A notable increase in OLR from 3.3 g/l.d to 6.3 g/l.d can be observed after 41 days, occurring at the same time as an increase in flow rate from 2.7 ml/min to 4.4 ml/min. Several low sCOD removal efficiencies were noted when the OLR increased, which can be observed on day 41 (14%) and day 42 (1%). When OLR decreased to 2.2 g/l.d on day 48, the sCOD removal efficiency increased as well (approx. 50%). Followingly, the sCOD removal efficiency was a negative 12% on day 55 after a gradual decrease in OLR from day 48 to day 55. The highest sCOD removal efficiencies were observed from day 62 (65%), when the OLR increased to 10.3 g/l.d. The sCOD removal efficiency then remained stable around approximately 60% during the rest of the experiment despite a decrease in OLR from 10.3 to 6.4 g/l.d.

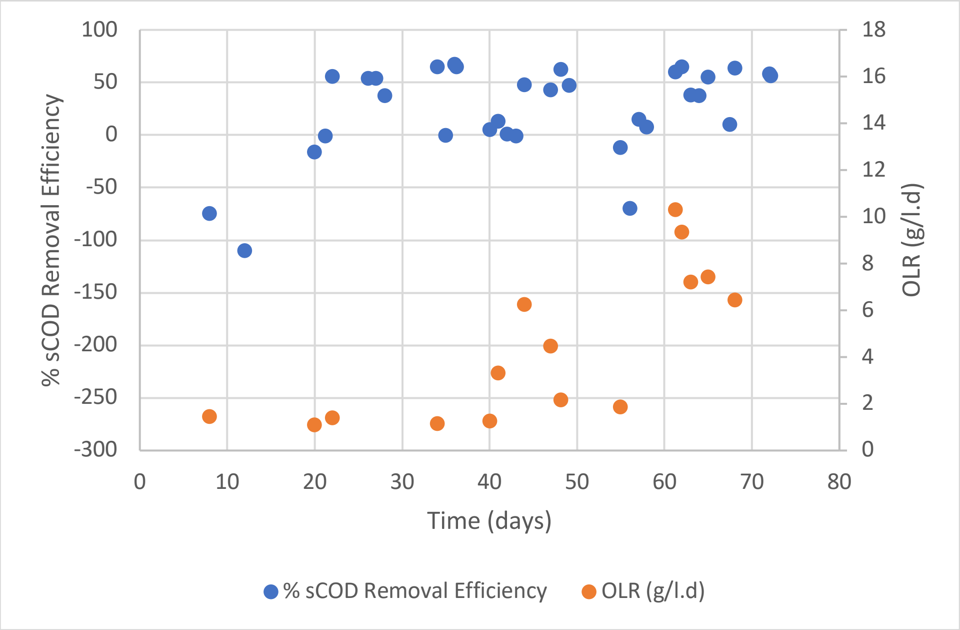

***Figure 4.2*** *Relationship between sCOD Removal Efficiency (%) and OLR (g/l.d) as a function of time.*

TSS removal efficiency (%) compared to tCOD removal efficiency (%) as functions of time are illustrated in Figure 4.3. The figure displays an improved tCOD removal efficiency over time. The highest tCOD removal efficiency was achieved on day 44 (79%), while the lowest removal efficiency was observed on day 20 (-35%). The average tCOD removal efficiency was 31%. The tCOD removal efficiency stabilizes from day 55 to day 72 at an average of 54%. The average TSS removal efficiency in the operating period was 14%, while the highest and lowest TSS removal efficiency was on day 43 (99%) and day 35 (-561%), respectively. Between day 34 and 36 there are several noteworthy datapoints indicating an average TSS removal efficiency of -452%. On day 40 there is a deviating data indicating tCOD removal efficiency of –153%. These observations suggest a significant deviation from the expected trend and warrant further investigation to understand the underlying causes and potential implications. Based on the considerable deviation demonstrated by these observations, they are excluded from the analysis.

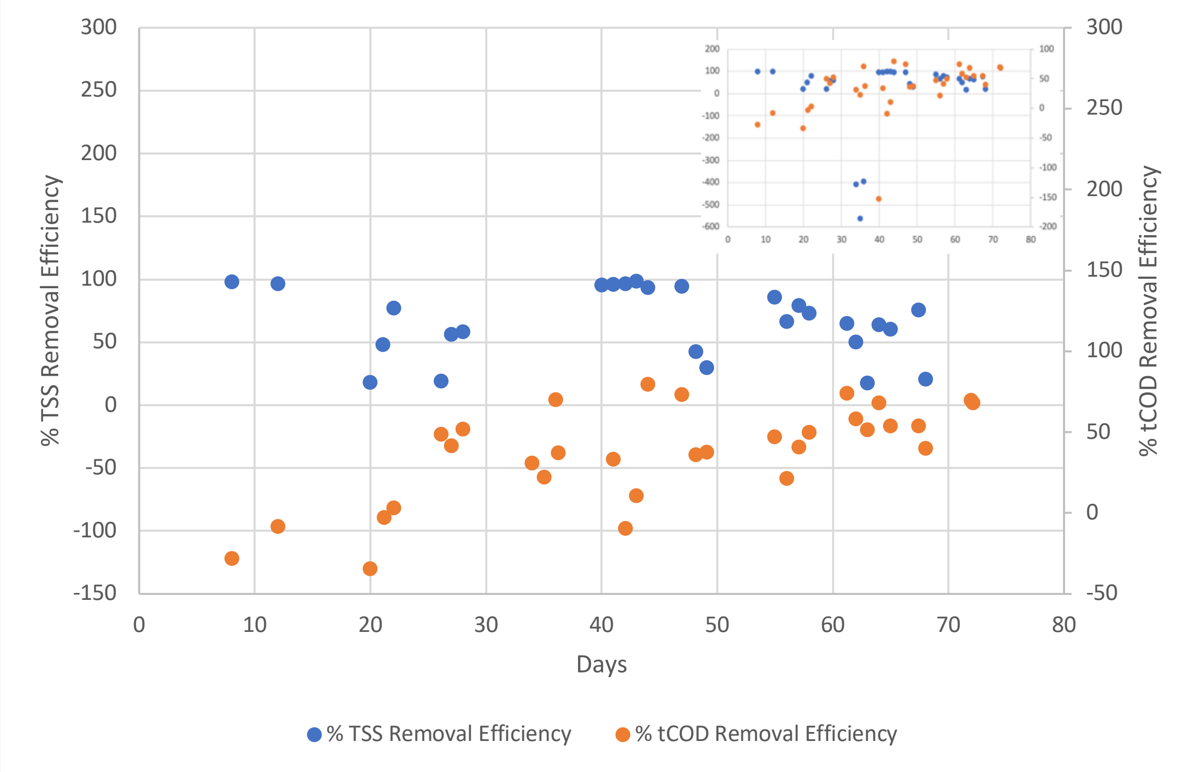

***Figure 4.3*** *The relationship between %TSS Removal Efficiency and %tCOD Removal Efficiency as functions of time of Reactor Vik.*

To determine the particular removal efficiency of COD (pCOD), the disparity between the tCOD and the sCOD was computed. Figure 4.4 illustrates the TSS removal efficiency (%) in comparison to the efficiency of pCOD removal (%) as functions of time. The average pCOD removal efficiency in the observation period was 239%. There are several deviating datapoint suggesting pCOD removal efficiencies of 4633% (day 20), 1733% (day 20), 1116% (day 27) and 1183% (day 28), and due to their significant deviation from the trend line, these data point will be excluded from the analysis. Towards the end of the observation period from day 55 to 72, the data demonstrates a discernible trend in which both TSS and pCOD removal efficiencies approach a stabilized value of approximately 68%.

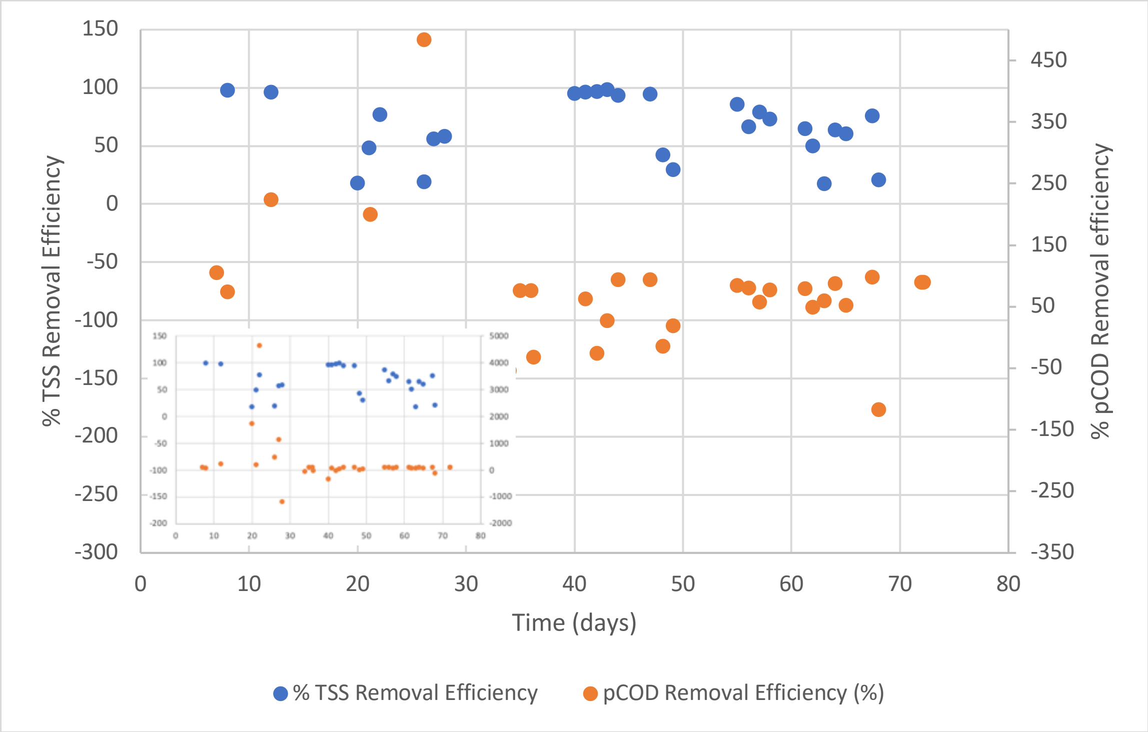

***Figure 4.4** The relationship between %TSS Removal Efficiency and %pCOD Removal Efficiency as functions of time of Reactor Vik.*

The sCOD profile efficiency (%) as a function of time for both influent and effluent for Reactor SNJ are displayed in Figure 4.5. The effluent sCOD concentrations exceeds the influent sCOD concentrations indicating a negative removal efficiency. Between day 12 to day 26 there are no datapoints, due to lack of wastewater in feed tank on day 12 which resulted in oxygen being pumped into the reactor and the granules being damaged. Thus, the granules were replaced on day 23, and measurements were continued on day 26. The average influent sCOD was about 505 mg/l, and effluent sCOD varied from 88 to 890 mg/l. From day 48 to day 61, a significant decrease in sCOD values for both influent and effluent were observed, from a concentration of 308 to 72 mg/l for the influent and from 400 to 140 mg/l for the effluent. The Hg free COD kit 09772, which required dilution of the wastewater samples, was used in this period. The average influent concentration when kit 09772 was used was 97 mg/l and the average effluent concentration was 109 mg/l. The average influent concentration when the other two kits was used, kit 09773 and 14541, was 504 mg/l and the average effluent concentration was 480 mg/l.

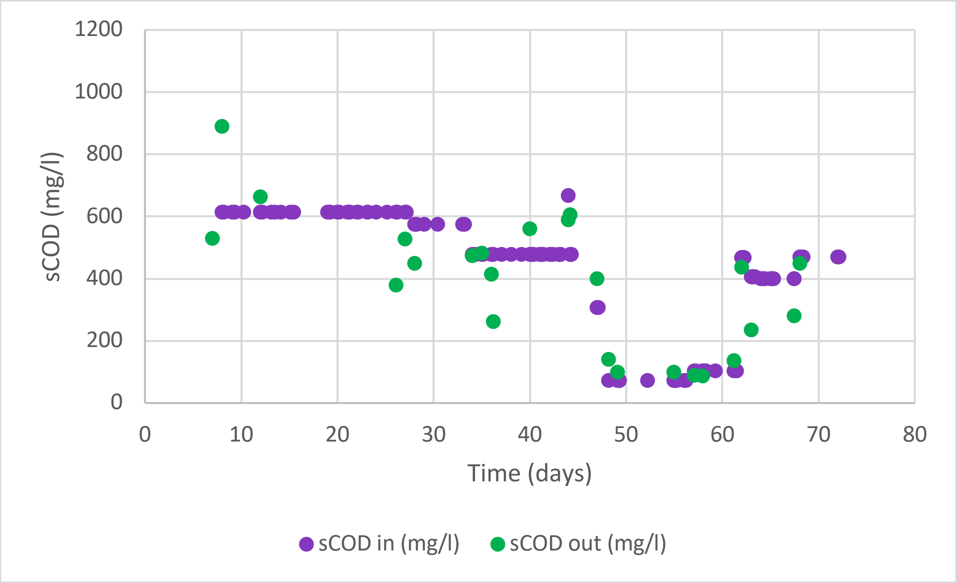

***Figure 4.5** Influent and effluent sCOD concentration (mg/l) as a function of time for Reactor SNJ.*

Figure 4.6 presents the relationship between sCOD profile efficiency (%) and OLR (g/l.d) as functions of time for Reactor SNJ. Throughout the course of the experiment, the OLR was consistently maintained at a low level within the range of 0 – 4.1 g/l.d, with the exception of day 44, which represents the initial data points following an increase in the flow rate from 2.7 ml/min to 5.0 ml/min. The increase of OLR on day 44 deviates heavily from the anticipated trend, and for this reason is excluded from the analysis. The highest sCOD removal efficiency is observed on day 36 (45%) and the lowest sCOD removal efficiency was on day 48 (-95%). The sCOD removal efficiency was low and mostly negative for the whole operating period, with an average of -3%. In general, there is a slight indication of a positive correlation between OLR and sCOD removal efficiency. An increase in OLR results in a slight increase in sCOD removal efficiency.

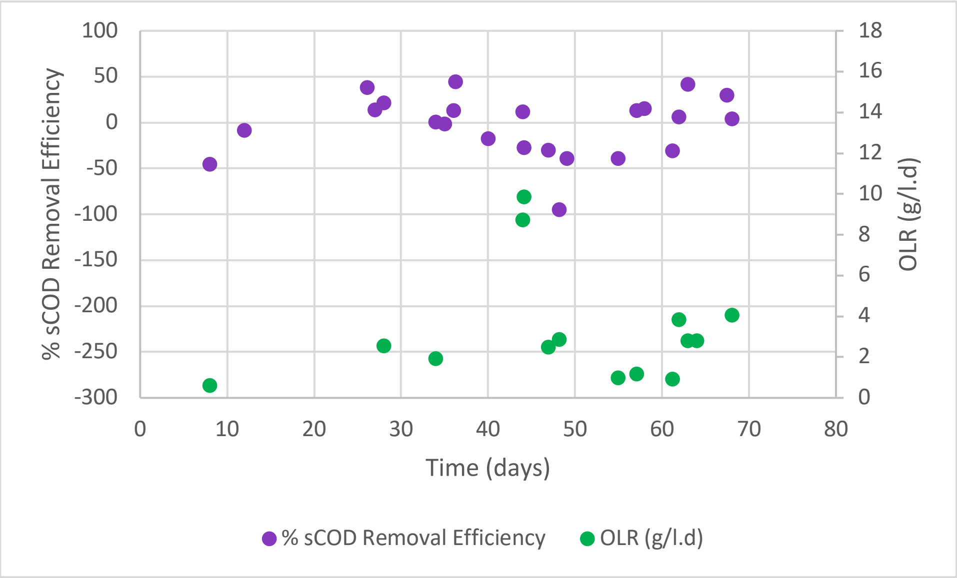

***Figure 4.6** Relationship between % sCOD Removal Efficiency and OLR (g/l.d) as a function of time for Reactor SNJ.*

TSS removal efficiency (%) compared to tCOD removal efficiency (%) as functions of time for Reactor SNJ are illustrated in Figure 4.7. The average TSS removal efficiency was 72% and the highest TSS removal efficiency was achieved on day 44 (99%), while the lowest TSS removal efficiency was observed at day 48 (27%). The efficiency of TSS removal exhibits a significant variability throughout the observation period, characterized by an initial decline from 89% to 41%, followed by an increase to 99%, a subsequent decrease to 27%, and a final rise to 91%. The TSS removal efficiency eventually achieves an average of 88% from day 58 to 68. The average tCOD removal efficiency was 16% and reached the maximum removal efficiency in the operating period on day 44 (69%), while the lowest tCOD removal efficiency was observed on day 40 (-64%). This abrupt change in the tCOD removal efficiency, coincides with a period during which there was a lack of measurement data for the COD concentration in the influent. Consequently, the effluent concentrations may not correspond to the influent concentration since they are not from the same feed tank. The average tCOD removal efficiency was 18% from day 58 to 68.

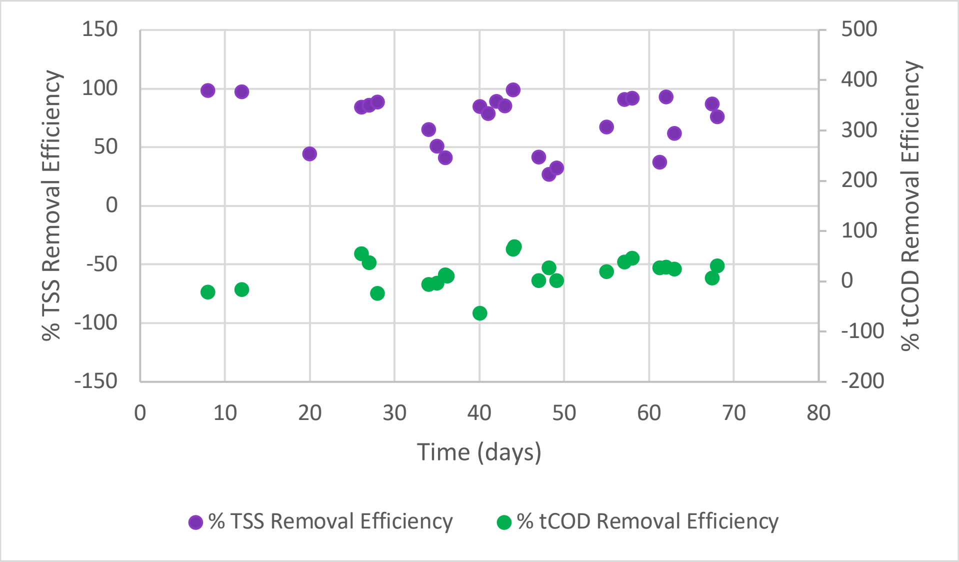

***Figure 4.7*** *The relationship between %TSS Removal Efficiency and %tCOD Removal Efficiency as functions of time of Reactor SNJ.*

The relationship between the TSS removal efficiency (%) and pCOD removal efficiency (%) for Reactor SNJ is displayed in Figure 4.8. The pCOD removal efficiency increases towards the end of the observation period. On day 36 and day 40 there are two abnormal data points deviating from the rest, which indicates that the pCOD removal efficiency was -3280% and -4500%, respectively. Due to the significant deviation exhibited by these datapoints, they will be excluded from the analysis. The average pCOD removal efficiency when these two datapoints are excluded is 68%.

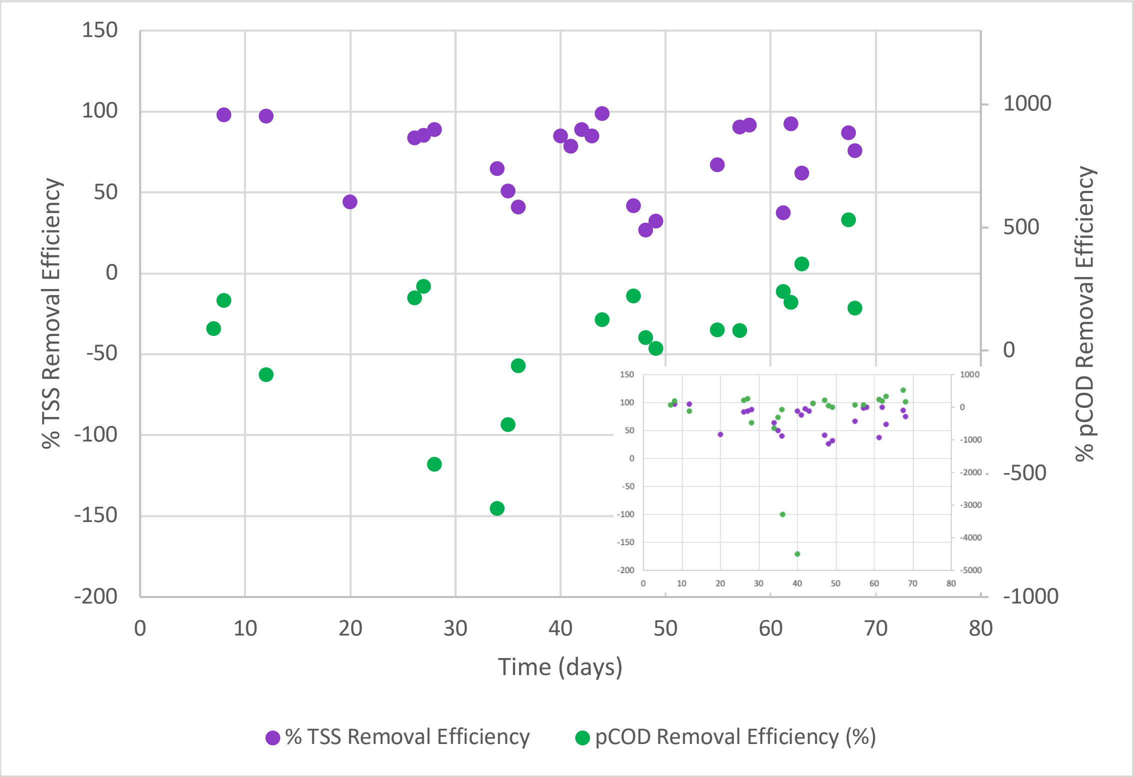

***Figure 4.8*** *pCOD Removal Efficiency (%) and TSS Removal Efficiency (%) as functions of time for Reactor SNJ.*

### 4.1.2 Methane Production

Besides the COD removal efficiency, the UASB reactors performance was also determined by investigating the methane produced. The composition of the biogas produced in Reactor Vik was measured throughout day 69 to day 70 and is illustrated in Figure 4.9. The measurements revealed that 54% of the gas present are methane, while the remaining 46% are carbon dioxide.

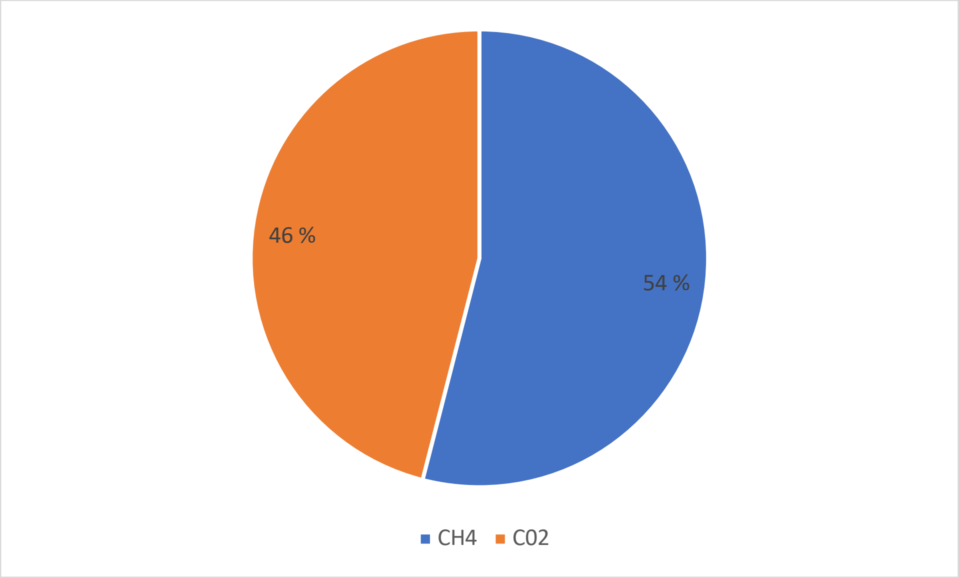

***Figure 4.9*** *Biogas composition of Reactor Vik.*

Figure 4.10 illustrates the relationship between the biogas formation rate (ml/d) and OLR (g/l.d) in Reactor Vik. In general, biogas production rate increases with increasing OLR. The highest biogas formation rate was on day 65 (1790 ml/d) when the OLR was 7.4 g/l.d. From the startup of the reactor until day 43 the biogas formation rate is highly variable. The average biogas formation rate in this period was 134 ml/d and the average OLR was 1.7 g/l.d. Furthermore, from day 43 and onwards, the biogas formation rate has a steadily increasing trend which corresponds to the increase in flow rate which led to an increase in OLR to 6.3 g/l.d. The biogas formation rate increased with 40 ml/d from day 43 and onwards. On day 57 there were complications with the reactor, as the tubes were clogged, resulting in a buildup of pressure within the reactor. The pressure created a vacuum in the tubes and the gas produced became trapped, resulting in hydrochloric acid (HCl) from the gas counter being drawn into the reactor. Phosphate buffer was added to the reactor to regulate the pH. The data obtained on day 57 deviate from the expected production levels, displaying irregular values in both lower and higher biogas production rates varying from 154 - 1243 ml/d.

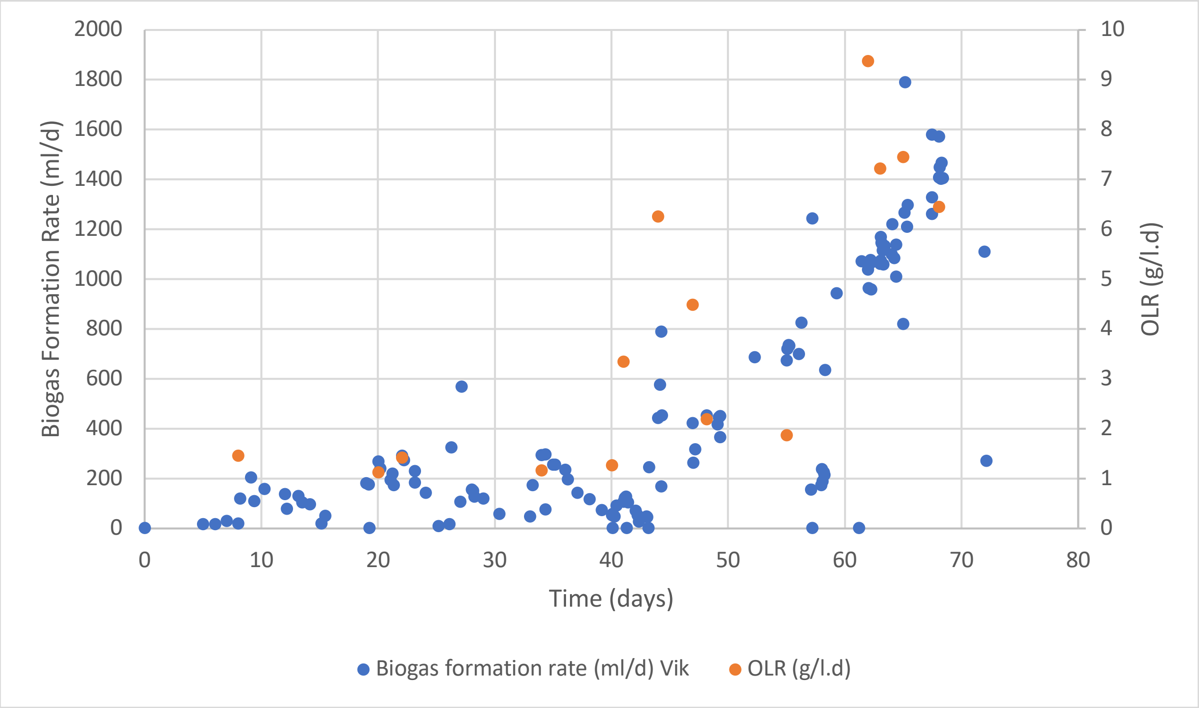

***Figure 4.10*** *Relationship between biogas formation rate (ml/d) and OLR (g/l.d over time) in Reactor Vik.*

Methane yield was determined from the massbalance of COD (Equation 4-1). The methane yield per tCOD in and sCOD in as functions of time is displayed in Figure 4.11, while the methane yield per tCOD removed and sCOD removed as functions of time in Reactor Vik is illustrated in Figure 4.12.

Methane yield per COD in:

$$Y = \frac{Q_{gas} \cdot \gamma_{CH_4/gas} \cdot \Upsilon_{COD/CH_4}}{Q_{liq} \cdot tCOD} \qquad Y = \frac{Q_{gas} \cdot \gamma_{CH_4/gas} \cdot \Upsilon_{COD/CH_4}}{Q_{liq} \cdot sCOD}$$

Methane yield per COD removed:

$$Y = \frac{Q_{gas} \cdot \gamma_{CH_4/gas} \cdot \Upsilon_{COD/CH_4}}{Q_{liq} \cdot tCODremoved} \qquad Y = \frac{Q_{gas} \cdot \gamma_{CH_4/gas} \cdot \Upsilon_{COD/CH_4}}{Q_{liq} \cdot sCOD}$$

Equation 4 -1

The observations where the methane yield per unit COD in exceeds 1 indicate potential errors or the absence of steady-state conditions within the reactor. In general, there is a slight trend of increase towards the end, and the majority of the collected data falls within the range of 0.1 to 1 in both figures. The average of the methane yield datas that falls within this range was 0.26 gCODCH$_4$/gtCODin, 0.36 gCODCH$_4$/gsCODin, 0.30 gCODCH$_4$/gtCOD removed and 0.41 gCODCH$_4$/gsCOD removed). On day 42, the flow rate was increased from 2.7 to 4.4 ml/min. From day 35 to day 42 there was a downward trend in methane yield, which subsequently experienced a turning point on day 42 and exhibited an upward trajectory, which can be seen in both figures. As mentioned previously, Reactor Vik was clogged on day 57, which led to abnormal methane yield values on this day (1.2 gCODCH$_4$/gtCODin, 3.0 gCODCH$_4$/gsCODin, 1.2 gCODCH$_4$/gtCOD removed and 4.1 gCODCH$_4$/gsCOD removed). The average methane yield based on the COD in the influent, exhibits lower values compared to the average methane yield based on the COD removed. This discrepancy can be attributed to the higher COD concentration in the influent compared to the concentration of COD that is effectively removed during the process. The average span of 10 days including standard errors is presented in Figure 4.12 as well.

The average methane yield based on COD removed at the end was 0.43 gCODCH4/gtCOD removed and 0.49 gCODCH4/gsCOD removed.

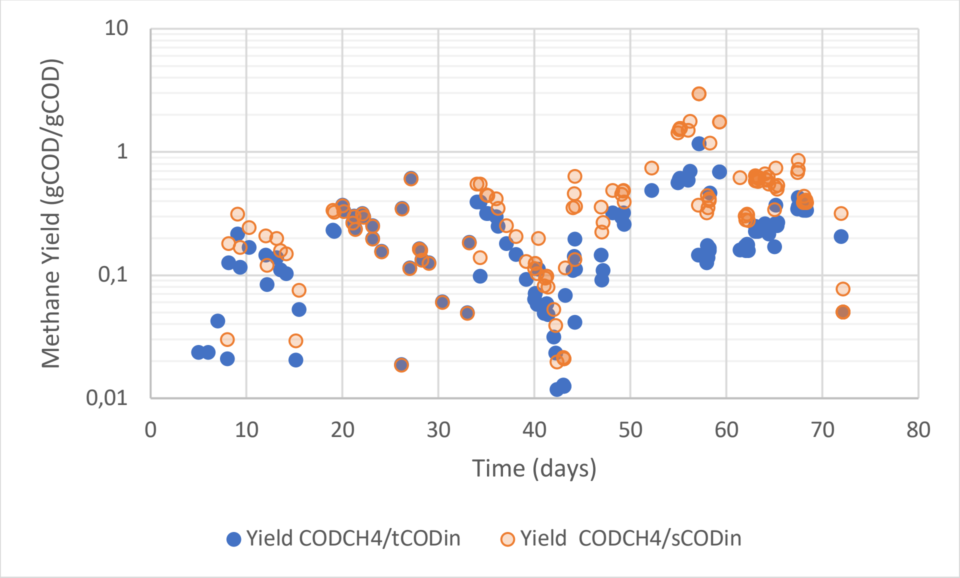

***Figure 4.11** Methane Yield per COD unit in as a function of time in Reactor Vik.*

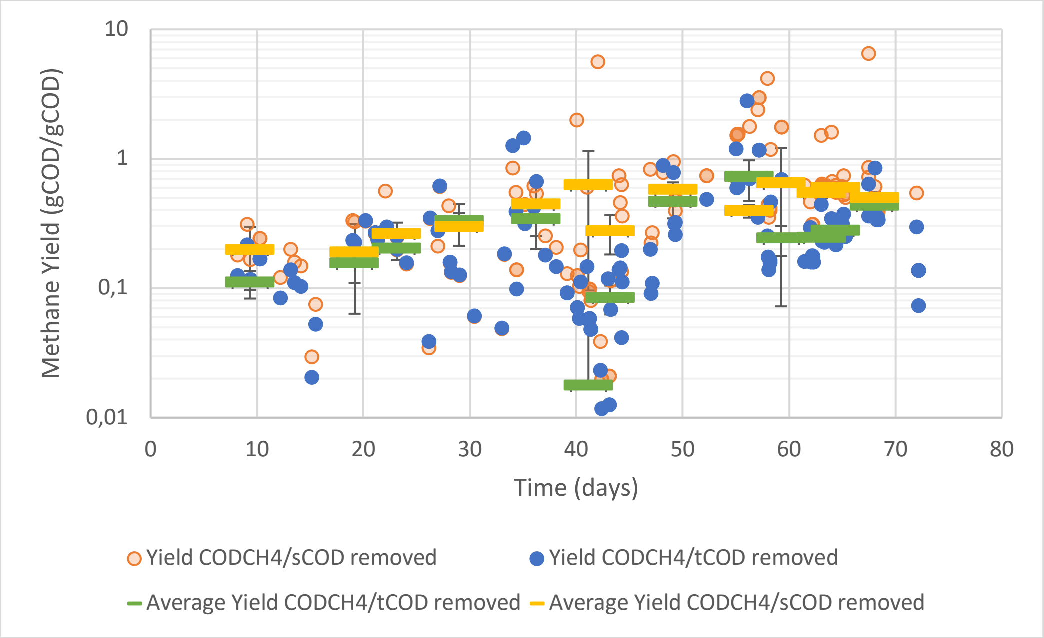

***Figure 4.12** Methane Yield per unit COD removed as a function of time in Reactor Vik including bars with standard errors representing the average over 10 days.*

Figure 4.13 illustrates the biogas formation rate (ml/d) as a function of time compared to OLR (g/l.d) for Reactor SNJ. The highest biogas production rates were observed on day 12 and 67 with rates of 1150 and 1337 ml/d, respectively. On day 12 the feed in the fridge ran out and oxygen was drawn into the reactor. On day 67 the granules rose to the surface and to physically push the granules back down into the reactor, the lid was removed which may have affected the gas counter. The overall average biogas production was 55 ml/d, and 34 ml/d excluding day 12 and 67. In general, the figure demonstrates a lack of significant biogas production over the examined period. The biogas composition was not determined for Reactor SNJ due to the lack of biogas production, and consequently the methane yield and COD balance was not calculated either.

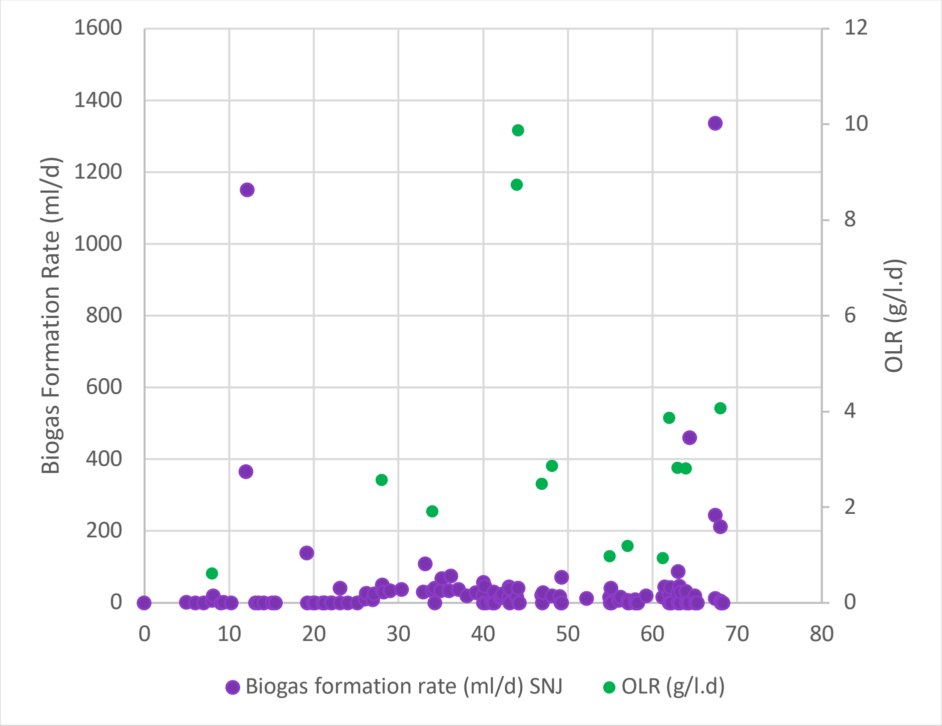

***Figure 4.13*** *Relationship between biogas formation rate (ml/d) and OLR (g/l.d) in Reactor SNJ.*

### 4.1.3 COD Balance and COD fraction

The COD balance is presented in this sub-chapter. COD balance was calculated based on total COD in the influent and effluent, and corresponding methane production as 1000 mg COD per 350 ml methane (Equation 4-2). The relationship between the OLR (g/l.d) and COD balance (%) based on total COD as functions of time for Reactor Vik is displayed in Figure 4.14.

$$COD\ massbalance\ (\%) = \frac{Q_{in}COD_{out} + Q_{gas}V_{gas} \cdot \frac{1000mgCOD}{350mlgas}}{Q_{in} \cdot COD_{in}} \times\ 100\%$$

Equation 4-2

Figure 4.14 displays the COD balance (%) based on total COD and OLR (g/l.d) as functions of time for Reactor Vik. The average span of 10 days including standard errors is presented in the figure as well. The averages grouped in 10-day intervals are as follows: 111%, 78%, 75% and 65%. The COD mass balance is expected to be a closed system, where the COD inputs should equal the COD outputs. During the observation period there are several datapoint exceeding a 100%, suggesting an imbalance where the COD output exceeds the COD input. Towards the end of the observation period the average COD balance was approximately 70%, and it seems stable despite the OLR increasing.

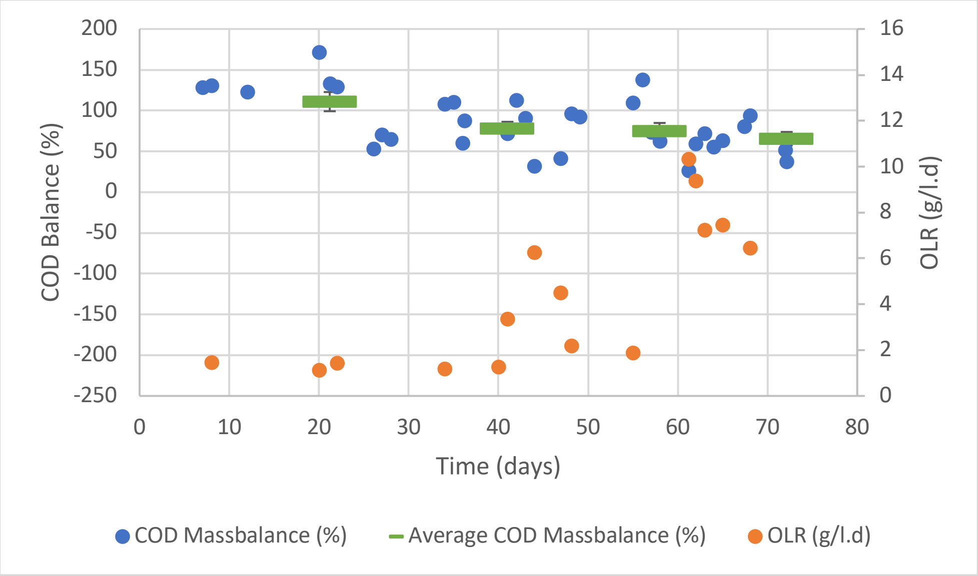

***Figure 4.1414** The COD balance (%) based on total COD and OLR (g/l.d) as functions of time of Reactor Vik. The bars display average COD massbalance (%) in the span of 10 days including standard errors.*

The average tCOD- and sCOD concentrations in the influent was 666 mg/l and 460 mg/l. The effluent tCOD- and sCOD concentrations was 413 mg/l and 301 mg/l on average. This means that the average soluable COD to total COD ratio is 69% for the influent and 73% for the effluent.

## 4.2 pH Variability, VFA composition- and specification, and sulfate- and chloride concentrations

The study focused on identifying critical factors that potentially might affect the performance of the UASB reactors. pH was measured frequently, and the VFA composition and specification, sulfate and chloride concentrations were measured on day 69.

### 4.2.1 pH

Figure 4.15 illustrates pH values from Reactor Vik of the influent and effluent as a function of time. The pH values range between 6.2 – 7.9 throughout the experiment, with an average pH of 6.7 ± 0.2 in the influent and 7.2 ± 0.3 in the effluent. The data clearly indicates that the pH values of the effluent were consistently higher than the influent pH levels.

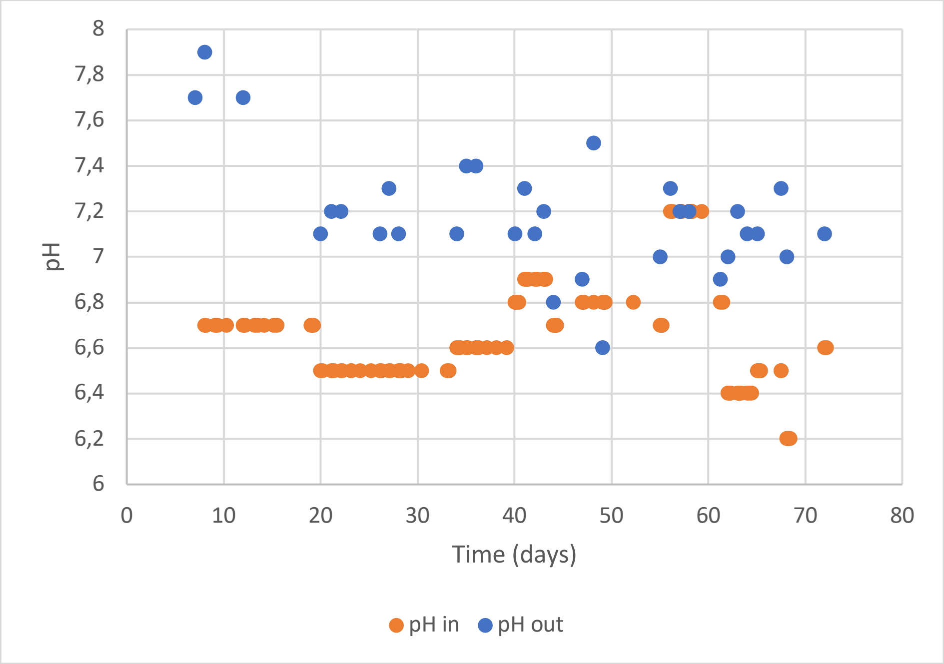

***Figure 4.1515*** *pH of influent and effluent of Reactor Vik.*

Figure 4.16 pH of influent and effluent of Reactor Vik. displays the pH readings for Reactor SNJ as a function of time. The pH values in Reactor SNJ were consistently higher than those measured in Reactor Vik. Throughout the experiment, the pH readings ranged between 6.9 - 7.8. Generally, the pH of the effluent was higher than the pH of the influent, with an average influent pH of 7.1 ± 0.2 and effluent pH of 7.3 ± 0.2.

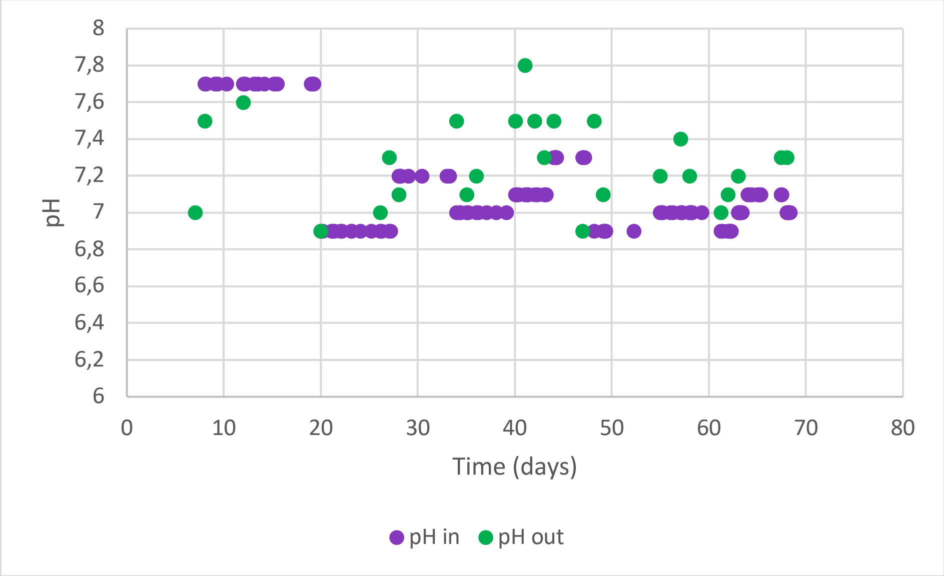

***Figure 4.1616** pH of influent and effluent of Reactor SNJ.*

### 4.2.2 Total VFA analysis using 5-point titration

On day 63 a total VFA analysis using 5-point titration was taken of the influent and effluent of both Reactors. Table 4.1 illustrates the results of the total VFA concentration for both Reactor Vik and Reactor SNJ. The effluent concentrations exceed the influent concentrations by approximately two times.

***Table 4.1** The total concentrations of VFA present in the influent and effluent of Reactor Vik and Reactor SNJ.*

| | **VIK** | | **SNJ** | |
|---|---|---|---|---|
| | In | Out | In | Out |
| **Total VFA (mgHAc/l)** | 40.5 | 76.5 | 13.5 | 32 |

### 4.2.3 VFA Specification, $SO_4^{2-}$ , $Cl^-$, using Ionic Chromatography

On the same day, day 63, a specification of the VFAs using Ionic Chromatography was taken. The concentrations of $SO_4^{2-}$ and $Cl^-$ were also measured. The distribution and concentration of VFAs in and out of the two reactors are displayed in Table 4.2.

***Table 4.2*** *Specification of the VFAs present in the influent and effluent of Reactor Vik and Reactor SNJ.*

| | **VIK** | | **SNJ** | |
|---|---|---|---|---|
| | Concentration (mgHAc/l) | | Concentration (mgHAc/l) | |
| | In | Out | In | Out |
| **Acetic acid** | 13.1 | 42.5 | 4.6 | 10.8 |
| **Propionic acid** | 7.9 | 14.8 | 2.1 | 5.9 |
| **Butyric acid** | 7.6 | 8.5 | 3.5 | 7.8 |
| **Other acids** | 11.9 | 10.7 | 3.3 | 7.5 |

The concentrations of the $SO_4^{2-}$ and $Cl^-$ in the influent and effluent of the reactors are displayed in Table 4.3. Note that the values for both sulfate and chloride are higher for Reactor SNJ than Reactor Vik, both in the influent and effluent.

***Table 4.3*** *Concentrations of the Sulfate and Chloride in the influent and effluent of Reactor Vik and Reactor SNJ.*

| | **VIK** | | **SNJ** | |
|---|---|---|---|---|
| | In | Out | In | Out |
| **$SO_4^{2-}$ Concentration (mM)** | 0.19 | 0.39 | 0.60 | 0.50 |
| **$Cl^-$ Concentration (mM)** | 1.78 | 1.78 | 8.36 | 8.43 |

## 4.3 Methodological- and random errors

In analytical measurements, there will always be some degree of uncertainty associated with the procedures and instruments employed, and as a result, both random and systematic errors may occur. The pH measurements may be subjected to some errors arising from several factors, including the accuracy of the pH electrode, temperature fluctuations, electrode drift, and variations in the buffer solutions used for calibration. There might also be multiple factors affecting the COD measurements. As mentioned previously, the usage of COD kit 09772 required dilution. Ideally, the COD measurements should fall within the upper limits of the range of the kit to achieve more accurate results. The dilution of the wastewater samples introduces a greater potential for error, where the original concentration of organic material in the sample may be under- or overestimated. The measurement accuracy of the pipette could impact the analysis and interpretation of the result.

Some of the kits used during the COD measurements had expired. An expired COD kit may contain degraded or inactive reagents, which can result in inaccurate or unreliable COD measurements. During the COD measurements, two different Hg-free COD kits (09772 and 09773) were used, as previously mentioned. These test kits measure $Cl^-$ at the same time as COD, which may have affected the results, especially on the wastewater samples from Reactor SNJ, as it has been confirmed that the influent contains a significant amount of chloride (8.36 M, measured on day 63). When using kit 09772, 50 mg/l $Cl^-$ can correspond to 10 mg/l COD, and while using kit 09773, 50 mg/l $Cl^-$ can correspond to 30 mg/l COD. The manuals for kits 09772 and 09773 state that wastewater samples containing more than 250 mg/l $Cl^-$ and 400 mg/l $Cl^-$, respectively, must be diluted with distilled water before COD determination. When using kit 14541 that contains Hg, the chloride is masked with mercury sulfate, and the manual for the kit specifies that the wastewater sample must have a concentration of at least 2000 mg/l $Cl^-$ to impact the COD measurements. However, the use of mercury-based reagents has been associated with environmental concerns. Consequently, when analyzing wastewater samples containing chloride, it is still recommended to use Hg-free kits. Nonetheless, it is advised to remove or neutralize the chloride before analyzing to achieve more precise results. This approach was not implemented during this experiment.

When wastewater samples are diluted with distilled water, the interfering elements present in the samples, such as chloride and sulfate, are also diluted. Consequently, these elements might not have the same impact on the COD measurements. Although the COD readings are adjusted afterwards by multiplying with the dilution factor, the interfering elements might not be having the same impact on the results. Thus, dilution of the samples will give more accurate COD values, compared to when COD measurements are taken using kits that do not require dilution.

During the experiment, the focus was to acquire several measurements over time rather than infrequent parallels. This was done to obtain more datapoints over time to be able to observe the development in COD removal and gas production in the reactors over an extended period. Parallel measurements were not taken due to limitations of the COD kits and time constraints on the experiment. Gathering datapoints gradually over time results in greater variation between the datapoints, which can be seen in the figures as fluctuations in the trend line. Collecting multiple measurements in parallel rather than over time would have reduced the variation in the data. Since parallel measurements were not taken, standard deviation is not reported in this study.

# 5 Discussion

This chapter discusses the experimental results. The discussion is divided into four sub-chapters; (a) reactor performance, including COD removal efficiency, methane production, and COD balance; (b) environmental factors including pH variability, VFA composition, and concentration of $SO_4^{2-}$ and $Cl^-$; (c) economy and energy recovery; and (d) hydrodynamic conditions, which provide an overview of the overall condition of the reactors.

## 5.1 Reactor performance

In this sub-chapter, the performance of the reactor will be evaluated and analyzed by investigating two main parameters: COD removal and methane production. These two parameters affect the effectiveness of anaerobic treatment for treating low- to medium strength municipal wastewater. In addition, the COD balance will be determined, and influencing factors will be discussed.

### 5.1.1 COD Removal Efficiency

COD concentrations of the influent and effluent of Reactor Vik as functions of time are shown in Figure 4.1. The consistently higher sCOD concentration in the influent compared to the effluent is a positive indication that Reactor Vik is effectively removing organic matter from the wastewater. The relationship between the sCOD removal efficiency and OLR is displayed in Figure 4.2. In general, the OLR and sCOD removal efficiency had a negative correlation. When the OLR increased, the sCOD removal efficiency decreased, and conversely, a decrease in OLR leads to an increase in sCOD removal efficiency. Furthermore, as the OLR increases, disturbances arise in the performance of the reactor, impacting stability and consequently reducing efficiency. At the end of the operating period the sCOD removal efficiency remained stable at approximately 60% despite the OLR declining, indicating that the reactor is capable of handling lower organic loads without a significant decrease in the treatment efficiency. This implies that the reactor has a certain level of flexibility and resilience to variations in organic load. The stable sCOD removal efficiency can also be an indication that the reactor is approaching a steady state. A sCOD removal efficiency of 60% falls short of the discharge limit set by the Norwegian Pollution Control Act, which requires a minimum of 75% COD removal of the wastewater received by the WWTP. If the reactor was implemented in a full-scale treating wastewater from IVAR Vik WWTP the effluent would need a post-treatment to fulfill the discharge limits. Temporal variations of the TSS removal efficiency compared to tCOD removal efficiency and pCOD removal efficiency are illustrated in Figure 4.3 and Figure 4.4, respectively. The average tCOD removal efficiency in the operating period was 31%, but the average tCOD removal efficiency at the end of the operating period was 54% (from day 55 – 72). This suggests that the reactor has improved tCOD removal efficiency towards the end. As the observation period approached the end, the TSS removal efficiency and the pCOD removal efficiency had an average value of 68% (from day 55 to 72). This may indicate that the purification process is effectively removing organic matter from both suspended solids and particulate compounds in the wastewater.

COD concentrations of the influent and effluent of Reactor SNJ as functions of time are displayed in Figure 4.5. While the wastewater from SNJ WWTP is considered low strength, the average influent sCOD concentration was 505 mg/l, which is higher than the average influent sCOD concentration of Vik, characterized as medium strength wastewater, which was 406 mg/l. This discrepancy is noteworthy and warrants further investigation. Furthermore, in the observation period the sCOD concentrations of the effluent are mostly higher than the influent. This indicates that the reactor has a negative sCOD removal efficiency, which can be observed in Figure 4.6, as the average sCOD removal efficiency was -3%. In general, there is a slight indication of a positive correlation between the OLR and sCOD removal efficiency. However, definitive conclusions cannot be drawn due to the fact that the flow rate was only altered once, generating uncertainty about the potential impact the OLR has on the sCOD removal efficiency. The TSS removal efficiency compared to the tCOD- and pCOD removal efficiency as functions of time are illustrated in Figure 4.7 and 4.8, respectively. The average TSS removal efficiency was 72% during the operating period, but the TSS removal efficiency exhibits a significant variability throughout the experiment. During methane production, ascending gas bubbles in the reactor entrain particulate matter, resulting in higher TSS in the effluent. Due to the low gas production in Reactor SNJ, the release of particulate matter is infrequent, and when it does occur, it happens abruptly and in large quantities. Consequently, the TSS removal efficiency has large fluctuations resembling waves, thereby causing variations in the clarity of the effluent, with periods of clear water followed by periods of cloudy water. The average tCOD removal efficiency in Reactor SNJ was 16%, and no significant improvement was observed towards the latter stages of the reactor operation. The pCOD removal efficiency has large variability all throughout the observation period. The data suggests that Reactor SNJ exhibits a great capability of removing TSS, but overall poor COD removal efficiency.

The results obtained from the experiment indicate that the COD concentration in the wastewater from SNJ WWTP is higher than the COD concentration in the wastewater from Vik WWTP. As previously stated, the wastewater from SNJ WWTP is a low strength wastewater, while the wastewater from Vik WWTP is a medium strength wastewater. Consequently, the COD measurements indicate inaccuracies. The Hg-free COD kits were most frequently employed, and as mentioned above, chloride is measured simultaneously with COD in these kits. Table 4.3 displays the chloride concentration of the influent and effluent in Reactor Vik and Reactor SNJ measured on day 69. The influent of Reactor SNJ was determined to contain 8.36 mM $Cl^-$, which corresponds to 296 mg/l. According to the manuals, this chloride concentration would result in an overestimated COD concentration of 59.2 mg/l for kit 09772 and 177.6 mg/l for kit 09773 for COD measurements of Reactor SNJ. Furthermore, the chloride concentration of 8.43 mM for the effluent in Reactor SNJ, which corresponds to 298 mg/l, would lead to an overestimated COD concentration of 60 mg/l when using kit 09772, and 178,8 mg/l when using kit 09773. Both influent and effluent in Reactor Vik was determined to contain 1.78 mM $Cl^-$, which corresponds to 63.1 mg/l. This chloride concentration would result in an overestimated COD concentration of 12.6 mg/l for kit 09772, and 37.86 mg/l for kit 09773 for COD measurements of Reactor Vik. Consequently, chloride exerts a substantial influence on the COD measurements, particularly for the wastewater originating from Reactor SNJ, leading to elevated COD values compared to the true concentrations. This could be the reason behind the considerably higher COD measurements in the wastewater from Reactor SNJ compared to the measurements in the wastewater from Reactor Vik.

### 5.1.2 Methane Production

In addition to the COD removal efficiency, the performance of the UASB reactors was also evaluated by analyzing the amount of biogas produced. The quality of the biogas was further determined by the proportion of methane in the biogas. The composition of the biogas produced in Reactor Vik, illustrated in Figure 4.9, measured throughout day 69 to day 70 revealed that 54% of the gas present are methane, while the remaining 46% are carbon dioxide. The correlation between the biogas formation rate and the OLR is displayed in Figure 4.10. Average biogas formation rate of Reactor Vik from day 0 to day 43 was 134 ml/d when the average OLR was 1.7 g/l.d. On day 41 the flow rate was increased from 2.7 ml/min to 4.4 ml/min, and consequently the OLR increased from 3.3 g/l.d to 6.3 g/l.d . The increase in OLR led to a gradual average increase of 40 ml/d in biogas formation rate. This increase in biogas formation rate kept on going throughout the rest of the observation period. The average flow rate in this period was 4.9 ± 0.6 ml/min, and the average OLR was 6.2 ± 1.6 g/l.d. The granules and particulate matter tended to clog the tubes which caused fluctuations in the flow rate. The variation in OLR can be attributed to the variability of the flow rate itself, as well as the inconsistent influent COD concentrations in the wastewater. The observations indicate that there is a positive correlation between the OLR and the biogas formation rate for Reactor Vik. This implies that the reactor demonstrates the ability to generate methane gas during the treatment of medium strength wastewater.

The methane yield in Reactor Vik based on both total and dissolved COD input and COD removed as functions of time is illustrated in Figure 4.11 and Figure 4.12. Methane yield refers to the amount of biogas produced per unit of organic matter added or removed in the reactor. This parameter holds significant value in evaluating the performance of a UASB reactor, as it reflects the ability of the reactor to convert organic matter into biogas through anaerobic digestion. Yields approaching one indicate better performance and greater efficiency in converting organic matter into biogas, as more biogas is produced per unit of organic matter added or removed in the reactor. The average methane yield based on the last 10 days of observation was 0.43 $gCODCH_4/gtCOD$ removed and 0.49 $gCODCH_4/gsCOD$ removed, and there is an indication of an increasing trend which could look promising over time. From Figure 4.11 and 4.12 there are several datapoints that exceed one which are considered unrealistic and assumed to be errors. In the reactor, particulate matter is retained through filtration. As these particles undergo degradation, they are subsequently transformed into methane, thereby leading to a high methane yield which can explain the fluctuations in the data of methane yield. On day 42, the flow rate was increased leading to low methane yields. The increase of flow rate corresponded to a reduced HRT, thereby limiting the contact time between the organic matter and the methanogenic bacteria leading to a lower methane yield. Additionally, increasing the flow rate may induce instability in the microbial community within the reactor, further contributing to the reduction in methane yield. On day 57, when the tubes became clogged and the feed was stopped, there was no removal of COD occurring. Consequently, upon restart, the biogas production rate will be low, and the anerobic bacteria will not have had sufficient time to respond to the input. Although removal will transpire during the restart, there will be no biogas production, as illustrated in Figure 4.10, resulting in a diminished yield.

The biogas formation rate compared to OLR as functions of time of Reactor SNJ is displayed in Figure 4.13. In general, the reactor exhibits a significant lack of biogas production over the examined period despite the variations in the OLR. The most important deviations in Reactor SNJ were observed on day 12 (1150 ml/d) and day 67 (1337 ml/d). On day 12 the feed in the fridge depleted in the afternoon and oxygen was drawn into the reactor during the entire night, leading to incorrect gas counter reading due to overcounting. On day 67 the reactor lid was opened to manually dislodge floating granules. This action potentially created a vacuum within the reactor upon lid replacement, leading to the simultaneous release of numerous gas bubbles. These datapoints are excluded from the analysis due to the substantial deviation resulted by these two events. The average biogas production was 34 ml/d, when excluding these two data points. The biogas composition was not determined for Reactor SNJ due to the lack of biogas production. This implies that there are factors complicating the biogas formation within the reactor, or that the reactor may not have the capability to produce methane gas when treating low strength wastewater.

Table 4.3 illustrates the concentrations of sulfate and chloride in the influent and effluent of both Reactor Vik and Reactor SNJ on day 69. The sulfate concentration in Reactor SNJ attributes to a production of hydrogen sulfide ($H_2S$), which can lead to problems in the anaerobic treatment. The concentration of sulfate in the influent and effluent of Reactor SNJ was 0.6 mM and 0.5 mM, which corresponds to 19.2 mg/l and 16.0 mg/l, respectively. This discrepancy corresponds to 0.1 mM sulfate, which is 3.4 mg/l $H_2S$ assuming everything converts to $H_2S$. The presence of $H_2S$ was also confirmed by strong odor from the wastewater. The hydrogen sulfide gas produced will accumulate in the reactor and the concentration of $H_2S$ will continue to increase. In the scenario where the reactor is producing methane, the $H_2S$ gas would leave the reactor together with methane. However, in the case of Reactor SNJ, where methane gas production is absent, the presence of $H_2S$ poses a problem. On the other hand, in Reactor Vik, the performance will not be significantly influenced by sulfate due to two factors; (1) the low concentration of sulfate present and; (2) the significant methane production in Reactor Vik. This might be one of the factors attributing to the lack of methane production in Reactor SNJ. An additional factor that might be contributing to the minimal production of methane is the presence of saline water. The salinity is considered to be inhibitory to the anaerobic treatment process. The concentration of chloride measured on day 69 in the influent of Reactor SNJ was 8.36 mM. The sea water intervention in the wastewater from SNJ WWTP is then estimated to be 1.44% based on a chloride concentration in seawater of 0.56 M. This proposition appears plausible as it corresponds to the prevailing conditions of springtime characterized by limited precipitation, thereby leading to negligible admixture of seawater from the bay.

### 5.1.3 COD Balance

The anaerobic reactor performance can be monitored by the COD balance which gives the operator vital information about the operational status of the system. The performance of the system is considering satisfactory at around 100%, indicating that the mass balance is closed. However, as displayed in Figure 4.14, in Reactor Vik the average COD balance towards the end of the observation period was 70%, which implies that there is an insufficient output compared to input. This occurrence may be attributed to the unaccounted loss of certain compounds or the accumulation of mass in the granules. In general, it is assumed that all gas produced is measured. However, a substantial amount of gas has been unaccounted for as it has been directed towards the outlet instead of being captured and measured by the gas counter. The mass balance also fails to account for the portion of the COD that is accumulated within the sludge blanket (Henze et al., 2008).

An additional commonly mentioned factor contributing to a discrepancy in the COD mass balance is the loss of electrons when they are associated with oxidizing anions such as $SO_4^{2-}$ and $NO_3^-$. To achieve closing of the COD balance, it is necessary to either consider all reduced gases or measure the concentration of electron acceptors. Moreover, the effluent may contain gases containing soluble COD, such as $H_2S$ (Safitri, 2016).

## 5.2 Environmental factors

The fermentation process results in formation of various VFAs which are then converted to methane in the methanogenesis stage. At the end of the experiment, on day 63, the concentration of the VFAs was measured using a 5-point titration method, as displayed in Table 4.1. This procedure was carried out with the purpose of verifying that the concentration remained within an acceptable level as exceeding this threshold would have resulted in the accumulation of acids, thereby causing an unstable and inefficient anaerobic process. Additionally, the specification of VFAs was conducted, as displayed in Table 4.2.

In Reactor Vik, acetic acid constitutes the primary portion of VFA production, with propionic acid being the subsequent component. The influent in Reactor Vik has a total VFA concentration of 40.5 mg/l, whereas the effluent exhibits a concentration of 76.5 mg/l. Specifically, the influent contains an acetic acid concentration of 13.1 mg/l (32% of the total VFA concentration in the influent), while the effluent demonstrates a concentration of 42.5 mg/l (56% of the total VFA concentration in the effluent). Acetic acid is known to be the most dominant VFA in anaerobic digestion and is a key precursor to methane production. A high concentration of acetic acid suggests that the hydrolysis and acidogenesis stages of anerobic digestion are proceeding efficiently, which is essential for the subsequent methanogenesis stage to be successful. The pH varied from 6.2 to 7.9 in Reactor Vik, with an average pH of 6.7 ± 0.2 in the influent and 7.2 ± 0.2 in the effluent. Acetic acid as the main constituent, along with a stable pH, may indicate a substantial methane production in the reactor (Henze et al., 2008).

In Reactor SNJ, the primary VFA production consists of acetic acid, followed by butyric acid. The influent in Reactor SNJ has a total VFA concentration of 13.5 mg/l, whereas the effluent exhibits a concentration of 32.0 mg/l. Specifically, the influent contains an acetic acid concentration of 4.6 mg/l (34% of the total VFA concentration in the influent), while the effluent displays a concentration of 10.8 mg/l (34% of the total VFA concentration in the effluent). The pH varied from 6.9 to 7.8 in reactor, with an average pH of 7.1 ± 0.2 in the influent and 7.3 ± 0.2 in the effluent. Acetic acid served as the primary constituent in Reactor SNJ as well. However, Reactor SNJ exhibits a lower VFA content, particularly in terms of acetic acid compared to Reactor Vik. This observation can contribute to the fact that Reactor SNJ generates less methane gas compared to Reactor Vik.

However, the concentrations of VFAs present in both reactors are sufficiently negligible to exert any significant toxic effects on the hydrogen-utilizing methanogenic bacteria. Furthermore, a pH within an optimal range is consistently maintained in both reactors throughout the examination period, implying that the presence of VFAs did not have an adverse effect on the environment within the reactors.

## 5.3 Economy and Energy Recovery

In this section, economic and energy recovery will be discussed. This section will present the estimation of electricity generation from the methane produced in Reactor Vik during the experiment. Due to the insufficient production of biogas in Reactor SNJ, the estimation of electricity generation from the generated methane in this reactor will not be conducted.

Anaerobic treatment systems have higher construction costs, but lower operating costs compared to aerobic treatment systems. From an economic point of view, the specific methane amounts and the biogas quality from anaerobic treatment are important process parameters related to economy and energy recovery. According to Henze et al., (2008), the methane generated in the anaerobic digestion has an energy content of approximately 13.5MJ per kg of COD removed. This energy can be captured and converted into electricity, with an electricity generation of 1.5 kWh per kg of COD removed, assuming an electric conversion efficiency of 40% (Henze et al., 2008). The experiment indicates that 69% of the total COD entering Reactor Vik is soluble COD. With a total COD concentration of 931 g/m$^3$ in the wastewater from IVAR Vik WWTP, it is inferred that the soluble COD content is 642 g/m$^3$. At the end of the experiment, the reactor exhibits an average sCOD removal efficiency of 60%. Consequently, it is estimated that 385 gCOD/m$^3$ can be converted into methane gas. According to theoretical calculations, the conversion of COD results in the generation of 0.35 l $CH_4$/g COD. Hence, the daily methane production is projected to reach 1083 m$^3$/d. Furthermore, the biogas composition analysis indicates that methane accounts for 54% of the total biogas generated during the experiment. Based on these results, the total biogas production potential at IVAR Vik WWTP is estimated to be 2000 $m^3$/d. IVAR Vik WWTP receives 8000 $m^3$ wastewater per day and achieves an estimated daily removal of 3083 kg COD. As a result, the energy generation will be 41.6 GJ per day, leading to the production of 4624 kWh per day of electricity. According to numbers from Statistisk Sentralbyrå, which is the national statistical institute of Norway, the average electricity consumption for a single-family house is stated as 20 230 kWh per year in 2012 (Statistisk Sentralbyrå, 2014). To put this into perspective, the results from the analysis of the laboratory-scale UASB reactor treating medium strength wastewater from IVAR Vik WWTP could potentially supply power to 83 households per year if the reactor was treating the amount of wastewater IVAR Vik WWTP receives.

## 5.4 Hydrodynamic Conditions

This section provides an overview of the operational state of the UASB reactors, including reactor dimensions and granule size which affect the reactor performance in Reactor Vik and Reactor SNJ.

As mentioned in the literature review, employing taller reactors with a high height/diameter ratio, along with effluent recirculation, proves advantageous for improved mixing and contact between wastewater and granules. In tall reactors, the gas loading and hydrostatic pressure at the bottom tend to be higher compared to short reactors (Kato, 1994; Lettinga, 1995). The height/diameter ratio of the reactors utilized in this experiment was 7.9 where the height was 540 mm and the diameter was 68 mm, which is a high height/diameter ratio. The reactors had sub-optimal internal mixing due to the dead spaces in the lower section of the reactor. The inlet and recirculation liquid entered the bottom side of the reactors rather than at the very bottom, resulting in inadequate distribution of the liquid.

Granule volumes of 50 – 60% v/v with respect to the UASB reactors were used. The size of the granules was 1.0 – 3.0 mm in diameter. According to Bhunia and Ghangrekar (2007), the specific methanogenic activity increased with the size of the bioparticles (Bhunia & Ghangrekar, 2007). On the other hand, Batstone and Keller (2001) reported that the biochemical activity was predominant only at the outer 200 – 300 μm layer of the granule for granules with higher diameters. Hence, larger sized granules may have a minimal contribution to the overall biological activity (Batstone & Keller, 2001). In a study conducted by van Lier (1996), it was observed that smaller granules exhibit higher mass transfer rates owing to their increased affinity for substrates. However, despite their higher specific activity, smaller granules tend to be weaker and more prone to washout from the system (van Lier, 1996). The specific up-flow velocity in the reactor causes granule segregation. Consequently, larger granules settle in the lower part, forming a stationary bed, while smaller, fluffier granules are located in the upper portion. During the observation period there were several events of granule washout in both reactors, but it occurred more frequently in Reactor SNJ. However, the most significant wash out event took place in Reactor Vik on day 57. This wash out resulted in a loss of biomass, roughly estimated to be 3 – 4 mm by visual examination of the reactor. The exact amount of biomass lost during each washout event is unknown.

Re-design of the laboratory scale reactors should be evaluated to prevent frequent events of granule washout. A larger volume reactor might reduce the likelihood of washout by providing more space for the granules to settle and establish a stable biomass bed. Reevaluation of granules size should also be considered to determine the appropriate size range that promotes effective settling and retention of the granules within the system for optimal performance.

# 6 Conclusions

In Reactor Vik the dissolved COD removal efficiency remained stable at approximately 60% towards the end of the observation period despite the OLR decreasing from 10.3 to 6.4 g/l.d. A COD removal of 60% does not fulfill the environmental standards and regulations set by the Norwegian Pollution Control Act (Forurensningsloven), which required a COD removal of 75% minimum, indicating a need of post-treatment. However, when the COD is removed anaerobically it produces methane which can be used as an energy source. The methane production rate in Reactor Vik had an increased rate of 40 ml/d following a single increment in flow rate. This observation suggests a potential for even greater methane production in the future as higher OLRs are applied. During the last 10 days of observation, Reactor Vik demonstrated an average methane yield of 0.43 $gCODCH_4/gtCOD$ removed and 0.49 $gCODCH_4/gsCOD$ removed. Furthermore, there was an observable improvement in methane yield over the course of the observation period, suggesting a promising trend in methane yield if continued over a longer duration. The COD balance of Reactor Vik towards the end of the operation period was 70%, implying an unaccounted loss of 30% of the initial COD. Under these conditions, approximately 4624 kWh/d of electricity and 41.6 GJ/d of energy could be recovered at IVAR Vik which has approximately 8000 $m^3/d$ of average hydraulic loading with an estimated daily removal of 3083 kgCOD based on sCOD to tCOD ratio of 69% with a tCOD concentration of 931 mg/l.

In Reactor SNJ the average dissolved COD removal efficiency was a negative 3%, indicating a negative removal rate. The methane production was virtually absent, while the average TSS removal efficiency was 72%. However, since the performance of the UASB reactor is based on COD removal and methane production, the effective removal of TSS will not have a significant impact on the overall assessment. The lack of methane production can be explained by many factors; (1) the reactor is treating low strength wastewater, which is not an ideal concentration for effective wastewater treatment by an UASB reactor and; (2) the high concentration present of inhibitors like hydrogen sulfide ($H_2S$) and salinity.

When deriving conclusions concerning the performance of the reactors, the potential errors regarding interference in the COD measurements were excluded. Treatment of medium-strength wastewater from IVAR Vik WWTP represents a feasible and promising alternative as pre-treatment for the aerobic biological treatment by reduction to approximately 250 - 300 mg/l soluble COD and converting the organic matter into economically valuable products as methane with 1080 $m^3/d$. When used as a pre-treatment the process can minimize the oxygen demand and surplus sludge formation in a subsequent aerobic post treatment. On the other hand, treatment of the wastewater from IVAR SNJ WWTP with an UASB reactor is not effective for methane generation or COD removal. However, based on this study no definitive conclusions can be drawn regarding the overall effectiveness of UASB reactors in treating low-strength wastewater.

# 7 Recommendations

Based on the results from this study, more conclusions could be provided by further studies and experimentations. Most importantly, research regarding the performance of UASB treating low strength wastewater should be further investigated. Specifically, the investigation should solely focus on the low influent COD concentration, without any significant interfering elements and inhibitors. This approach would enable a more comprehensive understanding of anaerobic treatment on low strength wastewater. Secondly, the presence and concentrations of potential inhibiting compounds should be monitored more frequently to detect and prevent complications for optimal reactor performance. By detecting potential interfering elements, their influence on analytical measurements would also be identified and diminished, for example the significant impact of chloride on the COD Cell test kits. Thirdly, the implementation of multiple parallels would contribute to increased reliability and efficiency of the system, thus reducing the risk of errors and operational disturbances. Finally, re-design of the laboratory scale reactor or increased recirculation flow distributions should be considered. According to Safitri (2016), to optimize the utilization of the entire reactor volume, an improved liquid distribution system is deemed necessary. Specifically, the feed mechanism and recirculation effluent should flow linearly from the bottom to the top of the reactor, in accordance with the height of the reactor, commencing from the lowermost section of the reactors. Various approaches have been suggested as potential solutions, including the utilization of different feed inlet devices, increasing the number of feed inlet points per square meter, or implementing higher superficial velocity (Safitri, 2016).

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# Appendixes

### Appendix 1: Daily data of Reactor Vik and Reactor SNJ

### Appendix 2: Data of VFA composition- and specification, concentrations of sulfate and chloride, and gas composition of Reactor Vik

# Appendix 1: Daily data of Reactor Vik and Reactor SNJ

## Reactor Vik

The daily measurement data of Reactor Vik are calculated and summarised in Table A.1.

***Table A.1** . Recapitulation of daily measurement of Reactor Vik*

| Dato | Klokkeslett | Biogas formation rate (ml/d) | Flow rate (ml/min) | tCOD in (mg/l) | OLR (g/l.d) | sCOD in (mg/l) | tCOD out (mg/l) | sCOD out (mg/l) | tCOD Removal Efficiency (%) | sCOD Removal Efficiency (%) | pH in | pH out | TSS in (mg/l) | TSS out (mg/l) | TSS Removal Efficiency (%) | HRT (h) | tCOD balance (%) | sCOD Balance (%) | Yield (g CODCH4/g tCODin) | Yield (g CODCH4/g sCODin) | Yield (g CODCH4/g tCOD removed) | Yield (g CODCH4/g sCOD removed) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.02.2023 | 08:00 | 0 | 1,49 | 500 | | | | | | | | | | | | | | | 0,00 | | | |
| 20.02.2023 | 08:00 | 16 | 1,49 | 500 | | | | | | | | | | | | | | | 0,02 | | | |
| 21.02.2023 | 08:00 | 16 | 1,49 | 500 | | | | | | | | | | | | | | | 0,02 | | | |
| 22.02.2023 | 08:00 | 30 | 1,49 | 500 | | | 618 | 646 | | | | 7,7 | | 2,89 | | | 127,85 | | 0,04 | | -0,18 | -0,03 |
| 23.02.2023 | 08:00 | 20 | 1,49 | 678 | 1,46 | 472 | 870 | 816 | -28,32 | -74,58 | 6,7 | 7,9 | 162 | 3 | 30,38 | 5,56 | 130,41 | 175,88 | 0,02 | 0,03 | -0,07 | -0,04 |
| 23.02.2023 | 12:00 | 118 | 1,49 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,13 | 0,18 | 0,13 | 0,18 |
| 24.02.2023 | 10:00 | 205 | 1,49 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,22 | 0,31 | 0,22 | 0,31 |
| 24.02.2023 | 16:30 | 110 | 1,49 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,12 | 0,17 | 0,12 | 0,17 |
| 25.02.2023 | 14:25 | 159 | 1,49 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,17 | 0,24 | 0,17 | 0,24 |
| 27.02.2023 | 08:00 | 137 | 1,49 | 678 | | 472 | 734 | 990 | -8,26 | -109,75 | 6,7 | 7,7 | 162 | 5,75 | 30,38 | 5,56 | 122,70 | 230,49 | 0,14 | 0,21 | -1,75 | -0,19 |
| 27.02.2023 | 12:00 | 79 | 1,49 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,08 | 0,12 | 0,08 | 0,12 |
| 28.02.2023 | 11:10 | 130 | 1,48 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,14 | 0,20 | 0,14 | 0,20 |
| 28.02.2023 | 20:15 | 103 | 1,48 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,11 | 0,16 | 0,11 | 0,16 |
| 01.03.2023 | 11:09 | 97 | 1,48 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,10 | 0,15 | 0,10 | 0,15 |
| 02.03.2023 | 11:00 | 19 | 1,48 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,02 | 0,03 | 0,02 | 0,03 |
| 02.03.2023 | 19:20 | 49 | 1,48 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,05 | 0,08 | 0,05 | 0,08 |
| 06.03.2023 | 07:30 | 181 | 1,23 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,23 | 0,33 | 0,23 | 0,33 |
| 06.03.2023 | 12:00 | 175 | 1,23 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,23 | 0,32 | 0,23 | 0,32 |
| 06.03.2023 | 14:00 | 2 | 1,23 | 678 | | 472 | | | | | 6,7 | | 162 | | | 5,56 | | | 0,00 | 0,00 | 0,00 | 0,00 |
| 07.03.2023 | 07:45 | 267 | 1,40 | 556 | 1,12 | 562 | 748 | 650 | -34,53 | -15,66 | 6,5 | 7,1 | 20,5 | 16,8 | -1,08 | 5,56 | 171,24 | 151,98 | 0,37 | 0,36 | -1,06 | -2,32 |
| 07.03.2023 | 12:00 | 241 | 1,40 | 556 | | 562 | | | | | 6,5 | | 20,5 | | | 5,56 | | | 0,33 | 0,33 | 0,33 | 0,33 |
| 08.03.2023 | 09:30 | 195 | 1,40 | 556 | | 562 | | | | | 6,5 | 7,2 | 20,5 | 10,63 | -1,08 | 5,56 | | | 0,27 | 0,27 | 0,27 | 0,27 |
| 08.03.2023 | 12:45 | 219 | 1,40 | 556 | | 562 | 572 | 566 | -2,88 | -0,71 | 6,5 | | 20,5 | | | 5,56 | 132,97 | 130,49 | 0,30 | 0,30 | -10,46 | -41,84 |
| 08.03.2023 | 16:00 | 172 | 1,40 | 556 | | 562 | | | | | 6,5 | | 20,5 | | | 5,56 | | | 0,24 | 0,23 | 0,24 | 0,23 |
| 09.03.2023 | 09:00 | 291 | 1,90 | 518 | 1,42 | 524 | 502 | 230 | 3,09 | 56,11 | 6,5 | 7,2 | 21,86 | 5 | -1,16 | 5,56 | 128,61 | 75,23 | 0,32 | 0,31 | 10,26 | 0,56 |
| 09.03.2023 | 12:15 | 272 | 1,90 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,30 | 0,29 | 0,30 | 0,29 |
| 10.03.2023 | 10:53 | 230 | 1,90 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,25 | 0,25 | 0,25 | 0,25 |
| 10.03.2023 | 11:46 | 182 | 1,90 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,20 | 0,20 | 0,20 | 0,20 |
| 11.03.2023 | 09:15 | 143 | 1,90 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,16 | 0,15 | 0,16 | 0,15 |
| 12.03.2023 | 12:14 | 9 | 1,90 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,01 | 0,01 | 0,01 | 0,01 |
| 13.03.2023 | 10:45 | 18 | 1,94 | 518 | | 524 | 265 | 242 | 48,84 | 53,82 | 6,5 | 7,1 | 21,86 | 17,69 | -1,16 | 5,56 | 53,03 | 48,04 | 0,02 | 0,02 | 0,04 | 0,03 |
| 13.03.2023 | 13:40 | 325 | 1,94 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,35 | 0,34 | 0,35 | 0,34 |
| 14.03.2023 | 08:07 | 107 | 1,92 | 518 | | 524 | 303 | 242 | 41,51 | 53,82 | 6,5 | 7,3 | 21,86 | 9,58 | -1,16 | 5,56 | 69,97 | 57,53 | 0,11 | 0,11 | 0,28 | 0,21 |
| 14.03.2023 | 11:20 | 567 | 1,92 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,61 | 0,60 | 0,61 | 0,60 |
| 15.03.2023 | 08:20 | 154 | 1,95 | 518 | | 524 | 250 | 327 | 51,74 | 37,60 | 6,5 | 7,1 | 21,86 | 9,17 | -1,16 | 5,56 | 64,61 | 78,57 | 0,16 | 0,16 | 0,32 | 0,43 |
| 15.03.2023 | 11:30 | 150 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,16 | 0,16 | 0,16 | 0,16 |
| 15.03.2023 | 14:00 | 126 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,13 | 0,13 | 0,13 | 0,13 |
| 16.03.2023 | 08:00 | 118 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,13 | 0,12 | 0,13 | 0,12 |
| 17.03.2023 | 17:20 | 57 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,06 | 0,06 | 0,06 | 0,06 |
| 20.03.2023 | 08:00 | 46 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,05 | 0,05 | 0,05 | 0,05 |
| 20.03.2023 | 13:00 | 174 | 1,95 | 518 | | 524 | | | | | 6,5 | | 21,86 | | | 5,56 | | | 0,18 | 0,18 | 0,18 | 0,18 |
| 21.03.2023 | 07:45 | 295 | 2,70 | 300 | 1,17 | 214 | 207 | 75 | 31 | 64,95 | 6,6 | 7,1 | 2,79 | 14,24 | 28,67 | 5,56 | 107,99 | 89,71 | 0,39 | 0,55 | 1,26 | 0,84 |
| 21.03.2023 | 15:30 | 295 | 2,70 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,39 | 0,55 | 0,39 | 0,55 |
| 21.03.2023 | 16:26 | 74 | 2,70 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,10 | 0,14 | 0,10 | 0,14 |
| 22.03.2023 | 08:12 | 256 | 2,88 | 300 | | 214 | 234 | 214 | 22 | 0 | 6,6 | 7,4 | 2,79 | 18,45 | 28,67 | 5,56 | 109,75 | 144,51 | 0,32 | 0,45 | 1,44 | 0,00 |
| 22.03.2023 | 11:37 | 254 | 2,88 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,32 | 0,44 | 0,32 | 0,44 |
| 23.03.2023 | 08:15 | 233 | 2,82 | 300 | | 214 | 90 | 70 | 70 | 67,29 | 6,6 | 7,4 | 2,79 | 13,87 | 28,67 | 5,56 | 59,56 | 74,15 | 0,30 | 0,41 | 0,42 | 0,62 |
| 23.03.2023 | 13:30 | 196 | 2,82 | 300 | | 214 | 188 | 75 | 37,33 | 64,95 | 6,6 | | 2,79 | | | 5,56 | 87,43 | 69,76 | 0,25 | 0,35 | 0,66 | 0,53 |
| 24.03.2023 | 10:08 | 142 | 2,82 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,18 | 0,25 | 0,18 | 0,25 |
| 25.03.2023 | 10:01 | 116 | 2,82 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,15 | 0,21 | 0,15 | 0,21 |
| 26.03.2023 | 11:09 | 72 | 2,82 | 300 | | 214 | | | | | 6,6 | | 2,79 | | | 5,56 | | | 0,09 | 0,13 | 0,09 | 0,13 |
| 27.03.2023 | 08:25 | 52 | 2,56 | 343 | 1,26 | 194 | 868 | 183 | -153,06 | 5,67 | 6,8 | 7,1 | 259 | 11,8 | 43,44 | 5,56 | 259,40 | 105,54 | 0,06 | 0,11 | -0,04 | 1,98 |
| 27.03.2023 | 09:47 | 58 | 2,56 | 343 | | 194 | | | | | 6,8 | | 259 | | | 5,56 | | | 0,07 | 0,12 | 0,07 | 0,12 |
| 27.03.2023 | 10:27 | 0 | 2,56 | 343 | | 194 | | | | | 6,8 | | 259 | | | 5,56 | | | 0,00 | 0,00 | 0,00 | 0,00 |
| 27.03.2023 | 13:47 | 47 | 2,56 | 343 | | 194 | | | | | 6,8 | | 259 | | | 5,56 | | | 0,06 | 0,10 | 0,06 | 0,10 |
| 27.03.2023 | 17:15 | 91 | 2,56 | 343 | | 194 | | | | | 6,8 | | 259 | | | 5,56 | | | 0,11 | 0,20 | 0,11 | 0,20 |
| 28.03.2023 | 08:15 | 105 | 2,6 | 892 | 3,34 | 533 | 596 | 461 | 33,18 | 13,51 | 6,9 | 7,3 | 244,69 | 9,66 | 40,25 | 2,78 | 71,68 | 94,63 | 0,05 | 0,08 | 0,15 | 0,60 |
| 28.03.2023 | 11:30 | 121 | 2,6 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,06 | 0,09 | 0,06 | 0,09 |
| 28.03.2023 | 14:00 | 126 | 2,6 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,06 | 0,10 | 0,06 | 0,10 |
| 28.03.2023 | 14:44 | 0 | 2,6 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,00 | 0,00 | 0,00 | 0,00 |
| 28.03.2023 | 17:05 | 103 | 2,6 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,05 | 0,08 | 0,05 | 0,08 |
| 29.03.2023 | 08:51 | 70 | 2,67 | 892 | | 533 | 978 | 528 | -9,64 | 0,94 | 6,9 | 7,1 | 244,69 | 8,33 | 40,25 | 2,78 | 112,78 | 104,31 | 0,03 | 0,05 | -0,33 | 5,60 |
| 29.03.2023 | 13:27 | 51 | 2,67 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,02 | 0,04 | 0,02 | 0,04 |
| 29.03.2023 | 16:30 | 26 | 2,67 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,01 | 0,02 | 0,01 | 0,02 |
| 30.03.2023 | 07:55 | 46 | 4,35 | 892 | | 533 | 796 | 536 | 10,76 | -0,56 | 6,9 | 7,2 | 244,69 | 3,34 | 40,25 | 2,78 | 90,51 | 102,69 | 0,01 | 0,02 | 0,12 | -3,78 |
| 30.03.2023 | 09:40 | 45 | 4,35 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,01 | 0,02 | 0,01 | 0,02 |
| 30.03.2023 | 11:38 | 0 | 4,35 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,00 | 0,00 | 0,00 | 0,00 |
| 30.03.2023 | 12:55 | 246 | 4,35 | 892 | | 533 | | | | | 6,9 | | 244,69 | | | 2,78 | | | 0,07 | 0,11 | 0,07 | 0,11 |
| 31.03.2023 | 07:40 | 442 | 4,29 | 1012 | 6,25 | 314 | 208 | 164 | 79,45 | 47,77 | 6,7 | 6,8 | 391,96 | 25,86 | 68,97 | 2,78 | 31,47 | 87,40 | 0,11 | 0,35 | 0,14 | 0,74 |
| 31.03.2023 | 11:30 | 577 | 4,29 | 1012 | | 314 | | | | | 6,7 | | 391,96 | | | 2,78 | | | 0,14 | 0,46 | 0,14 | 0,46 |
| 31.03.2023 | 13:23 | 168 | 4,29 | 1012 | | 314 | | | | | 6,7 | | 391,96 | | | 2,78 | | | 0,04 | 0,13 | 0,04 | 0,13 |
| 31.03.2023 | 13:35 | 790 | 4,29 | 1012 | | 314 | | | | | 6,7 | | 391,96 | | | 2,78 | | | 0,19 | 0,63 | 0,19 | 0,63 |
| 31.03.2023 | 15:00 | 451 | 4,29 | 1012 | | 314 | | | | | 6,7 | | 391,96 | | | 2,78 | | | 0,11 | 0,36 | 0,11 | 0,36 |
| 03.04.2023 | 07:00 | 422 | 3,99 | 780 | 4,48 | 318 | 208 | 180 | 73,33 | 43,40 | 6,8 | 6,9 | 218,5 | 11,7 | 59,23 | 2,78 | 41,19 | 92,22 | 0,15 | 0,36 | 0,20 | 0,82 |
| 03.04.2023 | 08:30 | 263 | 3,99 | 780 | | 318 | | | | | 6,8 | | 218,5 | | | 2,78 | | | 0,09 | 0,22 | 0,09 | 0,22 |
| 03.04.2023 | 11:15 | 316 | 3,99 | 780 | | 318 | | | | | 6,8 | | 218,5 | | | 2,78 | | | 0,11 | 0,27 | 0,11 | 0,27 |
| 04.04.2023 | 11:50 | 453 | 4,14 | 366 | 2,18 | 240 | 234 | 90 | 36,07 | 62,5 | 6,8 | 7,5 | 31,72 | 18,25 | 34,43 | 2,78 | 95,96 | 86,34 | 0,32 | 0,49 | 0,89 | 0,78 |
| 05.04.2023 | 10:10 | 417 | 4,14 | 366 | | 240 | 228 | 126 | 37,70 | 47,5 | 6,8 | 6,6 | 31,72 | 22,24 | 34,43 | 2,78 | 91,79 | 97,49 | 0,29 | 0,45 | 0,78 | 0,95 |
| 05.04.2023 | 12:50 | 444 | 4,14 | 366 | | 240 | | | | | 6,8 | | 31,72 | | | 2,78 | | | 0,31 | 0,48 | 0,31 | 0,48 |
| 05.04.2023 | 14:35 | 451 | 4,14 | 366 | | 240 | | | | | 6,8 | | 31,72 | | | 2,78 | | | 0,32 | 0,49 | 0,32 | 0,49 |
| 05.04.2023 | 15:40 | 364 | 4,14 | 366 | | 240 | | | | | 6,8 | | 31,72 | | | 2,78 | | | 0,26 | 0,39 | 0,26 | 0,39 |
| 08.04.2023 | 13:45 | 686 | 4,14 | 366 | | 240 | | | | | 6,8 | | 31,72 | | | 2,78 | | | 0,49 | 0,74 | 0,49 | 0,74 |
| 11.04.2023 | 07:25 | 675 | 4,9 | 264 | 1,86 | 104 | 140 | 116 | 46,97 | -11,54 | 6,7 | 7 | 101,97 | 14,29 | 60,61 | 2,78 | 108,90 | 253,37 | 0,56 | 1,42 | 1,19 | -12,29 |
| 11.04.2023 | 09:30 | 720 | 4,9 | 264 | | 104 | | | | | 6,7 | | 101,97 | | | 2,78 | | | 0,60 | 1,51 | 0,60 | 1,51 |
| 11.04.2023 | 11:26 | 735 | 4,9 | 264 | | 104 | | | | | 6,7 | | 101,97 | | | 2,78 | | | 0,61 | 1,55 | 0,61 | 1,55 |
| 11.04.2023 | 12:50 | 733 | 4,9 | 264 | | 104 | | | | | 6,7 | | 101,97 | | | 2,78 | | | 0,61 | 1,54 | 0,61 | 1,54 |
| 12.04.2023 | 08:48 | 700 | 4,82 | 264 | | 104 | 208 | 176 | 21,21 | -69,23 | 7,2 | 7,3 | 101,97 | 34,03 | 60,61 | 2,78 | 137,72 | 318,84 | 0,59 | 1,50 | 2,78 | -2,16 |
| 12.04.2023 | 13:47 | 824 | 4,82 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,69 | 1,76 | 0,69 | 1,76 |
| 13.04.2023 | 09:45 | 154 | 4,33 | 264 | | 104 | 156 | 88 | 40,91 | 15,38 | 7,2 | 7,2 | 101,97 | 21,4 | 60,61 | 2,78 | 73,55 | 121,31 | 0,14 | 0,37 | 0,35 | 2,39 |
| 13.04.2023 | 12:10 | 1243 | 4,33 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 1,17 | 2,96 | 1,17 | 2,96 |
| 13.04.2023 | 13:00 | 0 | 4,33 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,00 | 0,00 | 0,00 | 0,00 |
| 14.04.2023 | 07:20 | 172 | 5,56 | 264 | | 104 | 132 | 96 | 50 | 7,69 | 7,2 | 7,2 | 101,97 | 27,4 | 60,61 | 2,78 | 62,58 | 124,23 | 0,13 | 0,32 | 0,25 | 4,15 |
| 14.04.2023 | 08:20 | 237 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,17 | 0,44 | 0,17 | 0,44 |
| 14.04.2023 | 10:00 | 190 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,14 | 0,35 | 0,14 | 0,35 |
| 14.04.2023 | 12:48 | 226 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,16 | 0,42 | 0,16 | 0,42 |
| 14.04.2023 | 13:10 | 215 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,16 | 0,40 | 0,16 | 0,40 |
| 14.04.2023 | 14:40 | 636 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,46 | 1,18 | 0,46 | 1,18 |
| 15.04.2023 | 14:21 | 943 | 5,56 | 264 | | 104 | | | | | 7,2 | | 101,97 | | | 2,78 | | | 0,69 | 1,75 | 0,69 | 1,75 |
| 17.04.2023 | 13:20 | 0 | 4,82 | 1486 | 10,31 | 384 | 384 | 152 | 74,16 | 60,42 | 6,8 | 6,9 | 322,7 | 113,8 | 74,16 | 2,78 | 25,84 | 39,58 | 0,00 | 0,00 | 0,00 | 0,00 |
| 17.04.2023 | 18:39 | 1070 | 4,82 | 1486 | | 384 | | | | | 6,8 | | 322,7 | | | 2,78 | | | 0,16 | 0,62 | 0,16 | 0,62 |
| 18.04.2023 | 07:26 | 1037 | 4,99 | 1304 | 9,37 | 742 | 546 | 260 | 58,13 | 64,96 | 6,4 | 7 | 189 | 93,9 | 43,10 | 2,78 | 58,95 | 65,06 | 0,17 | 0,30 | 0,29 | 0,46 |
| 18.04.2023 | 08:30 | 962 | 4,99 | 1304 | | 742 | | | | | 6,4 | | 189 | | | 2,78 | | | 0,16 | 0,28 | 0,16 | 0,28 |
| 18.04.2023 | 12:10 | 1077 | 4,99 | 1304 | | 742 | | | | | 6,4 | | 189 | | | 2,78 | | | 0,18 | 0,31 | 0,18 | 0,31 |
| 18.04.2023 | 13:30 | 1066 | 4,99 | 1304 | | 742 | | | | | 6,4 | | 189 | | | 2,78 | | | 0,18 | 0,31 | 0,18 | 0,31 |
| 18.04.2023 | 14:15 | 957 | 4,99 | 1304 | | 742 | | | | | 6,4 | | 189 | | | 2,78 | | | 0,16 | 0,28 | 0,16 | 0,28 |
| 19.04.2023 | 07:40 | 1060 | 5,2 | 964 | 7,22 | 378 | 470 | 234 | 51,24 | 38,10 | 6,4 | 7,2 | 106,26 | 87,69 | 60,79 | 2,78 | 71,42 | 119,71 | 0,23 | 0,58 | 0,44 | 1,52 |
| 19.04.2023 | 08:57 | 1169 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,25 | 0,64 | 0,25 | 0,64 |
| 19.04.2023 | 09:50 | 1073 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,23 | 0,58 | 0,23 | 0,58 |
| 19.04.2023 | 11:21 | 1145 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,24 | 0,62 | 0,24 | 0,62 |
| 19.04.2023 | 12:15 | 1141 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,24 | 0,62 | 0,24 | 0,62 |
| 19.04.2023 | 13:57 | 1115 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,24 | 0,61 | 0,24 | 0,61 |
| 19.04.2023 | 15:00 | 1059 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,23 | 0,58 | 0,23 | 0,58 |
| 19.04.2023 | 17:30 | 1134 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,24 | 0,62 | 0,24 | 0,62 |
| 20.04.2023 | 07:48 | 1099 | 5,2 | 964 | | 378 | 308 | 236 | 68,05 | 37,57 | 6,4 | 7,1 | 106,26 | 38,6 | 60,79 | 2,78 | 55,44 | 122,33 | 0,23 | 0,60 | 0,35 | 1,59 |
| 20.04.2023 | 08:54 | 1220 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,26 | 0,67 | 0,26 | 0,67 |
| 20.04.2023 | 12:50 | 1084 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,23 | 0,59 | 0,23 | 0,59 |
| 20.04.2023 | 16:43 | 1139 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,24 | 0,62 | 0,24 | 0,62 |
| 20.04.2023 | 17:44 | 1010 | 5,2 | 964 | | 378 | | | | | 6,4 | | 106,26 | | | 2,78 | | | 0,22 | 0,55 | 0,22 | 0,55 |
| 21.04.2023 | 08:00 | 818 | 6,2 | 834 | 7,45 | 418 | 384 | 186 | 53,96 | 55,50 | 6,5 | 7,1 | 261,47 | 103,65 | 49,88 | 2,78 | 63,00 | 78,33 | 0,17 | 0,34 | 0,31 | 0,61 |
| 21.04.2023 | 10:20 | 1265 | 6,2 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,26 | 0,52 | 0,26 | 0,52 |
| 21.04.2023 | 10:57 | 1790 | 6,2 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,37 | 0,74 | 0,37 | 0,74 |
| 21.04.2023 | 15:00 | 1209 | 6,2 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,25 | 0,50 | 0,25 | 0,50 |
| 21.04.2023 | 16:10 | 1296 | 6,2 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,27 | 0,54 | 0,27 | 0,54 |
| 23.04.2023 | 18:37 | 1261 | 4,74 | 834 | | 418 | 384 | 374 | 53,96 | 10,53 | 6,5 | 7,3 | 261,47 | 63,37 | 49,88 | 2,78 | 80,23 | 157,68 | 0,34 | 0,68 | 0,63 | 6,48 |
| 23.04.2023 | 19:02 | 1327 | 4,74 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,36 | 0,72 | 0,36 | 0,72 |
| 23.04.2023 | 19:23 | 1579 | 4,74 | 834 | | 418 | | | | | 6,5 | | 261,47 | | | 2,78 | | | 0,43 | 0,85 | 0,43 | 0,85 |
| 24.04.2023 | 08:57 | 1408 | 4,96 | 902 | 6,44 | 782 | 542 | 282 | 39,91 | 63,94 | 6,2 | 7 | 85,31 | 67,67 | 13,30 | 2,78 | 93,80 | 74,94 | 0,34 | 0,39 | 0,84 | 0,61 |
| 24.04.2023 | 09:19 | 1571 | 4,96 | 902 | | 782 | | | | | 6,2 | | 85,31 | | | 2,78 | | | 0,38 | 0,43 | 0,38 | 0,43 |
| 24.04.2023 | 11:13 | 1449 | 4,96 | 902 | | 782 | | | | | 6,2 | | 85,31 | | | 2,78 | | | 0,35 | 0,40 | 0,35 | 0,40 |
| 24.04.2023 | 13:35 | 1401 | 4,96 | 902 | | 782 | | | | | 6,2 | | 85,31 | | | 2,78 | | | 0,34 | 0,39 | 0,34 | 0,39 |
| 24.04.2023 | 15:12 | 1465 | 4,96 | 902 | | 782 | | | | | 6,2 | | 85,31 | | | 2,78 | | | 0,35 | 0,40 | 0,35 | 0,40 |
| 24.04.2023 | 17:00 | 1404 | 4,96 | 902 | | 782 | | | | | 6,2 | | 85,31 | | | 2,78 | | | 0,34 | 0,39 | 0,34 | 0,39 |
| 28.04.2023 | 07:23 | 1109 | 5,14 | 1128 | | 728 | 344 | 302 | 69,50 | 58,52 | 6,6 | 7,1 | 85,31 | | | 2,78 | 50,98 | 73,23 | 0,20 | 0,32 | 0,29 | 0,54 |
| 28.04.2023 | 11:15 | 269 | 5,14 | 1128 | | 728 | 360 | 318 | 68,09 | 56,32 | 6,6 | | 85,31 | | | 2,78 | 36,89 | 51,40 | 0,05 | 0,08 | 0,07 | 0,14 |

## Reactor SNJ

The daily measurement data of Reactor SNJ are calculated and summarised in Table A.2.

***Table A.2.*** *Recapitulation of daily measurement of Reactor SNJ*

| Dato | Klokkeslett | Biogas formation rate (ml/d) | Flow rate (ml/min) | tCOD in (mg/l) | OLR (g/l.d) | sCOD in (mg/l) | tCOD out (mg/l) | sCOD out (mg/l) | tCOD Removal Efficiency (%) | sCOD Removal Efficiency (%) | pH in | pH out | TSS in (mg/l) | TSS out (mg/l) | TSS Removal Efficiency (%) | HRT (h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.02.2023 | 08:00 | 0 | 0,64 | 500 | | | | | | | | | | | | 16,67 |
| 20.02.2023 | 08:00 | 1 | 0,64 | 500 | | | | | | | | | | | | 16,67 |
| 21.02.2023 | 08:00 | 0 | 0,64 | 500 | | | | | | | | | | | | 16,67 |
| 22.02.2023 | 08:00 | 0 | 0,64 | 500 | | | 582 | 530 | | | | 7 | | 1,13 | | 16,67 |
| 23.02.2023 | 08:00 | 7 | 0,64 | 676 | 0,62 | 614 | 824 | 890 | -21,89 | -44,95 | 7,7 | 7,5 | 113 | 2 | 98,23 | 5,56 |
| 23.02.2023 | 12:00 | 20 | 0,62 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 24.02.2023 | 10:00 | 0 | 0,62 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 24.02.2023 | 16:30 | 1 | 0,62 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 25.02.2023 | 14:25 | 0 | 0,62 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 27.02.2023 | 08:00 | 366 | 0,62 | 676 | | 614 | 786 | 664 | -16,27 | -8,14 | 7,7 | 7,6 | 113 | 2,97 | 97,37 | 5,56 |
| 27.02.2023 | 12:00 | 1151 | 0,62 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 28.02.2023 | 11:10 | 0 | 0,63 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 28.02.2023 | 20:15 | 0 | 0,63 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 01.03.2023 | 11:09 | 0 | 0,63 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 02.03.2023 | 11:00 | 0 | 0,63 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 02.03.2023 | 19:20 | 0 | 0,63 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 06.03.2023 | 07:30 | 1426 | 5,02 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 06.03.2023 | 12:00 | 139 | 5,02 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 06.03.2023 | 14:00 | 0 | 5,02 | 676 | | 614 | | | | | 7,7 | | 113 | | | 5,56 |
| 07.03.2023 | 07:45 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | 6,9 | 25,3 | 14,1 | 44,27 | 5,56 |
| 07.03.2023 | 12:00 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 08.03.2023 | 09:30 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 08.03.2023 | 12:45 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 08.03.2023 | 16:00 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 09.03.2023 | 09:00 | | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 09.03.2023 | 12:15 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 10.03.2023 | 10:53 | 41 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 10.03.2023 | 11:46 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 11.03.2023 | 09:15 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 12.03.2023 | 12:14 | 0 | 5,02 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 13.03.2023 | 10:45 | 10 | 2,77 | 676 | | 614 | 307 | 379 | 54,59 | 38,27 | 6,9 | 7 | 25,3 | 4,06 | 83,95 | 5,56 |
| 13.03.2023 | 13:40 | 27 | 2,77 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 14.03.2023 | 08:07 | 8 | 1,97 | 676 | | 614 | 427 | 527 | 36,83 | 14,17 | 6,9 | 7,3 | 25,3 | 3,64 | 85,61 | 5,56 |
| 14.03.2023 | 11:20 | 24 | 1,97 | 676 | | 614 | | | | | 6,9 | | 25,3 | | | 5,56 |
| 15.03.2023 | 08:20 | 37 | 2,80 | 636 | 2,56 | 575 | 791 | 450 | -24,37 | 21,74 | 7,2 | 7,1 | 82,9 | 9,28 | 88,81 | 5,56 |
| 15.03.2023 | 11:30 | 49 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 15.03.2023 | 14:00 | 31 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 16.03.2023 | 08:00 | 35 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 17.03.2023 | 17:20 | 37 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 20.03.2023 | 08:00 | 31 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 20.03.2023 | 13:00 | 109 | 2,80 | 636 | | 575 | | | | | 7,2 | | 82,9 | | | 5,56 |
| 21.03.2023 | 07:45 | 33 | 2,76 | 483 | 1,92 | 478 | 511 | 474 | -5,80 | 0,84 | 7 | 7,5 | 33,19 | 11,64 | 64,93 | 5,56 |
| 21.03.2023 | 15:30 | 40 | 2,76 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 21.03.2023 | 16:26 | 0 | 2,76 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 22.03.2023 | 08:12 | 35 | 2,77 | 483 | | 478 | 503 | 483 | -4,14 | -1,05 | 7 | 7,1 | 33,19 | 16,3 | 50,89 | 5,56 |
| 22.03.2023 | 11:37 | 68 | 2,77 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 23.03.2023 | 08:15 | 34 | 2,76 | 483 | | 478 | 422 | 414 | 12,63 | 13,39 | 7 | 7,2 | 33,19 | 19,5 | 41,25 | 5,56 |
| 23.03.2023 | 13:30 | 74 | 2,76 | 483 | | 478 | 432 | 263 | 10,56 | 44,98 | 7 | | 33,19 | | | 5,56 |
| 24.03.2023 | 10:08 | 38 | 2,76 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 25.03.2023 | 10:01 | 20 | 2,76 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 26.03.2023 | 11:09 | 28 | 2,76 | 483 | | 478 | | | | | 7 | | 33,19 | | | 5,56 |
| 27.03.2023 | 08:25 | 22 | 2,61 | 483 | | 478 | 790 | 560 | -63,56 | -17,15 | 7,1 | 7,5 | 111 | 16,8 | 84,86 | 5,56 |
| 27.03.2023 | 09:47 | 57 | 2,61 | 483 | | 478 | | | | | 7,1 | | 111 | | | 5,56 |
| 27.03.2023 | 10:27 | 0 | 2,61 | 483 | | 478 | | | | | 7,1 | | 111 | | | 5,56 |
| 27.03.2023 | 13:47 | 47 | 2,61 | 483 | | 478 | | | | | 7,1 | | 111 | | | 5,56 |
| 27.03.2023 | 17:15 | 0 | 2,61 | 483 | | 478 | | | | | 7,1 | | 111 | | | 5,56 |
| 28.03.2023 | 08:15 | 5 | 2,63 | 483 | | 478 | | | | | 7,1 | 7,8 | 43,55 | 9,25 | 78,76 | 2,78 |
| 28.03.2023 | 11:30 | 0 | 2,63 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 28.03.2023 | 14:00 | 31 | 2,63 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 28.03.2023 | 14:44 | 0 | 2,63 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 28.03.2023 | 17:05 | 0 | 2,63 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 29.03.2023 | 08:51 | 15 | 2,72 | 483 | | 478 | | | | | 7,1 | 7,5 | 43,55 | 4,85 | 88,86 | 2,78 |
| 29.03.2023 | 13:27 | 17 | 2,72 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 29.03.2023 | 16:30 | 26 | 2,72 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 30.03.2023 | 07:55 | 15 | 5,03 | 483 | | 478 | | | | | 7,1 | 7,3 | 43,55 | 6,51 | 85,05 | 2,78 |
| 30.03.2023 | 09:40 | 45 | 5,03 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 30.03.2023 | 11:38 | 40 | 5,03 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 30.03.2023 | 12:55 | 0 | 5,03 | 483 | | 478 | | | | | 7,1 | | 43,55 | | | 2,78 |
| 31.03.2023 | 07:40 | 17 | 4,99 | 1216 | 8,74 | 668 | 446 | 590 | 63,32 | 11,68 | 7,3 | 7,5 | 549,09 | 6,04 | 98,90 | 2,78 |
| 31.03.2023 | 11:30 | 41 | 4,99 | 1374 | 9,87 | 478 | 422 | 606 | 69,29 | -26,78 | 7,3 | | 549,09 | | | 2,78 |
| 31.03.2023 | 13:23 | 0 | 4,99 | 1374 | | 478 | | | | | 7,3 | | 549,09 | | | 2,78 |
| 31.03.2023 | 13:35 | 0 | 4,99 | 1374 | | 478 | | | | | 7,3 | | 549,09 | | | 2,78 |
| 31.03.2023 | 15:00 | 0 | 4,99 | 1374 | | 478 | | | | | 7,3 | | 549,09 | | | 2,78 |
| 03.04.2023 | 07:00 | 21 | 4,92 | 352 | 2,49 | 308 | 346 | 400 | 1,70 | -29,87 | 7,3 | 6,9 | 12,6 | 7,34 | 41,75 | 2,78 |
| 03.04.2023 | 08:30 | 0 | 4,92 | 352 | | 308 | | | | | 7,3 | | 12,6 | | | 2,78 |
| 03.04.2023 | 11:15 | 28 | 4,92 | 352 | | 308 | | | | | 7,3 | | 12,6 | | | 2,78 |
| 04.04.2023 | 11:50 | 19 | 5,05 | 394 | 2,87 | 72 | 290 | 140 | 26,40 | -94,44 | 6,9 | 7,5 | 10,13 | 7,39 | 27,05 | 2,78 |
| 05.04.2023 | 10:10 | 17 | 5,05 | 394 | | 72 | 388 | 100 | 1,52 | -38,89 | 6,9 | 7,1 | 10,13 | 6,83 | 32,58 | 2,78 |
| 05.04.2023 | 12:50 | 0 | 5,05 | 394 | | 72 | | | | | 6,9 | | 10,13 | | | 2,78 |
| 05.04.2023 | 14:35 | 0 | 5,05 | 394 | | 72 | | | | | 6,9 | | 10,13 | | | 2,78 |
| 05.04.2023 | 15:40 | 72 | 5,05 | 394 | | 72 | | | | | 6,9 | | 10,13 | | | 2,78 |
| 08.04.2023 | 13:45 | 12 | 5,05 | 394 | | 72 | | | | | 6,9 | | 10,13 | | | 2,78 |
| 11.04.2023 | 07:25 | 15 | 5 | 136 | 0,98 | 72 | 110 | 100 | 19,12 | -38,89 | 7 | 7,2 | 33,13 | 10,9 | 67,10 | 2,78 |
| 11.04.2023 | 09:30 | 0 | 5 | 136 | | 72 | | | | | 7 | | 33,13 | | | 2,78 |
| 11.04.2023 | 11:26 | 40 | 5 | 136 | | 72 | | | | | 7 | | 33,13 | | | 2,78 |
| 11.04.2023 | 12:50 | 0 | 5 | 136 | | 72 | | | | | 7 | | 33,13 | | | 2,78 |
| 12.04.2023 | 08:48 | 8 | 5 | 136 | | 72 | | | | | 7 | | 33,13 | | | 2,78 |
| 12.04.2023 | 13:47 | 16 | 5 | 136 | | 72 | | | | | 7 | | 33,13 | | | 2,78 |
| 13.04.2023 | 09:45 | 8 | 5,04 | 164 | 1,19 | 104 | 100 | 90 | 39,02 | 13,46 | 7 | 7,4 | 73,12 | 6,88 | 90,59 | 2,78 |
| 13.04.2023 | 12:10 | 0 | 5,04 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 13.04.2023 | 13:00 | 0 | 5,04 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 14.04.2023 | 07:20 | 9 | 4,45 | 164 | | 104 | 88 | 88 | 46,34 | 15,38 | 7 | 7,2 | 73,12 | 6,1 | 91,66 | 2,78 |
| 14.04.2023 | 08:20 | 0 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 14.04.2023 | 10:00 | 0 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 14.04.2023 | 12:48 | 0 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 14.04.2023 | 13:10 | 0 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 14.04.2023 | 14:40 | 0 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 15.04.2023 | 14:21 | 20 | 4,45 | 164 | | 104 | | | | | 7 | | 73,12 | | | 2,78 |
| 17.04.2023 | 13:20 | 17 | 4,93 | 132 | 0,94 | 104 | 96 | 136 | 27,27 | -30,77 | 6,9 | 7 | 19,2 | 12 | 37,50 | 2,78 |
| 17.04.2023 | 18:39 | 45 | 4,93 | 132 | | 104 | | | | | 6,9 | | 19,2 | | | 2,78 |
| 18.04.2023 | 07:26 | 24 | 5,08 | 528 | 3,86 | 468 | 380 | 438 | 28,03 | 6,41 | 6,9 | 7,1 | 40,8 | 2,97 | 92,72 | 2,78 |
| 18.04.2023 | 08:30 | 0 | 5,08 | 528 | | 468 | | | | | 6,9 | | 40,8 | | | 2,78 |
| 18.04.2023 | 12:10 | 43 | 5,08 | 528 | | 468 | | | | | 6,9 | | 40,8 | | | 2,78 |
| 18.04.2023 | 13:30 | 0 | 5,08 | 528 | | 468 | | | | | 6,9 | | 40,8 | | | 2,78 |
| 18.04.2023 | 14:15 | 0 | 5,08 | 528 | | 468 | | | | | 6,9 | | 40,8 | | | 2,78 |
| 19.04.2023 | 07:40 | 27 | 5,1 | 384 | 2,82 | 406 | 292 | 236 | 23,96 | 41,87 | 7 | 7,2 | 20,13 | 7,67 | 61,90 | 2,78 |
| 19.04.2023 | 08:57 | 0 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 09:50 | 88 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 11:21 | 0 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 12:15 | 0 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 13:57 | 46 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 15:00 | 0 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 19.04.2023 | 17:30 | 31 | 5,1 | 384 | | 406 | | | | | 7 | | 20,13 | | | 2,78 |
| 20.04.2023 | 07:48 | 33 | 5,1 | 382 | 2,81 | 400 | | | | | 7,1 | | 20,13 | | | 2,78 |
| 20.04.2023 | 08:54 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 20,13 | | | 2,78 |
| 20.04.2023 | 12:50 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 20,13 | | | 2,78 |
| 20.04.2023 | 16:43 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 20,13 | | | 2,78 |
| 20.04.2023 | 17:44 | 460 | 5,1 | 382 | | 400 | | | | | 7,1 | | 20,13 | | | 2,78 |
| 21.04.2023 | 08:00 | 20 | 5,1 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 21.04.2023 | 10:20 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 21.04.2023 | 10:57 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 21.04.2023 | 15:00 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 21.04.2023 | 16:10 | 0 | 5,1 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 23.04.2023 | 18:37 | 12 | 5,07 | 382 | | 400 | 358 | 280 | 6,28 | 30,00 | 7,1 | 7,3 | 66,06 | 8,7 | 86,83 | 2,78 |
| 23.04.2023 | 19:02 | 245 | 5,07 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 23.04.2023 | 19:23 | 1337 | 5,07 | 382 | | 400 | | | | | 7,1 | | 66,06 | | | 2,78 |
| 24.04.2023 | 08:57 | 6 | 5,09 | 556 | 4,08 | 470 | 386 | 450 | 30,58 | 4,26 | 7 | 7,3 | 26,9 | 6,44 | 76,06 | 2,78 |
| 24.04.2023 | 09:19 | 213 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 24.04.2023 | 11:13 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 24.04.2023 | 13:35 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 24.04.2023 | 15:12 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 24.04.2023 | 17:00 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 28.04.2023 | 07:23 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |
| 28.04.2023 | 11:15 | 0 | 5,09 | 556 | | 470 | | | | | 7 | | 26,9 | | | 2,78 |

## Appendix 2: Data of VFA composition- and specification, concentrations of sulfate and chloride, and gas composition of Reactor Vik

***Table A.3.*** *Total VFA analysis using 5 points titration*

| | **VIK** | | **SNJ** | |
|---|---|---|---|---|
| | **In** | **Out** | **In** | **Out** |
| **Total VFA (mgHAc/l)** | 40,5 | 76,5 | 13,5 | 32 |

***Table A.4.*** *VFA Specification, SO4, Cl, using IC*

| | **Vik** | | **SNJ** | |
|---|---|---|---|---|
| | **Concentration (mgHAc/l)** | | **Concentration (mgHAc/l)** | |
| **VFA** | **In** | **Out** | **In** | **Out** |
| **Acetic acid** | 13,1 | 42,5 | 4,6 | 10,8 |
| **Propionic acid** | 7,9 | 14,8 | 2,1 | 5,9 |
| **Butyric acid** | 7,6 | 8,5 | 3,5 | 7,8 |
| **Other acids** | 11,9 | 10,7 | 3,3 | 7,5 |

***Table A.5.*** *Concentrations of sulfate and chloride*

| | **Vik** | | **SNJ** | |
|---|---|---|---|---|
| | **In** | **Out** | **In** | **Out** |
| **SO4 Concentration (M)** | 0,19 | 0,39 | 0,60 | 0,50 |
| **Cl Concentration (M)** | 1,78 | 1,78 | 8,36 | 8,43 |

***Table A.6.*** *Gas composition of Reactor Vik*

| **Gas** | **%** |
|---|---|
| Methane | 54 |
| Carbon dioxide | 46 |